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Circular Dichroism of *N*-Acetyl-L-amino Acid Methylamides with Aromatic Side Groups

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The circular dichroism (CD) and its temperature dependence were studied on *N*-acetyl-L-phenylalanine methylamide, *N*-acetyl-L-tyrosine methylamide, and *N*-acetyl-L-tryptophan methylamide, as model molecules of aromatic amino acid residues in proteins. The CD spectra indicated that the increase in temperature and the decrease in solvent polarity give a similar effect on the amide $\pi^* \leftarrow n$ transition. The CD behavior of the tryptophan derivative was different from that of the phenylalanine and tyrosine derivatives, suggesting that the bulkier indolyl side group interacts with the peptide backbone in a different way. The noticeable temperature dependence of the CD intensities in the 250—300 nm region observed for the phenylalanine and tryptophan derivatives suggested the coexistence of various conformers at higher temperatures.

Protein molecules contain chromophoric groups such as amides, disulfides, and aromatic groups. Although the amides and aromatic groups themselves possess planes of symmetry, they are made optically active by the asymmetric configuration of the amino acid residues and the asymmetric arrangement of these chromophoric groups. Accordingly, the optical activity depends on the conformation of the groups surrounding the chromophores. Studies on circular dichroism (CD) of model molecules of proteins, containing aromatic side groups are, therefore, important in understanding the relation between the conformation and the optical rotation of the amide and aromatic groups. The CD spectra of phenylalanine, tyrosine, tryptophan, and their derivatives of *N*-acetyl amino acid amides and *N*-acetyl amino acid ethyl esters have been studied by several investigators. However, since amino and carboxyl groups of amino acids are involved in forming the peptide bonds of protein main chains, the most appropriate model molecules of amino acid residues in proteins are *N*-acetyl amino acid methylamides ($CH_3CONHCHRCONHCH_3$). Accordingly, in the present study, the CD and ultraviolet absorption spectra of *N*-acetyl-L-phenylalanine methylamide (AcPheMA), *N*-acetyl-L-tyrosine methylamide (AcTyrMA), and *N*-acetyl-L-tryptophan methylamide (AcTrpMA) have been measured in a temperature range between −70 and 50 °C.

The CD spectra of *N*-acetyl-L-phenylalaninamide (AcPheA), *N*-acetyl-L-tyrosinamide (AcTyrA), and *N*-acetyl-L-tryptophanamide (AcTrpA), and their related compounds have previously been studied by Shiraki,[1] and Strickland *et al.*[2-7] Strickland *et al.* measured the CD spectra at 25 and −196 °C and analyzed the vibrational structure. They suggested that the observed temperature dependence of the spectra was related to the shift of equilibrium of various conformers. In the present study, temperature dependence of the CD spectra of AcPheMA, AcTyrMA, and AcTrpMA was studied in a wavelength region between 200 and 300 nm in aqueous, methanol, and 1,4-dioxane solutions, and a change in intensity of the CD bands assignable to the amide and aromatic chromophores was examined. The results were also compared with those obtained for the primary amides of AcPheA, AcTyrA, and AcTrpA.

Experimental

AcPheMA, AcTyrMA, and AcTrpMA were prepared from the corresponding *N*-acetyl amino acid ethyl esters by the following method. Each of the esters was dissolved in methanol. Vapor of methylamine was then blown into the methanol solution until concentration of methylamine was about 25%. After being kept for 2 or 3 d, the reacting solution of *N*-acetyl-L-phenylalanine ethyl ester with methylamine yielded white needle crystals of AcPheMA, which was then recrystallized from methanol. For the derivatives of L-tyrosine and L-tryptophan, methylamine and methanol were evaporated after the reaction to give pale yellow powders. Repeated recrystallization from a mixture of ethanol and hexane gave white powders of AcTyrMA or AcTrpMA. Elemental analyses gave the following results. AcPheMA. Found: C, 65.48; H, 7.51; N, 12.94%. Calcd for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72%. AcTyrMA. Found: C, 61.01; H, 7.01; N, 12.01%. Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86%. AcTrpMA. Found: C, 64.70; H, 6.80; N, 16.15%. Calcd for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.20%.

The CD spectra were measured with a JASCO J-20 spectropolarimeter and the ultraviolet absorption spectra with a Hitachi 124 spectrophotometer. Molar ellipticity $[\theta]$, in 10^{-2} ° M^{-1} cm^{-1} = ° cm^2 $dmol^{-1}$, was calculated by a relationship, $[\theta]=3300\ (\varepsilon_l-\varepsilon_r)$, where ε_l and ε_r are molar absorption coefficients for the left- and right-circularly polarized lights, respectively. The CD intensity was calibrated against the intensity ($\varepsilon_l-\varepsilon_r=2.20$ M^{-1} cm^{-1})** of the 290.5 nm band of an aqueous solution of (+)-10-camphorsulfonic acid. Sample cells of path lengths of 5 and 10 mm were used for a wavelength region of 230—300 nm, while cells of path lengths of 0.1 and 1 mm were used for a region of 200—250 nm to reduce absorptions of solvents. The path length of the 0.1 mm cell was corrected by the ultraviolet absorption of a standard aqueous solution of potassium chromate.

The temperature of the sample cells was controlled with two kinds of constant-temperature apparatus.[8] For the measurements at −70 °C, a low-temperature cell was used

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** 1 M = 1 mol dm^{-3}.

with Dry Ice-acetone as coolant. Inside the low-temperature cell, a sealed sample cell was fixed to cooled copper block. The outer cell was kept vacuum to prevent the sample cell from condensation of water vapor. For temperatures higher than −10 °C, a brass-block cell holder was used. Through the brass block, externally temperature-controlled water or ethanol was circulated to keep the cell holder at constant temperature. Nitrogen gas was blown onto the sample-cell surface to avoid water-vapor condensation for temperatures below room temperature.

Results and Discussion

N-Acetyl-L-phenylalanine Methylamide (AcPheMA). The CD spectra of AcPheMA measured in methanol at various temperatures are shown in Figs. 1 and 2. The ultraviolet absorption spectrum measured in methanol at 22 °C is very similar to the absorption spectrum of AcPheA in methanol–glycerol at 25 °C (Fig. 1 of Ref. 2). The CD spectral pattern in the wavelength region longer than 250 nm are essentially the same for the temperature range from −7 to 57 °C; three negative CD bands assignable to the phenyl group were observed at 255, 261.5, and 267.5 nm. The intensities of the three CD bands increased as temperature was lowered. At −70 °C, these bands have intensities threefold stronger than those at 57 °C, and three positive CD bands appeared at 251.5, 257.5, and 264 nm. Also, two shoulders appeared at 260 and 266 nm in the CD spectrum measured at −70 °C, but no corresponding absorption bands were detected at 22 °C. These spectral features of AcPheMA resemble those of AcPheA measured by Strickland *et al.*[2] The difference in intensity of the CD vibronic bands observed at −70 °C and higher temperatures suggests the coexistence of various conformers at higher

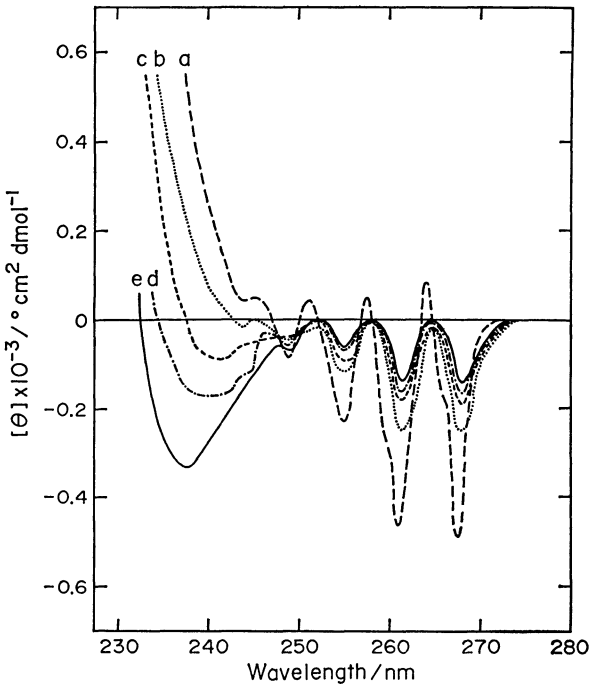

Fig. 1. CD spectra of *N*-acetyl-L-phenylalanine methylamide (AcPheMA) in methanol.
a: −70 °C, b: −7 °C, c: 22 °C, d: 40 °C, e: 57 °C.

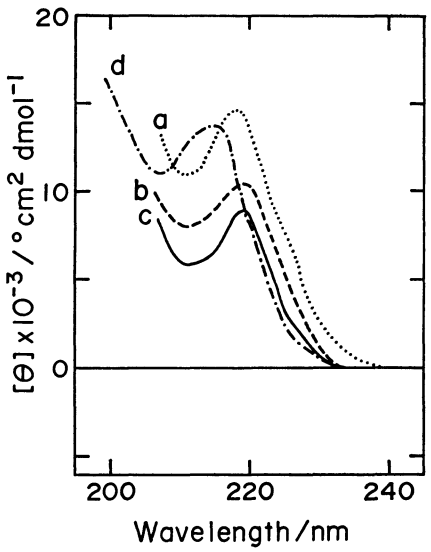

Fig. 2. CD spectra of *N*-acetyl-L-phenylalanine methylamide (AcPheMA) in methanol (a—c) and in water (d).
a: −5 °C, b: 22 °C, c: 40 °C, d: 22 °C in water.

temperatures, as in the case of AcPheA.

The CD bands observed in the 240—270 nm region are assigned to the vibrational structure of the $^1B_2 \leftarrow {}^1A_1$ electronic transition of the phenyl group. The vibronic assignments of the absorption and CD bands of AcPheMA were attempted in the light of the analysis for AcPheA.[2] The observed bands and their possible assignments are summarized in Table 1. The 0-0 band and the bands associated with the 0+930 cm^{-1} mode have a negative CD sign, while the 0+520 cm^{-1} progression observed only at −70 °C has a positive sign. The shoulders at 260 and 266 nm belong to the 0+180 cm^{-1} progression. The 0+180 cm^{-1} transition may be assigned to the B_1 vibrational mode, since this vibrational transition is electronically forbidden (total wavefunction is A_2). The absorption bands of the 0+180 cm^{-1} progression were, in fact, observed for neither AcPheMA nor AcPheA. The CD bands in the 240—250 nm region are likely to be distorted by overlapping tails of the phenyl $^1A_1 \leftarrow {}^1A_1$ and amide $\pi^* \leftarrow$n bands with band centers around 220 nm. This effect will be discussed later in detail.

In the CD spectrum of the methanol solution, a negative extremum was observed at 235—250 nm (Fig. 1). As temperature was raised, the intensity of this extremum increased with a blue shift, contrary to the $^1B_2 \leftarrow {}^1A_1$ bands whose intensities decreased. Judging from the band shape and the wavelength, this band is not likely to be due to the phenyl $^1B_2 \leftarrow {}^1A_1$ vibronic transition. In fact, no absorption band was observed in this wavelength region. This band at 235—250 nm is probably an apparent extremum formed as a residual difference between the overlapping tails of the shorter-wavelength CD bands assignable to the phenyl $^1A_1 \leftarrow {}^1A_1$ and amide $\pi^* \leftarrow$n transitions with the CD signs different from each other. Accordingly, temperature dependence of the CD spectrum in the 200—240 nm region was examined (Fig. 2). The CD extremum at about 220 nm for the methanol

Table 1. Vibronic bands of the phenyl $^1B_2 \leftarrow {}^1A_1$ transition for *N*-acetyl-L-phenylalanine methylamide (AcPheMA)

Absorption (at 22 °C)		Circular dichroism (at −70 °C)			Vibronic wave number	Possible assignment[a,b]	Symmetry[b]
Wavelength nm	Intensity	Wavelength nm	Sign	Intensity	cm^{-1}	cm^{-1}	
267.5	Weak	267.5	Negative	Strong	0−0	0−0	A_1
		266		Shoulder	0+210	0+180	(B_1)
264	Medium	264	Positive	Weak	0+500	0+520	B_2
261	Shoulder	261	Negative	Strong	0+930	0+930	A_1
		260		Shoulder	0+1080	0+930+180	(B_1)
258	Strong	257.5	Positive	Weak	0+1450	0+930+520	B_2
		255	Negative	Medium	0+1835	0+2×930	A_1
252.5	Medium				0+2220	(0+2×930+180)	(B_1)
		251.5	Positive	Weak	0+2380	(0+2×930+520)	(B_2)
247.5	Weak	248	Negative	Weak	0+2940	(0+3×930)	(A_1)
		245	Positive	Weak	0+3435	(0+3×930+520)	(B_2)

a) Wave numbers given are those for *N*-acetyl-L-phenylalaninamide (Ref. 2). b) Assignment and symmetry in parentheses are tentative.

solution showed appreciable dependence on temperature; its intensity increased about 1.5 times as temperature was lowered from 40 to −5 °C. This band was also found to be sensitive to the solvent. The CD intensity in water is about 40% stronger than in methanol, as seen in Fig. 2. Similar spectral changes have been noted by Barel and Glazer[9] for AcPheA in water and ethylene glycol–water (9:1). These authors considered that this spectral change was due to the change in solvent polarity and that the intensity decrease of the 220 nm band and the intensity increase of the 240 nm band were related to the dependence of the amide $\pi^* \leftarrow n$ optical rotation on the solvent polarity. Thus, the solvent-polarity dependence and the temperature dependence of the CD spectrum of AcPheMA is quite similar in the 200—250 nm region.

In order to examine temperature dependence of the amide $\pi^* \leftarrow n$ CD band free from the overlap of other bands, the CD spectrum of *N*-acetyl-L-leucine methylamide (AcLeuMA), $CH_3CONHCH(CH_2CH(CH_3)_2)CONHCH_3$, was measured in methanol at 0, 22, and 40 °C (Fig. 3). The spectral change with the increase in temperature resembles the spectral change with the decrease in solvent polarity.[10] Similar observations have been reported of the temperature dependence of the CD spectrum for *N*-acetyl-L-alanine ethylamide[11] and of the solvent dependence for *N*-acetyl-L-alaninamide.[9] Accordingly, it may be concluded that the increase in temperature and the decrease in solvent polarity give a similar effect on the amide $\pi^* \leftarrow n$ transition. In order to confirm the above conclusion, the CD spectra of AcPheMA in water and 1,4-dioxane, whose polarities are higher and lower, respectively, than methanol, have also been measured. The aqueous-solution spectrum did not show a remarkable change in the 240 nm region as temperature was raised. This quite small spectral change may be due to the 215 nm positive band whose intensity is strong enough to screen the intensity change of the amide $\pi^* \leftarrow n$ band. In 1,4-dioxane, on the other hand, a very strong negative CD band was

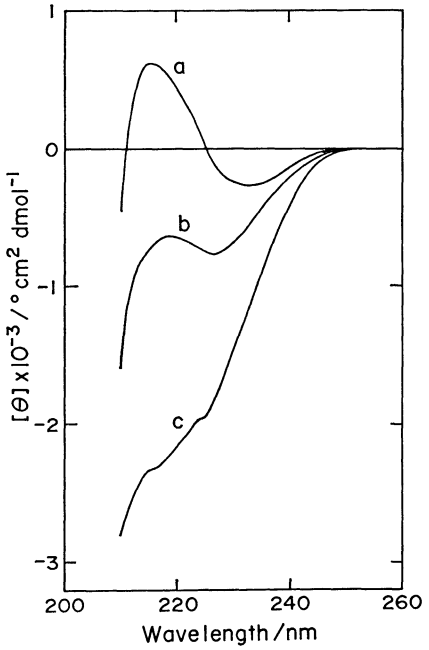

Fig. 3. CD spectra of *N*-acetyl-L-leucine methylamide (AcLeuMA) in methanol.
a: 0 °C, b: 22 °C, c: 40 °C.

observed at 233 nm (Fig. 4), which was blue-shifted with progressive intensity enhancement from 240 nm in methanol. These spectral observations indicate that the apparent negative CD extremum of AcPheMA around 240 nm is formed as a result of the overlap of the two CD bands with different signs. The CD spectra of AcPheA have been measured by Shiraki[1] in water, methanol, and 1,4-dioxane. The spectral changes with solvent are similar to those for AcPheMA studied in the present work, but the spectral patterns of AcPheA resemble the AcPheMA spectra measured at higher temperatures.

From the above considerations of the temperature and solvent dependence of the CD spectra, it is sug-

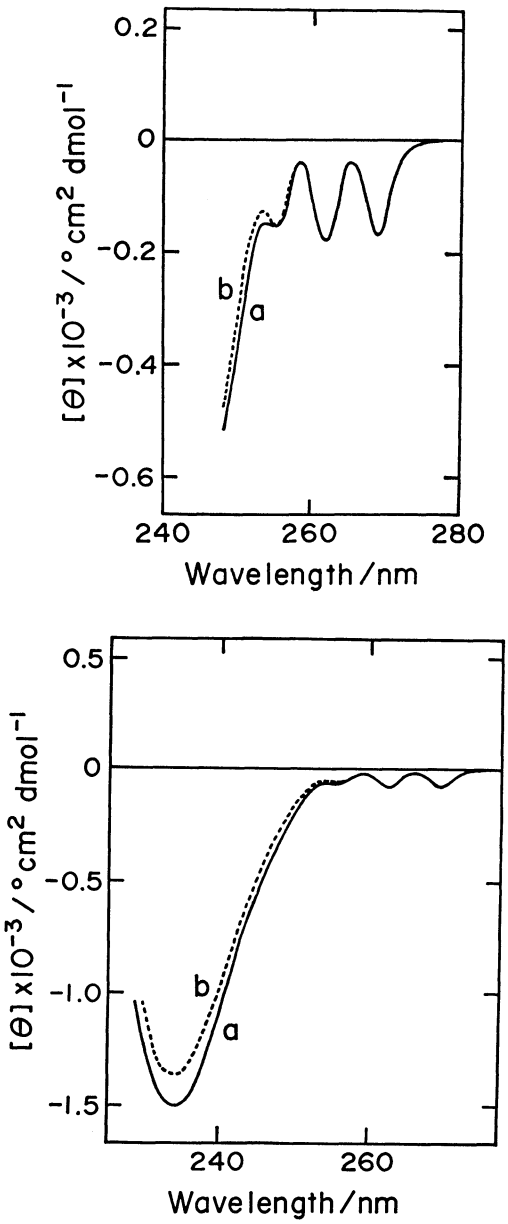

Fig. 4. CD spectra of *N*-acetyl-L-phenylalanine methylamide (AcPheMA) in 1,4-dioxane.
a: 20 °C, b: 40 °C.

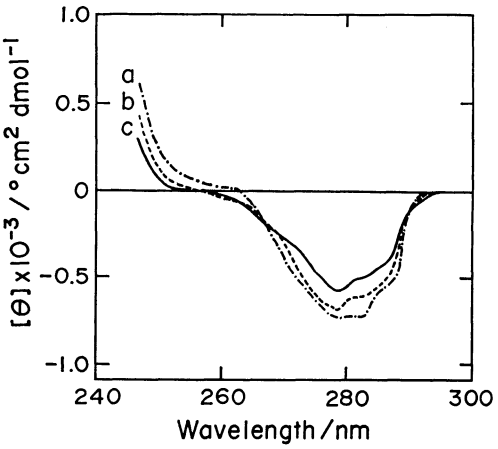

Fig. 5. CD spectra of *N*-acetyl-L-tyrosine methylamide (AcTyrMA) in methanol.
a: −70 °C, b: −7 °C, c: 22 °C.

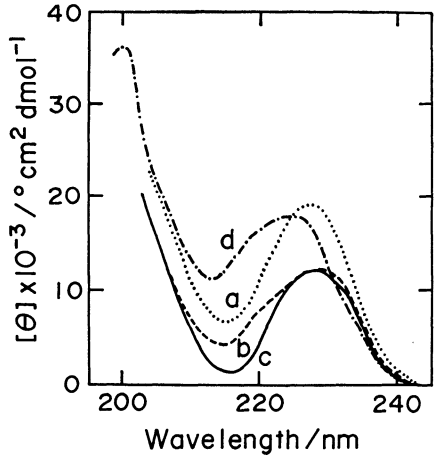

Fig. 6. CD spectra of *N*-acetyl-L-tyrosine methylamide (AcTyrMA) in methanol (a—c) and in water (d).
a: −7 °C, b: 22 °C, c: 40 °C, d: 22 °C in water.

gested that the hydrogen bonds between the amide group of AcPheMA and solvent molecules affect the amide $\pi^*\leftarrow$n transition. However, further studies are necessary to conclude whether the hydrogen bonding causes the conformational change or only the change of the electronic state of the amide group.

N-Acetyl-L-tyrosine Methylamide (AcTyrMA). The CD spectra of AcTyrMA measured in methanol at various temperatures are shown in Figs. 5 and 6. In the wavelength region of 260—290 nm, a negative extremum was observed at 278.5 nm with shoulders at 282.5 and 287 nm at room temperature. These bands are assigned to the vibrational structure of the phenolic $^1B_2\leftarrow{}^1A_1$ transition, similarly to the 240—270 nm bands of AcPheMA assigned to the phenyl $^1B_2\leftarrow{}^1A_1$ transition. The 278.5 and 282.5 nm CD bands of AcTyrMA correspond to the absorption bands at 277 and 283 nm observed with the same condition. The 287 nm CD shoulder, with no counterpart in the absorption spectrum, may be assigned to the 0-0 transition, with reference to the assignment of the 286 nm CD band of AcTyrA to the 0-0 transition.[3] As seen in Fig. 5, the shoulders at 282.5 and 287 nm became better resolved when temperature was lowered to −70 °C, and the peak intensity of the former was comparable to the intensity of the main peak at 278.5 nm. However, no remarkable spectral change with temperature was observed in this wavelength region. The CD spectrum at 40 °C was essentially the same as the spectrum at 22 °C. Thus, the temperature dependence of the spectral feature from −70 to 40 °C is much less than that for AcPheMA or AcTrpMA. The CD spectra in water and 1,4-dioxane were also similar to the spectrum of the methanol solution, with no appreciable spectral change with temperature. The resemblance of the CD and absorption spectra of AcTyrMA and other L-tyrosine derivatives in this wavelength region is accounted for by the reasoning that all of the vibronic bands have the same CD sign[3]

and that the rotational strength is nearly proportional to the dipole strength. In *N*-acetyl-L-tyrosine ethyl ester (AcTyrEE), the CD sign of this vibronic progression is different between the methanol and 1,4-dioxane solutions.[3] The fact that the very little variation of the CD spectra of AcTyrMA measured for various conditions suggests that the mobility of the *p*-hydroxyphenyl groups in AcTyrMA is more restricted than in AcTyrEE.

In the wavelength region shorter than 250 nm, a positive CD band appeared at 227.5 nm in methanol (Fig. 6), corresponding to the strong absorption band at 226 nm (ε_{max}=12000 M^{-1} cm^{-1}). This band is probably due to the phenolic $^1A_1 \leftarrow {}^1A_1$ transition. The aqueous-solution CD spectrum exhibits a peak at 225 nm (blue-shifted by 2.5 nm) with an intensity about 50% stronger than in methanol. The intensity of this CD band in methanol increased as temperature was lowered, similarly to the temperature dependence of the 220 nm CD band of AcPheMA. This spectral change is ascribed to the dependence of the $\pi^* \leftarrow$n transition on temperature. This is supported by the observation that in a 1,4-dioxane–methanol mixture (2:1, v/v) an apparent negative CD band appeared around 246 nm at 55 °C, but no distinct peak at 17 °C (Fig. 7). These observations of the spectral changes with temperature may be compared with the solvent effect on the CD spectra of AcTyrA observed in water, methanol, and 1,4-dioxane.[1] The effects of the increase in temperature and the decrease in solvent polarity resemble closely each other, as in AcPheMA.

N-Acetyl-L-tryptophan Methylamide (AcTrpMA). The CD spectra of AcTrpMA measured in methanol at various temperatures are shown in Figs. 8 and 9. It should be mentioned that in AcTrpMA the absorption strength relative to the rotational strength is much stronger than in AcPheMA or AcTyrMA. Accordingly, the solution concentration and the path length were limited in the measurements, giving very weak CD signals and consequently low signal-to-noise ratios. As temperature was lowered to −70 °C, the CD intensity in the longer-wavelength region increased progressively and the vibronic bands were well resolved (Fig. 8). The vibronic bands in this region are assigned to the $^1L_a \leftarrow {}^1A$ and $^1L_b \leftarrow {}^1A$ transitions of the indolyl side group. The low-temperature spectrum has

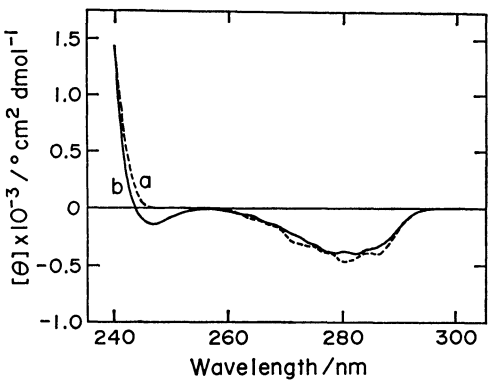

Fig. 7. CD spectra of *N*-acetyl-L-tyrosine methylamide (AcTyrMA) in 1,4-dioxane–methanol (2:1, v/v).
a: 17 °C, b: 55 °C.

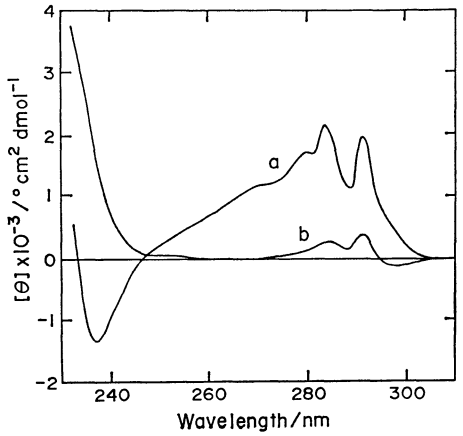

Fig. 8. CD spectra of *N*-acetyl-L-tryptophan methylamide (AcTrpMA) in methanol.
a: −70 °C, b: 22 °C.

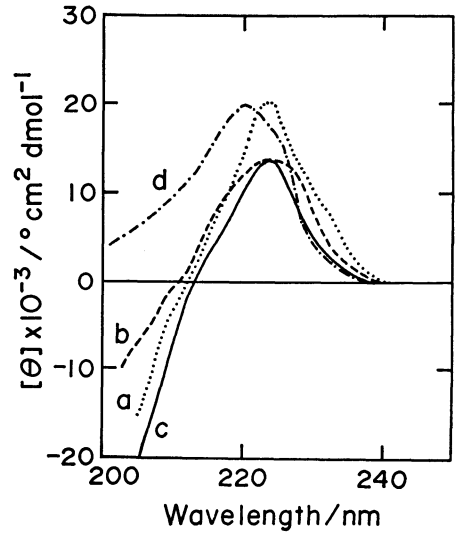

Fig. 9. CD spectra of *N*-acetyl-L-tryptophan methylamide (AcTrpMA) in methanol (a—c) and in water (d).
a: −5 °C, b: 22 °C, c: 40 °C, d: 22 °C in water.

two distinct positive peaks at 283.5 and 291 nm and a shoulder at 280 nm. An additional shoulder was observed around 270 nm at low temperature. These spectral features resemble those of the CD spectrum of AcTrpA.[5] The peaks of AcTrpMA at 283.5 and 291 nm are assigned to the 0+850 cm^{-1} $^1L_b \leftarrow {}^1A$ band and the 0—0 $^1L_b \leftarrow {}^1A$ band, respectively, with reference to the assignment of the corresponding CD bands of AcTrpA at 283 and 290 nm.[5] The noticeable difference in the CD spectra at room and lower temperatures suggests the coexistence of various conformers at higher temperatures, as in the case of AcPheMA. The CD spectrum of AcTrpMA in water in the 240—300 nm region resembles the aqueous-solution spectrum of L-tryptophan with unblocked amide and carboxyl groups;[12] three positive peaks with similar intensities were observed at 272, 281, and 291 nm.

In the spectral region of 200—240 nm, a positive CD band appeared at 223.5 nm in methanol and at 220 nm in water (Fig. 9), corresponding to the strong absorption band (ε_{max}=45000 M^{-1} cm^{-1}) at 221.5 nm

in methanol. With the decrease in temperature, the intensity of this CD band increased but the peak position remained almost unshifted, indicating that this band of AcTrpMA is due largely to the transition of the indolyl side group ($^1L_b \leftarrow {}^1A$ transition). A noticeable spectral feature in Fig. 9 is that the CD curve of the methanol solution crosses over the base line at about 210 nm to the negative region. This observation shows the presence of a negative CD band in the shorter-wavelength region, in striking contrast to an anticipated positive band for AcPheMA (Fig. 2) or AcTyrMA (Fig. 6). Another spectral feature to remark for AcTrpMA is the appearance of a negative band at 237 nm at −70 °C. This spectral change appears to be opposite to the temperature effect on the apparent band of AcPheMA or AcTyrMA observed at 230—240 nm. However, the band at 237 nm is also ascribable to the overlap of two CD bands with different signs, as suggested by its wavelength. These peculiar spectral patterns for AcTrpMA lead to an inference that the CD behavior of the amide $\pi^* \leftarrow n$ and $\pi^* \leftarrow \pi$ transitions is different from that for AcPheMA or AcTyrMA. The difference is probably related to the bulkier indolyl group, which may interact with the peptide backbone in a way different from the phenyl or *p*-hydroxyphenyl group.

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