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VIBRATIONAL CIRCULAR DICHROISM OF OLIGOPEPTIDES

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Osaka University

VIBRATIONAL CIRCULAR DICHROISM OF OLIGOPEPTIDES

A Doctoral Thesis

by

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Osaka University

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Chapter I

General Introduction

I-1 Vibrational Circular Dichroism

Molecular optical activity originates from different responce of left versus right circularly polarized light to chromophores chirally arranged in a molecule. In other words, observable optical activity is directly correlated to molecular geometry especially to the chiral structure of a molecule.

A common technique for probing molecular optical activity is circular dichroism (CD) spectroscopy. This is the measurement of the difference between the absorption of left and right circularly polarized light ($\Delta A = A_L - A_R$).

The integrated intensity of a CD band is related to the rotational strength, R^a, in unit of $(esu \cdot cm)^2$ as following,

$$R^a = 2.296 \times 10^{-39} \left[\int \Delta \varepsilon / \nu \, d\nu \right]$$

where $\Delta \varepsilon = \varepsilon_L - \varepsilon_R$ in molar extinction coefficient and ν is the frequency of a transition from the ground state $|0\rangle$ to the excited state $|a\rangle$. R^a is quantum mechanically expressed as

$$R^a = \mathrm{Im} \{ \langle 0|\mu|a\rangle\langle a|M|0\rangle \}$$

where μ is the electric and M is the magnetic dipole moment operators, and Im indicates the imaginary part of the complex quantity.

Before the 1970s, CD spectroscopy was limited to the visible (VIS) and ultraviolet (UV) regions and thus was used to study

electronic transitions of molecules. Therefore, organic molecules and biopolymers containing chromophores, such as carbonyl or aromatic groups, could be main targets in the structural studies. Most organic molecules have only a few accessible chromophores in the UV region which usually give rise to broad and featureless absorption bands, often resulting in a considerably overlapped obscure envelope. On the other hand, all optically active molecules have a lot of absorption bands in infrared (IR) region. Furthermore, these vibrational transitions are well resolved and relatively easily assignable. CD spectroscopy based on vibrational transitions in the IR region, which is now commonly called vibrational circular dichoroism (VCD), can be used for probing optical activity of most of all molecules and can provide much more structural information compared to VIS·UV-CD spectroscopy.

I-2 Structure of Polypeptides

It is a basic problem in biochemistry that structure and function of biomolecules are intimately related. Many works have been undertaken to determine the tertiary structure of proteins and nucleic acids in order to understand how and why they exhibit their biological activities.

The tertiary structure of polypeptides and proteins have been determined by X-ray diffraction, NMR, IR, and Raman spectroscopies and UV·VIS-CD. Among them, information from the crystal structures is straightforward. The tertiary structures of proteins in crystals are found to be composed of folding of secondary structure units. The typical secondary structures are helices and β-sheets (Fig. I-1).

The α-helix has 3.6 residues per turn, and has been found in a great number of proteins. Each peptide N-H group is hydrogen-bonded to the oxygen of a peptide C=O group four residues down the chain (Fig. I-2). The C=O groups lie parallel to the helix axis and point almost directly at the N-H groups, and therefore the hydrogen bonding formed between the groups is fairly stable. Another stable helix is, the 3_{10}-helix, in which the carbonyl oxygen is bonded to a N-H group located one amide group closer than in the α-helix (Fig. I-2). Short length 3_{10}-helices have recently been found in hemoglobin and lysozyme. For the β-sheet there are two ways of the arrangements of the β type extended strands, parallel and antiparallel. Antiparallel arrangement is more common. The other parts in folded polypep-

tides are often classified into random coil. However sometimes some local regularity is found. At the connections of folded β-strands or two α-helices, there often appear turn structures. There are several types of turn structures (Fig. I-3). The terms β and γ-turn have more restricted definitions and describe turns of four and three residues, respectively. These turns may be stabilized by an intramolecular hydrogen bond; in β-turn, the C=O of the first residue is hydrogen bonded to the N-H of the 4th residue, while in γ-turn, the C=O of the first residue is hydrogen bonded to the N-H of the third residue (Fig. I-2).

To maintain the secondary structure, an essential stabilizing factor is hydrogen bonding. Hydrogen bonding in helices and turn structure has intramolecular nature, while that of β-sheets is intermolecular type bonding. Intermolecular hydrogen bonding is generally stronger than intramolecular one, since the intramolecular hydrogen bonding is often distorted due to steric restriction. For the definition of intramolecular hydrogen bonding the number of atoms involved in the closed circles are denoted like C_5 and C_7.

Moreover, one of the determining factors of stereochemical protein structure arises from the arrangement of each amino acids which have characteristic respective chemical behavior. These amino acids are linked by peptide groups (-CONH-). The C-N bond in the peptide bond is rigid and cannot rotate freely, except the case of the imide linkage of the proline residue.

I-3 The Purpose of This Thesis

UV·VIS-CD spectroscopy has been extensively used to evaluate the contents of secondary structure in proteins and polypeptides. However, the electronic absorption bands in UV·VIS region consist of usually overlapped, broad, and featureless bands and consequently it interferes with the observation of well resolved CD signals, if any. The vibrational absorption bands in IR region are much more resolved and straightforward to assign and interpret. Vibrational spectroscopy by infrared absorption and Raman scattering has been utilized to structural studies of molecules based on mainly its characteristic frequency dependency on structure. VCD spectroscopy offers more pronounced conformational sensitivity because of its two-signed nature. Thus, VCD studies on biopolymers should be complement and extend valuable insight into the stereo-structure of polypeptides that has been provided by UV·VIS-CD and vibrational spectroscopies. The recent advance in the instrumentation made biopolymer-oriented VCD studies possible. VCD spectroscopy is now recognized as a valuable tool for the analysis of the secondary structure of proteins and oligopeptides.

UV·VIS-CD has been a fairly powerful method for structure determination of polypeptides and proteins, if they contain regular and repeated secondary structures like α-helix and β-sheets. However it becomes powerless when there is a distribution of several types of conformations or peptides are so small that regular secondary structure cannot be expected. Turn

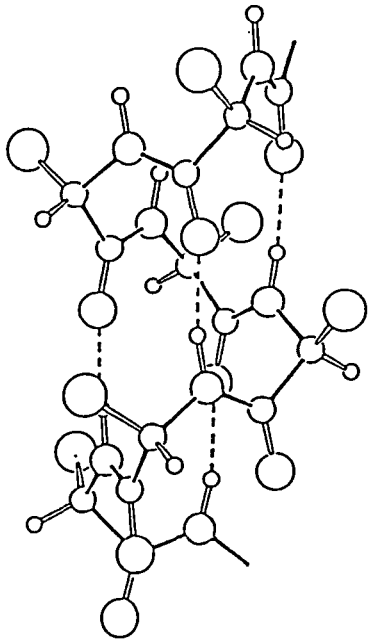
structure, for example, is also important to construct three dimentional structure of peptides and proteins. VCD may be sensitive to such local conformation as turn structure from its nature and also should be sensitive to the structure of oligopeptides. In this thesis (Chapter IV, V, and VI) the author aims to establish a correlation between the features of VCD bands and local conformations of oligopeptides. If it is successful, VCD may be used for the structure analysis of oligopeptides and smaller proteins.

Conventional CD in UV·VIS region is now used routinely, but one may encounter some difficulties in VCD measurements, because VCD is a weak phenomenon. Technologies of the spectroscopy in the IR region are inferior to those of the UV·VIS region. Rotational strength (R^a) for vibrational transitions is expected to be three to four orders of magnitude smaller than that for electronic transition because of its dependence on electronic and magnetic dipole moments. Therefore, special elaboration is necessary to obtain a good VCD spectrum. In Chapters II and III, technical methods devised in the present study will be discussed for VCD measurements by the use of dispersive and Fourier transform (FT) spectrophotometers.

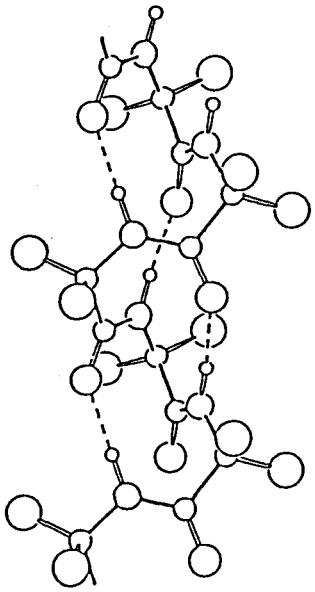
VCD spectroscopy is also applied to study the structure of biologically active cyclic peptides (e.g., Valinomycin, Gramicidin S, and their derivatives) in order to obtain new information on the conformation of the peptides where fixed turn structures are presumed to be involved. In Chapter VII, the analyses of VCD spectra of these biologically active peptides is described.

The summary of the established correlation between VCD feature and turn structures will be given in Chapter VIII.

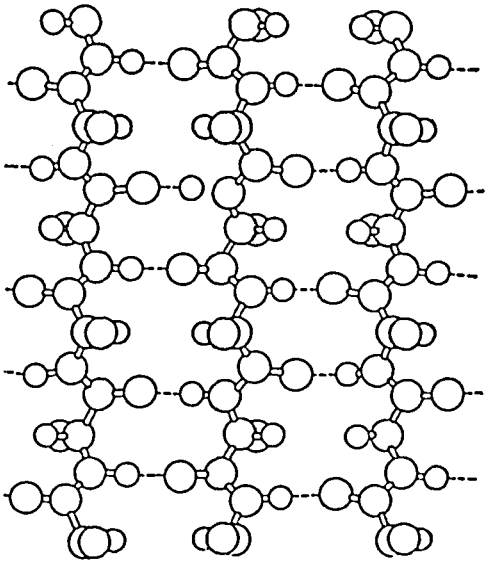
α–helix structure



3_{10}–helix structure



Antiparallel β–sheet structure



Parallel β–sheet structure

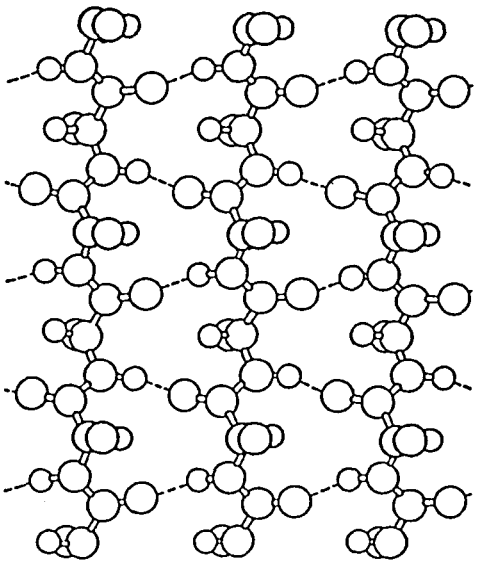


Fig.I-1 Diagrams of several types of secondary structures (Hydrogen bonds are indicated by dotted lines. Side chains are shown in the L configuration.)

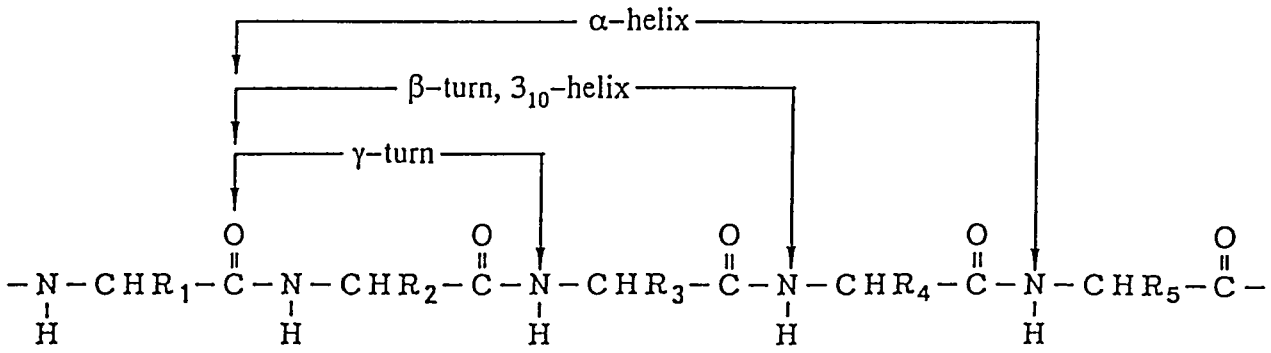
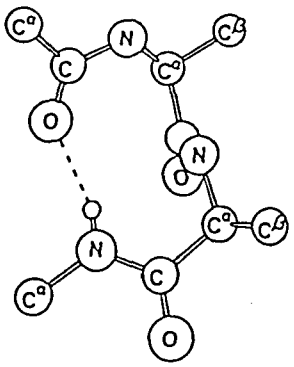
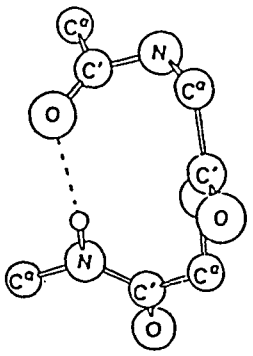


Fig.I-2 Hydrogen bonding in different types of peptide: γ-turn, β-turn, 3_{10}-helix, and α-helix.

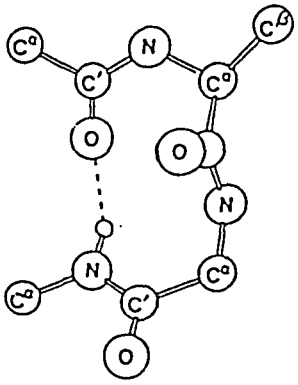
Type I β–Turn



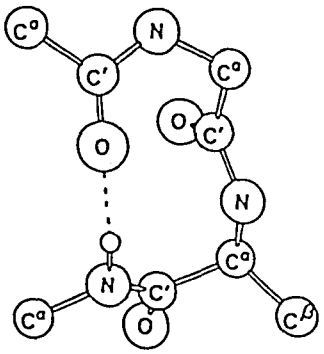
Type I' β–Turn



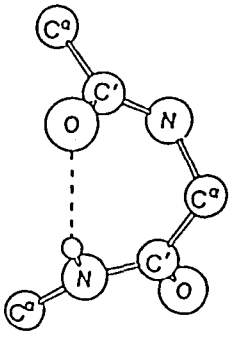
Type II β–Turn



Type II' β–Turn



γ–Turn



Inverse γ–Turn

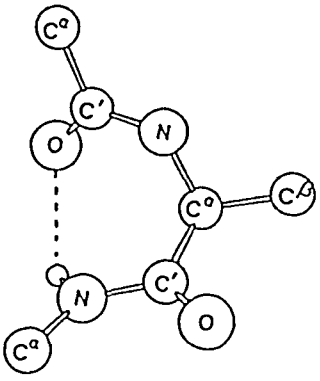


Fig.I-3 Diagrams of several turn structures.
(Hydrogen bond are indicated by dotted line. The configuration of the residues shown are listed on the figure.)

CHAPTER II

Instrumentation

II-1 Dispersive Infrared Spectropolarimeter

As mentioned in the previous chapter, ΔA in vibrational circular dichroism (VCD) is a very small quantity. In order to observe this small effect, sophisticated techniques must be employed: the highest throughput optics, the most sensitive detector, and electronics with the least noise and widest dynamic ranges as possible. In addition, instruments have to be optimized to suppress artifacts which may mask the small desired signal. Several research groups have developed instrumentations on VCD measurements along the line mentioned above. In this study, JASCO (Japan Spectroscopic Co., Ltd.) J-200E dispersive infrared spectropolarimeter was used for VCD measurement in the wavenumber region of 4000-2000 cm^{-1}, which is a modified instrument of a JASCO J-200D near-infrared spectropolarimeter[1]. The optical diagram is shown in Fig. II-1.

A 300 watt Xenon lamp, which is efficient in the near-infrared region, is used as light source. The light beam from this Xe source is modulated at a frequency of ω_c=60 Hz by a mechanical chopper CH. The low-pass filter F is used for removing the higher order diffraction light. The grating G with 300 lines/mm blazed at 3 μm was used in the VCD measurement of the N-H and C-H streching region. The monochromatic light emerging from the monochromator is converted to a linearly polarized light through a wire-grid polarizer PO. The following photo elastic modulator PEM constructed with CaF_2 convert the linearly polarized light into left- and right-circularly polarized lights sinusoidally at

ω_m=58KHz. The radiation transmitting through a sample is focused on the InSb detector DE (Barnes Co.). This InSb detector is operated at liquid nitrogen temperature and covers the wavenumber region of 10000 to 2000 cm^{-1}. The signal produced at the detector is processed by electronics schematically outlined in Fig. II-2. The low frequency component, called as DC signal, modulated by the mechanical chopper (60 Hz) and the high frequency component, called as AC signal, modulated by PEM (58 KHz) are separately amplified with lock-in amplifiers. Finally, the normarization by ratio of AC and DC signals, I_{AC}/I_{DC}, provides the signal proportional to ΔA. This dispersive infrared spectropolarimeter is interfaced to a personal computer (EPSON PC-286V) which allows data acquisition through the interface (JASCO IF-500). The optical unit was constantly flushed with nitrogen gas for reducting interference by atmospheric H_2O absorption.

Infrared detector have a slight polarization sensitivity which gives rise to artifact signal in VCD spectra. Generally these artifacts have arised from imperfect alignment, optical anisotropy of sample cell, and multiply reflected light beams. Artifacts were seldom observed in this spectropolarimeter. But the line spectra from the Xe spectrum caused some difficulty in normalization for VCD measurement. This artifact strongly depend on the slit S2 width as shown in Fig. II-3. Moderate focusing has been used whenever the measurements were carried out in order to be better efficiency of the beam incident on the detector. But the every instrumental parameter was hold the same condition during the measurements of two enantiomers.

II-2 Fourier Transform Infrared Spectropolarimeter

It is well know that Fourier transformed infrared (FTIR) spectroscopy provides significant advantages over conventional dispersive spectroscopic techniques[2,3]. These advantages are described as Fellgett's advantage[4] of spectral multiplexing, Jacquinot's advantage[5] of increased radiative throughput due to the absence of slits, and Conne's advantage[6] of high accuracy in wavelength determination owing to the use of the laser. With the advent of fast Fourier transform algorithms[7,8], the interferogram can be transformed to yield the desired spectrum in a very short time, thus it is possible to obtain infrared spectra over a wide spectral range in considerably shorter time than the time at the use of dispersive instruments.

Over the past two or three decades the use of Fourier transform (FT) methods has brought about sensitivity-improvements in various spectroscopies, and the FT based-technique has been applied to varieties of fields. A great improvement in sensitivity in VCD spectroscopy is expected by introducing FT technique, owing to its multiplex advantage and throughput advantage. The use of FT technique with employing wide-range detector will become effective in VCD spectroscopy.

In this study the JASCO IRF-10 interferometer was used for a circular dichroism measurement. The optical diagram is shown in Fig. II-4. A highly bright Globar lamp is used for the light source. The light beam emerging through the aperture AP is modulated by a Michelson interferometer. Then the output is

passed through a polarizer and modulator that produce the polarization modulation of the beam at the frequency (ω_m) of the modulator drive. The beam passed through a sample is focused onto the MCT detector. The signal thus produced on the detector is preamplified and then processed as shown in Fig. II-5. The DC interferogram is obtained through the standard FT-IR electronics typically found in a standard instrument. On the other hand, the AC interferogram is obtained through a lock-in amplifier of the reference frequency ω_m with a sufficiently short time constant. These two interferograms must be accumulated separately. The VCD spectrum ΔA is calculated from the ratio of the AC signal to the DC signal obtained by fast FT algorithms of these two interferograms. This FT-IR-CD instrument was interfaced to a personal computer (EPSON PC-286V) which allows data acquisition and data processing.

VCD spectra obtained by this FT-IR instrument were not free from artifacts that have been reported by other workers[9,10]. One of the artifacts observed in VCD measurements is baseline offset and/or sloped baseline. This artifact is attributable to a slight imperfection of optical system; such as residual static birefringence in any optics of detector, lens, modulator or sample cell, and retardation introduced by reflection etc. This artifact can be reduced by careful optical alignment. In typical FT-IR interferogram the intensity of the centerburst at zero optical path difference is observed as the strongest peak in the interferogram. The AC interferogram ideally does not exhibit a centerburst as such and sometime has a difficulty to pick up the

center of interferogram. The baseline offset due to optical imperfection gives rise to strong centerburst in the AC interferogram. The other unavoidable problem in VCD spectroscopy is absorption artifact, that is, a false peak which appears in VCD at the absorption band. The origin of this artifact is not clear but its appearance is somewhat dependent on the absorbance intensity. Too strong absorption means little detectable energy on the detector resulting in a low S/N level in VCD spectrum. Therefore, the VCD measurement for bands at strong absorption intensities (Abs.>0.8 OD) should be avoided. The estimation of the baseline from VCD spectra for two enantiometric samples is the most preferable as shown in Fig. II-6.

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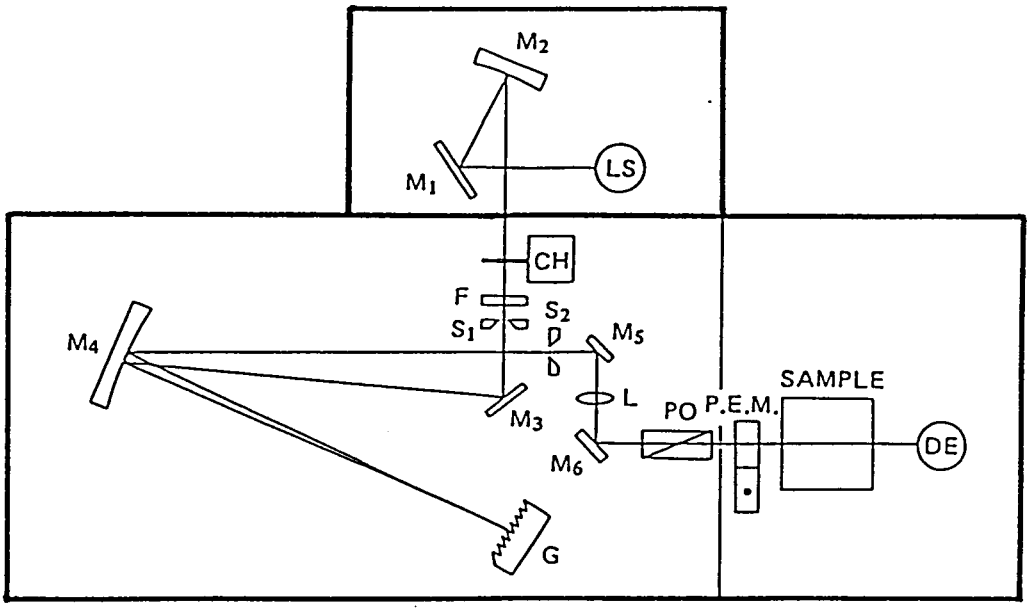


LS	: Light Source
M_1,M_3,M_5,M_6	: Plane Mirror
M_2	: Spherical Mirror
M_4	: Collimator Mirror
CH	: Chopper
F	: Filter
S_1	: Entrance Slit
S_2	: Exit Slit
G	: Grating
L	: Lens
PO	: Polarizer
P.E.M.	: Piezo Elastic Modulator
DE	: Detector

Fig.II-1 Optical diagram of JASCO J-200E infrared spectropolarimeter

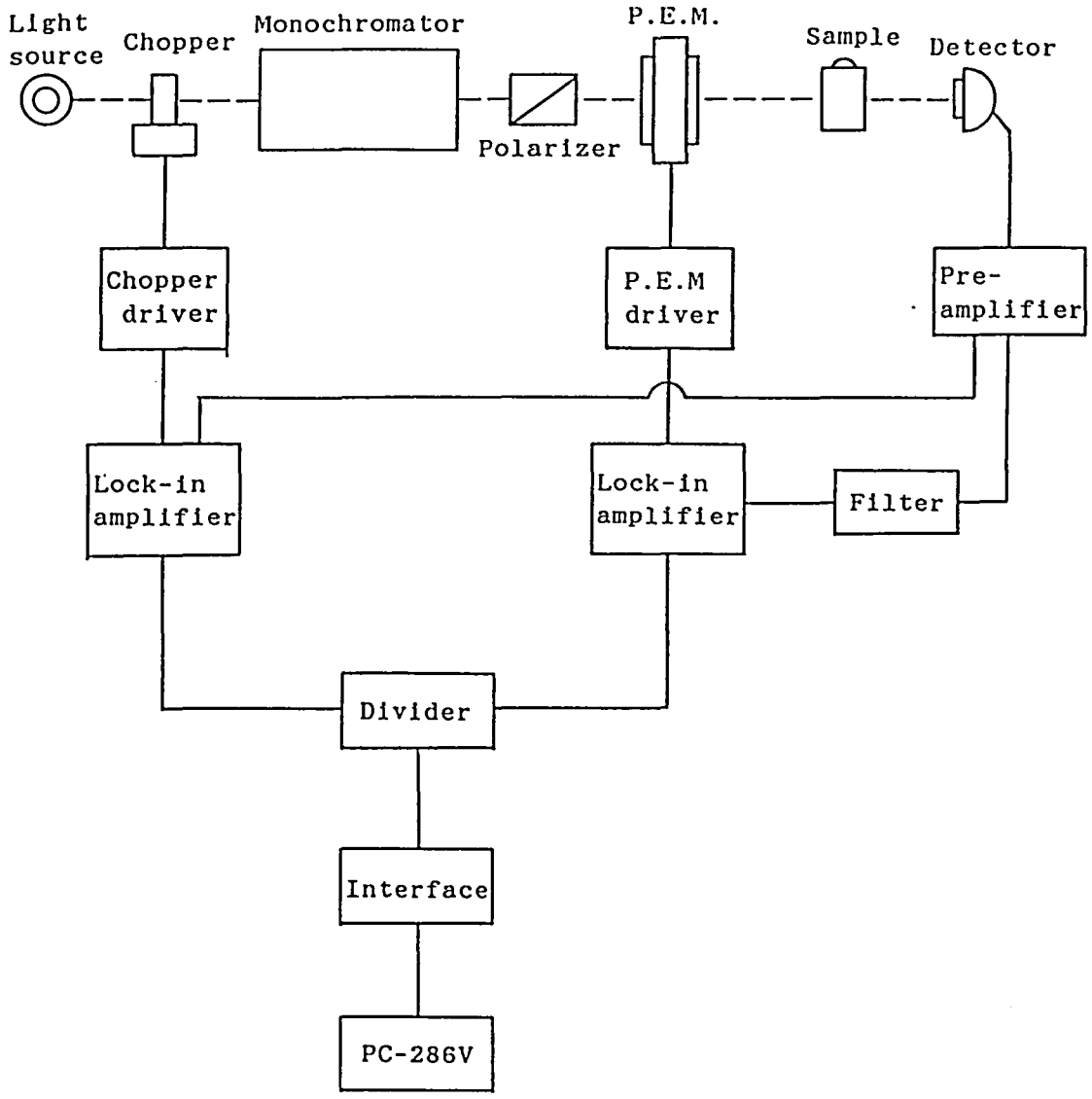


Fig.II-2 Electronic block diagram of JASCO J-200E infrared spectropolarimeter

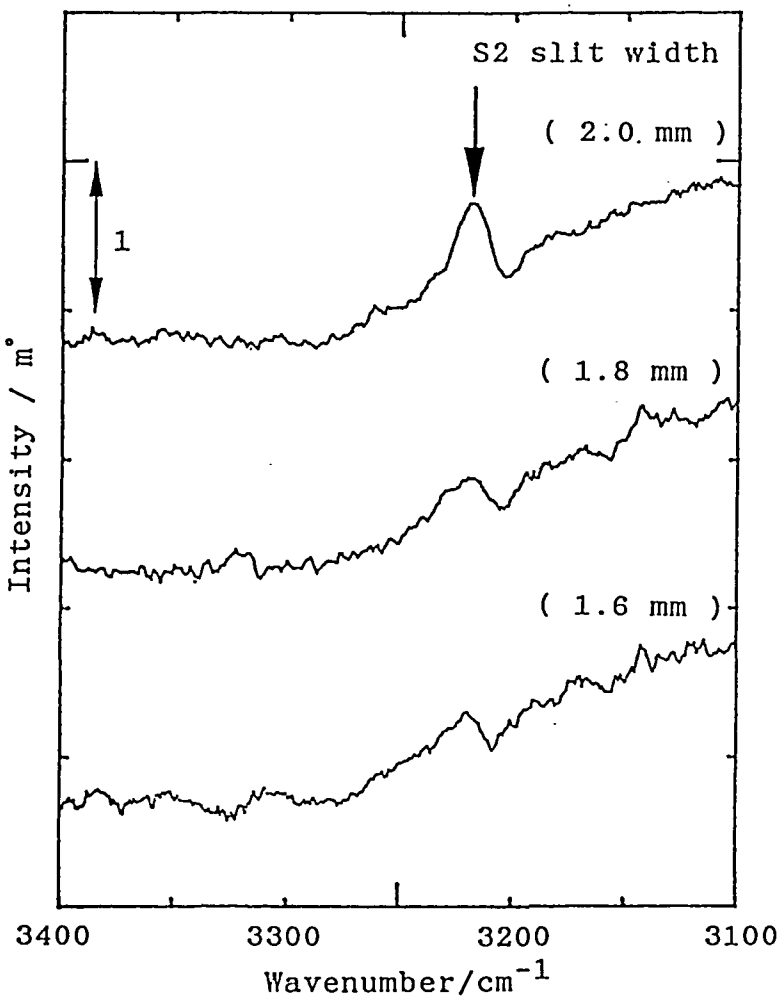
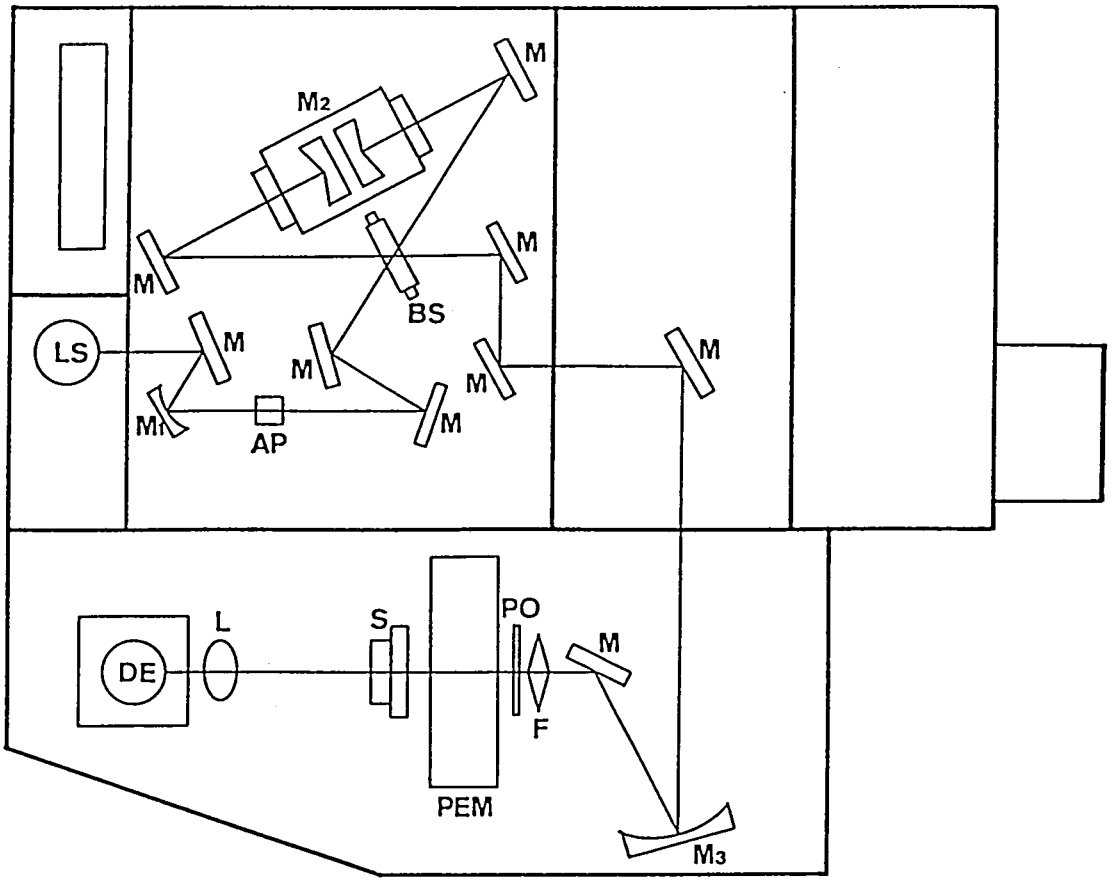


Fig.II-3 VCD baseline and slit width.

Sample is neat $CDCl_3$ at 0.1 mm pathlength. Experimental conditions: sensitivity 0.5m°/cm, time constant 1 sec, and scan speed 100 cm^{-1}/min. The arrow indicates the line from the Xe lamp.



LS : Light Source
M : Plane Mirror
M_1 : Spherical Mirror
M_2 : Movable Mirror
M_3 : Collimator Mirror
AP : Aperture
BS : Beam Splitter
F : Optical Filter
PO : Polarizer
PEM : Piezo Elastic Modulator
S : Sample
L : Lens
DE : Detector

Fig.II-4 Optical diagram of JASCO IRF-10 infrared spectropolarimeter

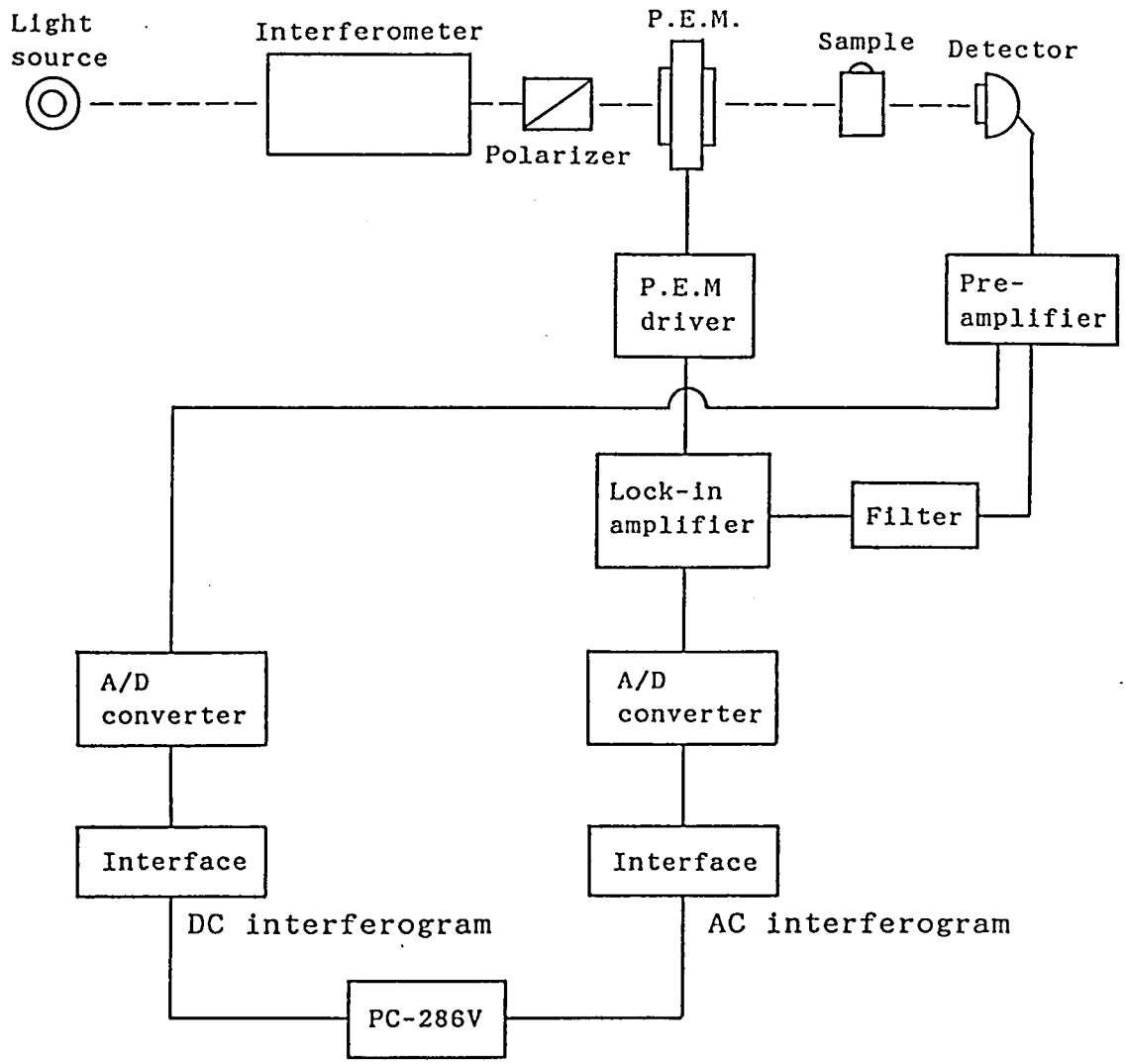


Fig.II-5 Electronic block diagram of JASCO IRF-10 infrared spectropolarimeter

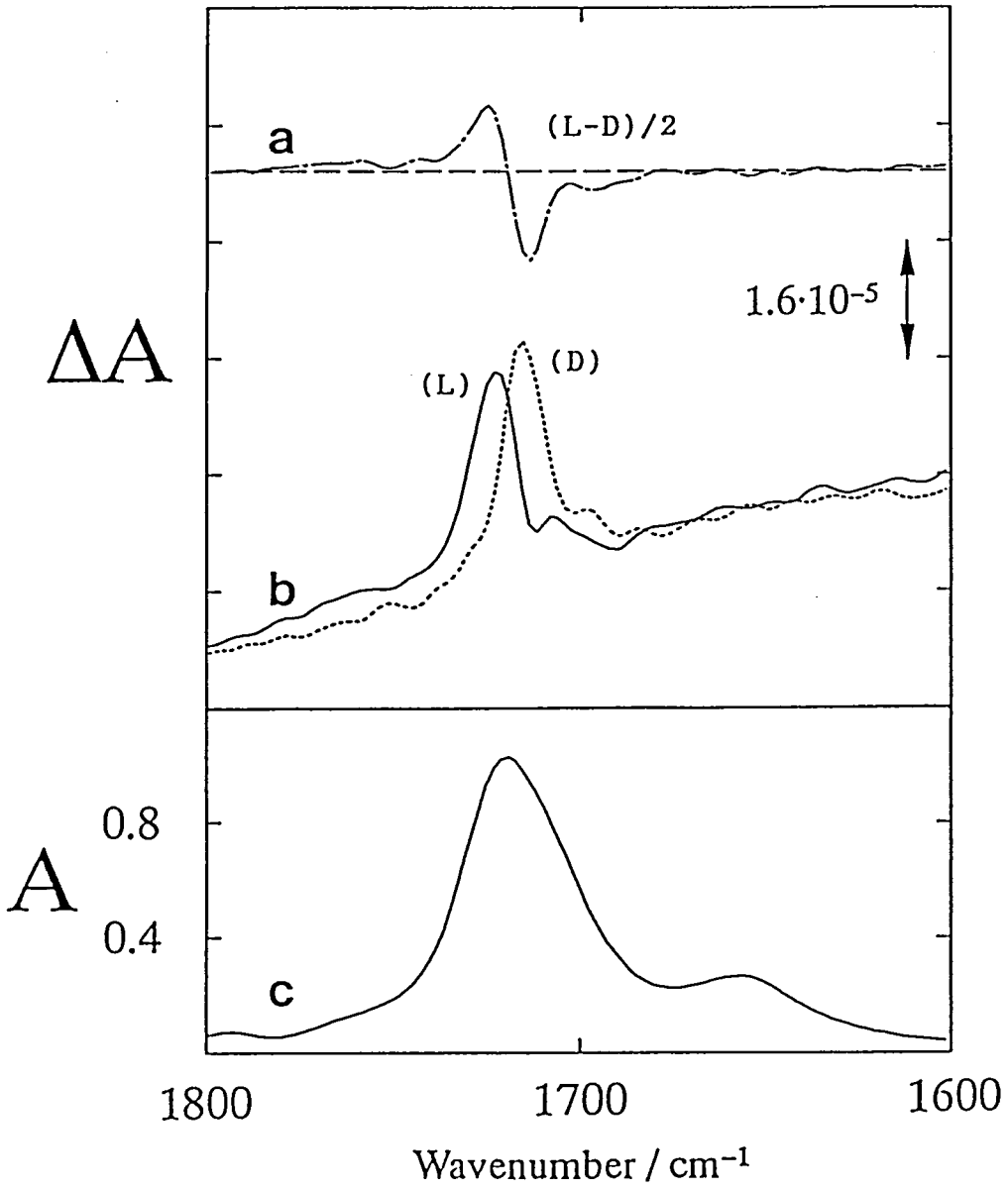


Fig.II-6 VCD (a,b) and absorption (c) spectra of N-(t)-butoxycarbonyl-(L,D)-alanine in the carbonyl streching region (0.28 M $CDCl_3$ solution; path. 0.05 mm) measured under high artifact conditions. (a) is the average of two enantiomer scans and (b) illustrates the raw data.

Chapter III

Methods

III-1 Experimental Parameters

When a linearly polarized light passes through an optically active medium with a length of l' cm, the transmitted light is generally elliptically polarized light as shown in Fig. III-1. The ellipticity θ is obtained from the major and minor axes of the ellipse of the electric vector,

$$\tan\theta = (b/a). \qquad ---(1)$$

The differential absorption of left and right circularly polarized light ($\Delta A = A_L - A_R$) called circular dichroism (CD), is related to θ.

$$\tan\theta = (10^{(-1/2)AR} - 10^{(-1/2)AL}) / (10^{(-1/2)AR} + 10^{(-1/2)AL})$$
$$= \tan \{(\ln10)(A_L - A_R)/4 \}$$
$$= \tanh (2.303\ \Delta A/4) \qquad ---(2)$$

On the other hand, molar absorption coefficient ε is given by

$$\varepsilon = A / C'l' \qquad ---(3)$$

where C' is the concentration of the medium in moles per liter. Then the molar circular dichroism $\Delta\varepsilon$ is related to the ellipticity θ.

$$\tan\theta = \tanh (2.303\ \Delta\varepsilon C'l'/4) \qquad ---(4)$$

Taking account of $\theta << 1$ the ellipticity θ is given by,

$$\theta = \ln10\ \Delta A/4 = 2.303\ \Delta\varepsilon C'l'/4 \qquad ---(5)$$

Sometimes CD intensity is expressed in terms of molecular ellipticity $[\theta]$,

$$[\theta] = (18000 / \pi)(\theta / C'l')$$
$$= (4500\ \ln10 / \pi)\Delta\varepsilon$$
$$= 3300\Delta\varepsilon \qquad ---(6)$$

The anisotropy facter g given by

$$g = (\Delta\varepsilon / \varepsilon) = (\Delta A / A)$$

is also of interest for analysis of VCD spectra because it indicates a criterion on the difficulty of CD measurement, the larger anisotropy the easier in measurement.

III-2 Spectra Measurements

The IR spectra of polypeptides and proteins exhibit several characteristic absorption bands (so-called 'amide bands') associated with the peptide (trans -CONH-) group.[1] Nine amide bands are generally referred as amide A, B, I to VII, whose frequency ranges and the major contributions are summarized in Table III-1. The amide I (predominantly C=O stretching) and amide II (predominantly NH bending) bands have been frequently used for analyses of secondary structure of proteins and polypeptides. The amide A band (mainly N-H stretching) have less interference from other vibrational modes. Most of polypeptide backbone conformations are maintained through inter and intra-molecular hydrogen bonds between the carbonyl oxygen and the amide hydrogen atoms. Therefore, the analyses of amide A, I, and II bands are important for the structural study of proteins and peptides. Sometimes one has some difficulty to determine a conformation on the basis of IR absorption spectrum alone due to too small frequency difference to differentiate. However CD spectroscopy has more enhanced sensitivity in signal separation because of its two-signed nature. In this thesis the analyses of IR and VCD spectra have been focused on the amide A and amide I band regions.

Water is often used as a standard solvent in studies of biomolecules. In aqueous media, amide A band is unable to be observed owing to interference by strong intensity of the O-H streching band of H_2O. Moreover, inter- and intra-molecular hydrogen bonds in the model peptides in aqueous media are weak-

ened to be observed because of competition with the intermolecular hydrogen bonding between solute and solvent water molecule. Apolar solvents such as carbon tetrachloride (CCl_4) and chloroform ($CHCl_3$) do not interact strongly with solute molecules and are apropriate for the study of hydrogen bonding. Dimethyl sulfoxide (DMSO) displays a strong polar character and is widely used for various investigations. It is of particular interest to compare the conformational behavior of some simple oligopeptides when dissolved in these various solvents. The deuterated solvents (such as $CDCl_3$, DMSO-d_6 etc.) were used in the present study because of good transparency in the frequency region of amide A and amide I bands.

IR absorption spectra were measured on a JASCO (Japan Spectroscopic Co., Ltd.) A-3 infrared spectrometer. This infrared spectrometer was interfaced to a personal computer (NEC PC-9801) which allows data acquisition.

When one measures IR absorption of a sample, a reference cell which is full of solvent is set in luminous flux of reference in order to subtract absorption of the solvent. Mainly, CaF_2 cell (with a pathlength of 0.05mm, 0.1mm, 0.2mm, and 0.5mm)were employed in concentrated solution because KBr cell is damaged by polar solvent such as DMSO. Quartz cell (with a pathlength of 1mm, 2mm, 5mm, 1cm, 2cm, and 5cm) were also used in measurements of the amide A region in dilute solution. Ordinally, absorbance of sample including solvent was kept less than one to obtain reliable VCD spectra.

Techniques in measurement and sampling for VCD experiments

are, in principle, almost similar to IR aborption experiments. Practically it takes a longer time owing to requirement to use a long time constant and/or repeated accumulation in ordrer to get a VCD spectrum with a resonable S/N ratio. In order to remove artifacts as much as possible, the baseline was evaluated from the average of VCD for a pair of optical antipodes measured under exactly the same conditions (cell, pathlength, concentration, temperature etc.). In other words, VCD spectra were obtained from the difference of the spectra of two enantiomers, i.e. $(A_{L\ form}-A_{D\ form})/2$. In the case where it is impossible to get a pair of optical antipodes, the baseline was taken from the VCD of recemic sample with absorption spectrum similar to that of each optically active sample in a given spectral region .

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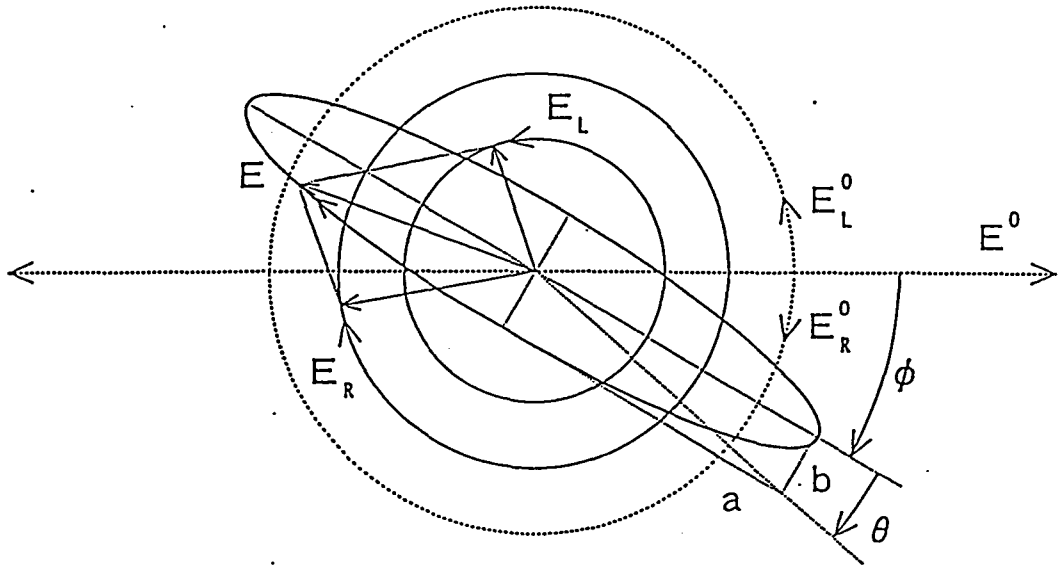
Table III-1. Frequencies and assignments of amide group bands.

Band	Usual frequency range (cm^{-1})	Approximate description[a]	
Amide A	∿3300	N-H	(3300)
Amide B	∿3100	N-H str (in Fermi resonance with 2 x amide II)	(3100)
Amide I	1597-1680	C=O str, N-H def, C-N str	(1650)
Amide II	1480-1575	C-N str, N-H def	(1560)
Amide III	1229-1301	C-N str, N-H def	(1300)
Amide IV	625-767	O=C-N def	(625)
Amide V	640-800	N-H def	(725)
Amide VI	537-606	C=O def	(600)
Amide VII	200	C-N tor	(200)

[a]Based upon normal coordinate calculations for the frequencies observed in N-methylacetamide (given in parentheses). Amides IV to VII are out-of-plane modes, i.e., out of the plane of the CONH grouping, and others are in-plane modes. Abbreviations: str, stretching; def, deformation; tor, torsion.



$\phi = \pi \nu (n_L - n_R) \ell$

$E_{R/L} = E^0_{R/L} 10^{-A_{R/L}/2}$

$a = E_L + E_R$, $b = E_L - E_R$

$\tan\theta = b/a = \tanh(\ell n 10 \cdot (A_L - A_R)/4) = \tanh(2.303 \Delta A/4)$

Fig.III-1. Elliptical polarization, specified by the angle ϕ, resolved into coherent right and left circular polarizations of different amplitude.

Chapter IV

VCD of Blocked Linear Dipeptides

(C_5 Conformation and Dimer Formation)

SUMMARY

Vibrational circular dichroism (VCD) and IR absorption spectra in the amide A, I, and II regions were measured for several blocked dipeptides of fundamental amino acids. The following dipeptides were synthesized: R'-CO-Aa-NH-R'' (R'=Me and $^{(t)}$Bu; Aa=Ala, Leu, Val and Phe; R''=Me, $^{(i)}$Bu and (neo)Pe).

The analyses of VCD and absorption spectra including their concentration dependence indicate that VCD spectra for the amide A band are quite sensitive to the state of hydrogen bonds as well as the conformation of oligopeptides. In Ac-Ala-NHMe, Piv-Ala-NHMe and Ac-Phe-NHMe, the C_5 conformer predominant in dilute solution gives rise to positive VCD for the hydrogen bond free N-H stretching band at the highest frequency side, and negative VCD for the intramolecularly hydrogen bonded N-H stretching band. The C_5-C_5 dimer formed in concentrated solution exhibits a negative-positive couplet pattern characteristic of dimer formation. In Ac-Ala-NH$^{(i)}$Bu, Ac-Ala-NH(neo)Pe and Ac-Leu-NHMe, hydrogen-bonded associates higher than dimer are formed and give an additional negative VCD band at the lower frequency side of the hydrogen-bonded absorption band in very concentrated solution.

It is concluded that the VCD spectra for the amide A band can be utilized to differentiate the aggregation modes in oligopeptides as well as to specify the local conformation.

INTRODUCTION

It has been recognized that intra-residue interactions as well as longer-range inter-residue interactions play an essential role in determining the peptide conformations in proteins.[1-3] N-acetyl-N'-methylamide derivatives of amino acids (referred here as blocked dipeptides) have been widely used as the simplest model peptides in order to understand the conformations observed in proteins[4-16]. Blocked dipeptides have been widely used in many theoretical and experimental studies in order to study the contribution of the side chains in amino acid residues to the stabilization of the secondary structure of proteins[17-20].

In this chapter, VCD and IR absorption spectra of blocked dipeptides of several amino acids (alanine, leucine, phenylalanine and valine) were measured in apolar solvents and analysed. An interpretation of characteristic feature of the VCD spectra were proposed.

EXPERIMENTAL

The blocked dipeptides investigated in this chapter are

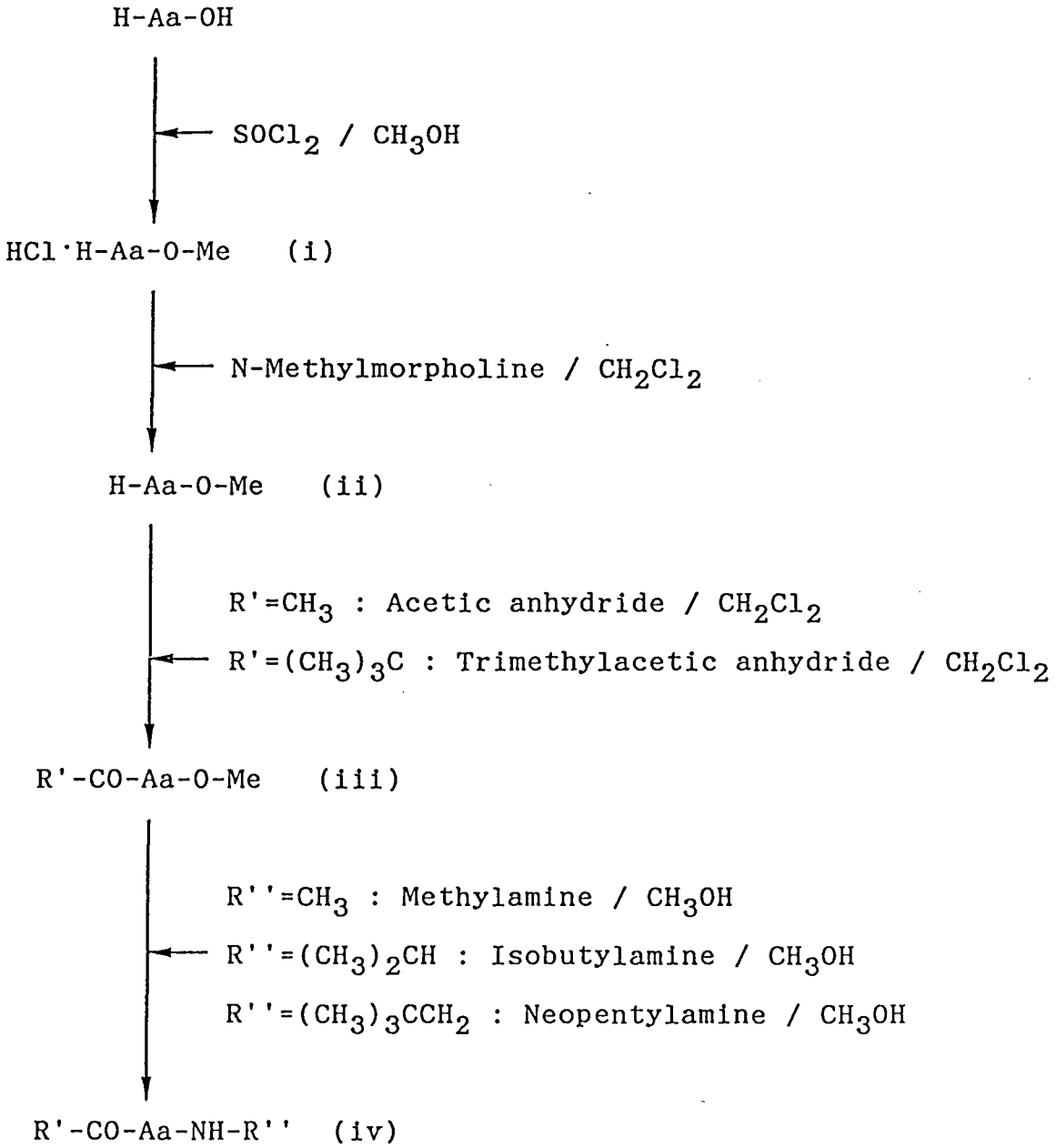
1) Ac-(L,D)Ala-NHMe
2) Piv-(L,D)Ala-NHMe
3) Ac-(L,D)Ala-NH$^{(i)}$Bu
4) Ac-(L,D)Ala-NH(neo)Pe
5) Ac-(L,D)Leu-NHMe

6) Ac-(L,D)Phe-NHMe

7) Ac-(L,D)Val-NHMe

where Ac=CH_3CO-, Piv=$(CH_3)_3CCO$-, Me=CH_3, $^{(i)}$Bu=$(CH_3)_2CHCH_2$-, (neo)Pe=$(CH_3)_3CCH_2$-. These amino acid residues (Ala, Leu, Phe, Val) are all soluble in apolar solvent.

All derivatives (R'-CO-Aa-NH-R'' ; Aa = Ala, Leu, Phe, and Val residues) were synthesized according to the following scheme:



L-(or D-) amino acid methylester hydrochloride (i) was prepared from L-(or D-) amino acid by the application of the method by Brenner and Huber.[21] The methylester hydrochloride was dissolved in dichloromethane and neutralized by N-methylmorpholine to obtain compound (ii). N-acetyl methylester derivatives (iii) were obtained by the procedure of Goodman and Langsam.[22] These products were added to alkylamine in a flask cooled in an ice bath. The reaction mixture was kept at 5 ^{o}C for several days. Finally, solvent was evaporated and the residue, i.e., N-acetyl-alanine-N'-methylamide (iv), was recrystallized. The chemical purity was checked by silica TLC and ^{1}H-NMR. Most reagents with high quality were purchased and used without further purification.

VCD spectra were recorded on a JASCO J-200E-1 infrared spectropolarimeter and a JASCO IRF-10 interferometer equipped with a VCD optical bench. Spectral grade CCl_4 and $CDCl_3$ were employed as solvent and dried with molecular sieves for 24 hr before the use.

RESULTS

< Blocked Ala dipeptide >

The IR absorption and VCD spectra of Ac-Ala-NHMe in $CDCl_3$ are shown in Fig. IV-1. The band intensity at 3450 cm^{-1} increases while the intensity of 3315 cm^{-1} band diminishes with dilution from 0.1 to 0.001 M. Obviously the former is due to the free

N-H stretching vibration and the latter is due to the intermolecularly hydrogen-bonded one. The hydrogen bonded N-H stretching band at 3315 cm^{-1} in concentrated solution exhibits the negative-positive couplet from the high frequency side in the VCD spectrum. This couplet and a negative VCD at 3425 cm^{-1} diminish with dilution and finally positive VCD bands at 3450 and 3380 cm^{-1} are observed in dilute solution. Low solubility prevents IR and VCD measurements in CCl_4.

The spectra of Piv-Ala-NHMe are shown in Fig. IV-2 at various concentrations in CCl_4 and $CDCl_3$. Three distinct bands are observed at 3465, 3425 and 3325 cm^{-1} in CCl_4 solution. The absorption band at 3465 cm^{-1} is assigned to the free N-H stretching vibration and the broad feature centered at 3325 cm^{-1} is attributable to the intermolecularly hydrogen-bonded N-H stretching band from the concentration dependence of intensity, although the measurement for dilute solution lower than 0.0005 M was not possible. The free N-H stretching band at 3465 cm^{-1} exhibits a positive VCD in dilute solution. In concentrated solution a negative VCD band at 3425 cm^{-1} and a negative-positive couplet for the intermolecularly hydrogen-bonded N-H stretching band at 3325 cm^{-1} is similar to those of Ac-Ala-NHMe.

Figs. IV-3 and IV-4 show the IR absorption and VCD spectra of Ac-Ala-NH$^{(i)}$Bu and Ac-Ala-NH(neo)Pe, respectively. Both peptides show qualitatively similar concentration dependence in absorption and VCD spectra. The absorption band for the intermolecularly hydrogen-bonded N-H stretching vibration shifts to low frequency as well as increases in intensity with concentra-

tion. The corresponding VCD shows a negative-positive couplet with the increase of concentration and finally a new negative peak appears at the low frequency side of the absorption band with further increase of concentration.

The IR absorption and VCD spectra of Ac-Ala-NHMe in the amide I and II regions are shown in Fig. IV-5. Two distinct bands are observed at 1652 cm^{-1} and 1512 cm^{-1} in 0.2 M $CDCl_3$ solution. Obviously, the former is due to the amide I band and the latter is due to the amide II band. The amide I VCD shows a characteristic positive-negative couplet and the amide II exhibits a positive monosignate band at 1530 cm^{-1}.

< Blocked Leu, Phe, and Leu dipeptides >

The IR absorption and VCD spectra of Ac-Leu-NHMe are shown in Fig. IV-6. The absorption peak of hydrogen-bonded N-H stretching vibration shifts to lower frequency with concentration. The negative-positive couplet in VCD for the intermolecularly hydrogen-bonded N-H stretching grows up with the increase of concentration at first and a new negative band at the low frequency side appears for a highly concentrated solution in CCl_4.

The IR absorption and VCD spectra of Ac-Phe-NHMe and Ac-Val-NHMe in $CDCl_3$ solution are shown in Figs. IV-7 and IV-8, respectively. Two major peaks are assigned to the free and hydrogen-bonded N-H stretching bands, respectively from the higher frequency side. The intermolecularly hydrogen-bonded N-H band at 3320 cm^{-1} shows a negative-positive couplet in VCD. The N-H stretching vibration in monomer species gives positive bands at 3460 and 3430 cm^{-1} for Ac-Val-NHMe, and positive and negative

bands at 3460 and 3430 cm^{-1}, respectively, for Ac-Phe-NHMe.

DISCUSSION

The conformation of blocked dipeptide Ac-Ala-NHMe was studied by means of X-ray[23] diffraction, IR[8,10,17,18,20,24-26], and ^1H-NMR[17,18,20]. From these investigations, the existence of a stable conformation of C_5 has been proposed in apolar solvents. As shown in Fig. IV-9(A) the C_5 conformer has a semiextended form with an intramolecular hydrogen bond of five-membered ring between the N-H group and the C=O group of the amino acid residue.

Neel[26] has ascribed the 3420 cm^{-1} band to intramolecularly hydrogen-bonded five-membered N-H streching vibration in the AAM. Cung et al[17]. proposed a scheme in which two C_5 monomers exist in equilibrium with an antiparallel intramolecularly hydrogen-bonded dimer as shown in Fig. IV-9(B). Asakura et al[20]. also noted a similar C_5-C_5 self-association of alanine containing blocked dipeptide in $CDCl_3$ by NMR spectroscopy.

In Ac-Ala-NHMe and Piv-Ala-NHMe (Figs. IV-1 and IV-2) the negative-positive couplet VCD for the intermolecularly hydrogen-bonded N-H stretching vibration is ascribed to C_5-C_5 hydrogen-bonded dimer as illustrated in Fig. IV-9. The positive VCD band for the N-H stretching vibration at the highest frequency at 3450 cm^{-1}, whose absorption intensity increases with dilution, is due to the hydrogen bond free N-H in monomer species. The negative VCD band at 3420 cm^{-1}, corresponds to the C_5 intramolecularly

hydrogen-bonded N-H stretching vibration. It also grows with concentration and it can be said that the C_5 conformation is stabilized by dimer formation.

The absorption maximum for the intermolecularly hydrogen-bonded N-H stretching of Ac-Ala-NH$^{(i)}$Bu (Fig. IV-3), Ac-Ala-NH(neo)Pe (Fig. IV-4) and Ac-Leu-NHMe (Fig. IV-6) shows a lower frequency shift with concentration. This continuous red shift is ascribed to the formation of hydrogen-bonded polymers of trimer or higher heteromer. Previous IR and X-ray works on Ac-Leu-NHMe showed that Ac-Leu-NHMe forms somewhat distorted β-parallel type structure stabilized by intermolecular hydrogen bonds (Fig. I-1)[19]. Similar type of association is expected for the concentrated solution of Ac-Leu-NHMe. The negative VCD band at the lowest frequency of the hydrogen-bonded N-H is due to this hydrogen-bonded polymer species.

The N-H stretching band of the intermolecularly hydrogen-bonded species exhibits the characteristic VCD, originating from the degenerate coupled oscillator mechanism.[27] The hydrogen-bonded dimer has a pair of equivalent chromophores at the position vectors R_a and R_b. The wave-functions for the isolated oscillator are expressed as $\phi^v{}_a$ and $\phi^v{}_b$ where v denotes the vibrational quantum number. The ground state wave function Ψ_o for the whole system is

$$\Psi_o = \phi^o{}_a \phi^o{}_b \qquad \text{---(1)}$$

and the degenerate first excited states in the zeroth order generate the two split states $\Psi_{\pm}$ by the interaction (Fig. VI-10).

$$\Psi_{\pm} = (\phi^1{}_a \phi^0{}_b \pm \phi^0{}_a \phi^1{}_b)\ /2^{1/2} \qquad \text{---(2)}$$

Denoting the electric transition moments of chromophores as μ_a and μ_b, the magnetic moments, $\mathbf{m}_a$ and $\mathbf{m}_b$ produced by those electric dipole moments are expressed as

$$\mathbf{m}_{a/b} = i(\omega/2c)(\mathbf{R}_{a/b} \cdot \mu_{a/b}) \qquad ---(3)$$

Then the dipole strength $D_{\pm}$ and rotational strength $R_{\pm}$ are derived as

$$D_{\pm} = |(\mu_a \pm \mu_b)/2^{1/2}|^2 \qquad ---(4)$$

$$R_{\pm} = \mathrm{Im}\,[\mu_{\pm} \cdot \mathbf{m}_{\pm}]$$

$$= \mp(\omega/4c)(\mu_a \times \mu_b)\,R_{ab} \qquad ---(5)$$

where $R_{ab}=R_b-R_a$ is the position vector from the chromophore a to b. The dipole interaction energy V_{ab} gives the energy splitting by $2V_{ab}$.

$$E_{\pm} = E_{1,0} \pm V_{ab} \qquad ---(6)$$

$$V_{ab} = \{(\mu_a \cdot \mu_b) - 3(\mu_a \cdot R_{ab})(\mu_b \cdot R_{ab})/R^2_{ab}\}/R^3_{ab} \qquad ---(7)$$

As seen in Eq (5) the degenerate coupled oscillator gives rise to a pair of CD band equal in magnitude and opposite in sign. This CD patter is called 'couplet'. Its magnitude and sign depend on the geometrical arrangement of the chromophores.

The C_5-C_5 dimer shows the couplet of negative-positive from the high frequency side for the hydrogen-bonded N-H stretching band. If the energy splitting is assumed to originate only from the dipole-dipole interaction, the energy of the symmetric state, E_+, is lower than that of antisymmetric state, E_-, (i.e. $E_+<E_-$) because of $V_{ab}<0$ for nearly antiparallel arrangement of the N-H stretching vibration transition moments. Consequently the negative-positive couplet leads to the conclusion of $(\mu_a \times \mu_b) \cdot R_{ab}<0$ and the relative arrangement of the N-H bonds (Fig. VI-11) with

negative dihedral angle. The VCD of hydrogen-bonded oligomers result in the complicated pattern because of the superposition of VCD of various oligomeric species.

The VCD pattern at concentrated solution of Ac-Phe-NHMe (Fig. IV-7) similar to that of Ac-Ala-NHMe (Fig. IV-1) indicates the C_5-C_5 hydrogen-bonded dimer formation in Ac-Phe-NHMe. These results are in agreement with the result of theoretical calculation by Abe et al.[16] The VCD at concentrated solution of Ac-Val-NHMe (Fig. IV-8) exhibits a positive band at 3430 cm^{-1} in contrast to the negative band in the other dipeptides. It indicates that Ac-Val-NHMe hardly takes the C_5-C_5 dimer structure owing to the steric hindrance by the bulky side chain.

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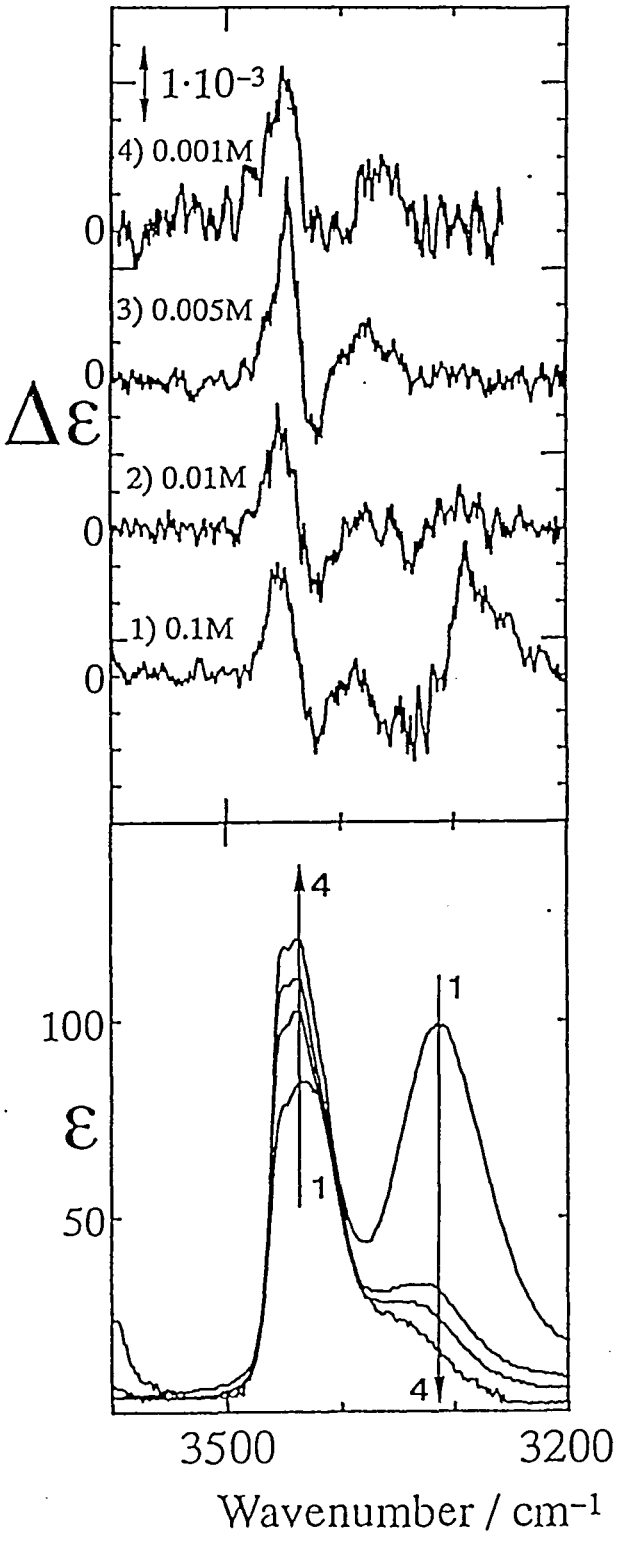


Fig.IV-1 VCD and IR absorption spectra of Ac-Ala-NHMe at various concentration in $CDCl_3$ solution.

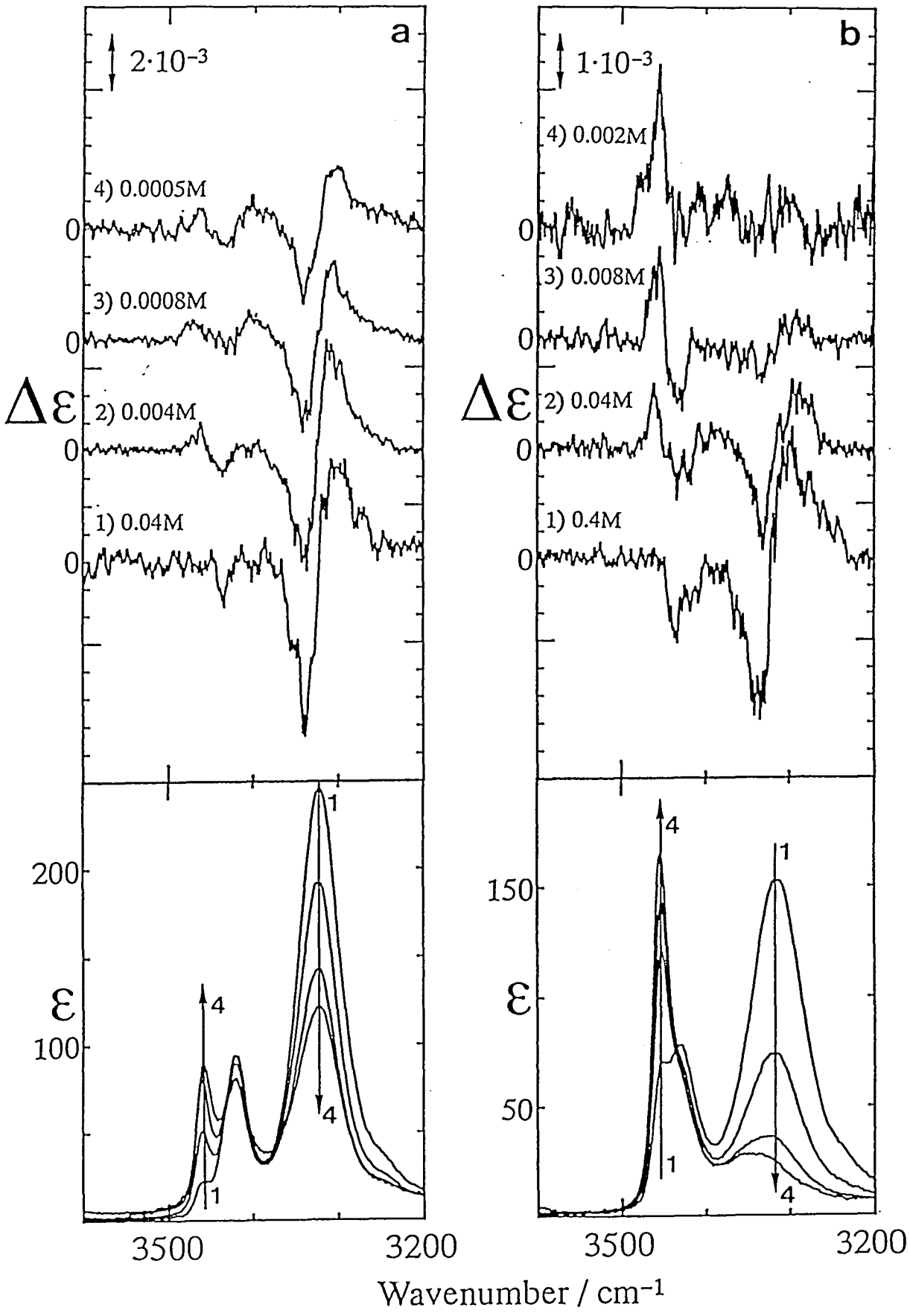


Fig.IV-2 VCD and IR absorption spectra of Piv-Ala-NHMe at various concentration in CCl_4 (a), $CDCl_3$ (b) solution.

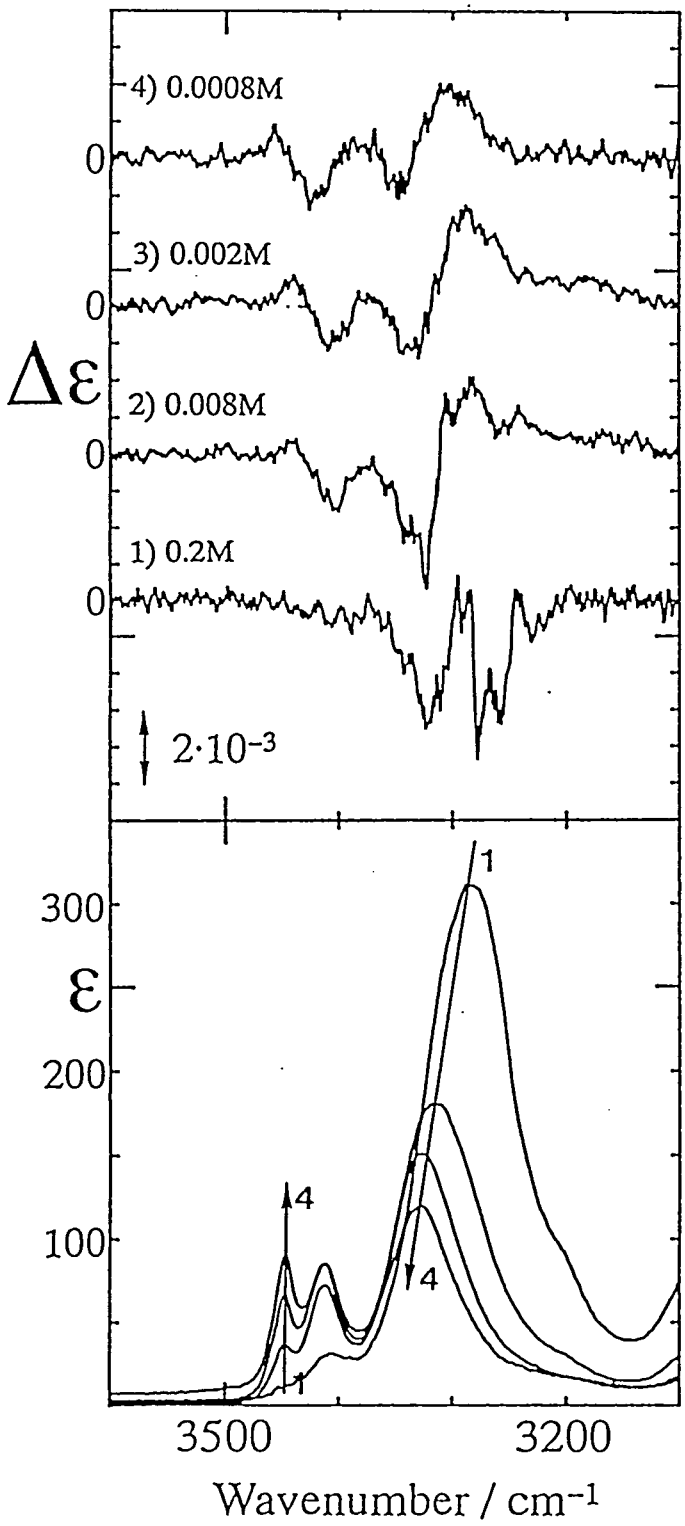


Fig.IV-3(a) VCD and IR absorption spectra of Ac-Ala-NH$^{(i)}$Bu at various concentration in CCl_4 solution.

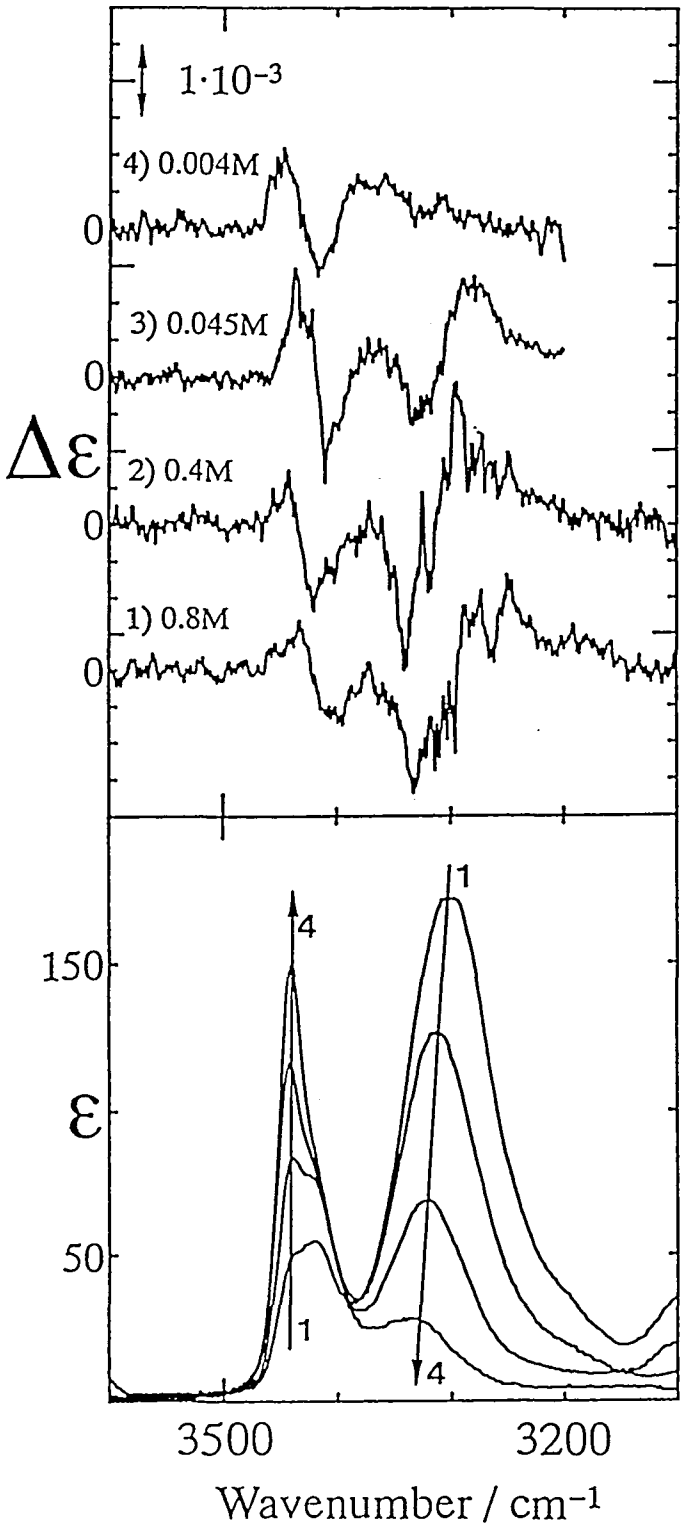


Fig.IV-3(b) (Continuation) $CDCl_3$ solution.

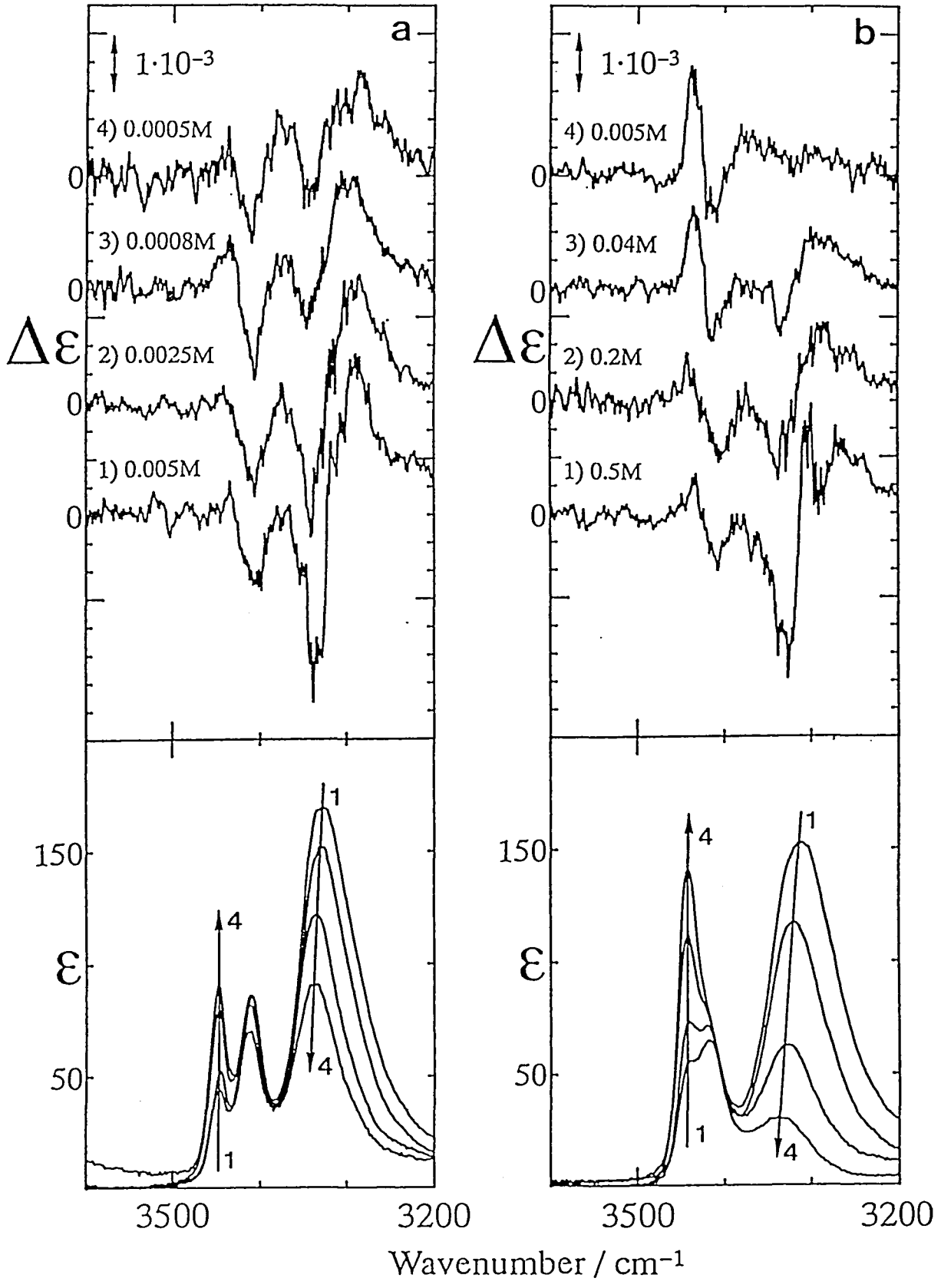


Fig.IV-4 VCD and IR absorption spectra of Ac-Ala-NH(neo)Pe at various concentration in CCl_4 (a), $CDCl_3$ (b) solution.

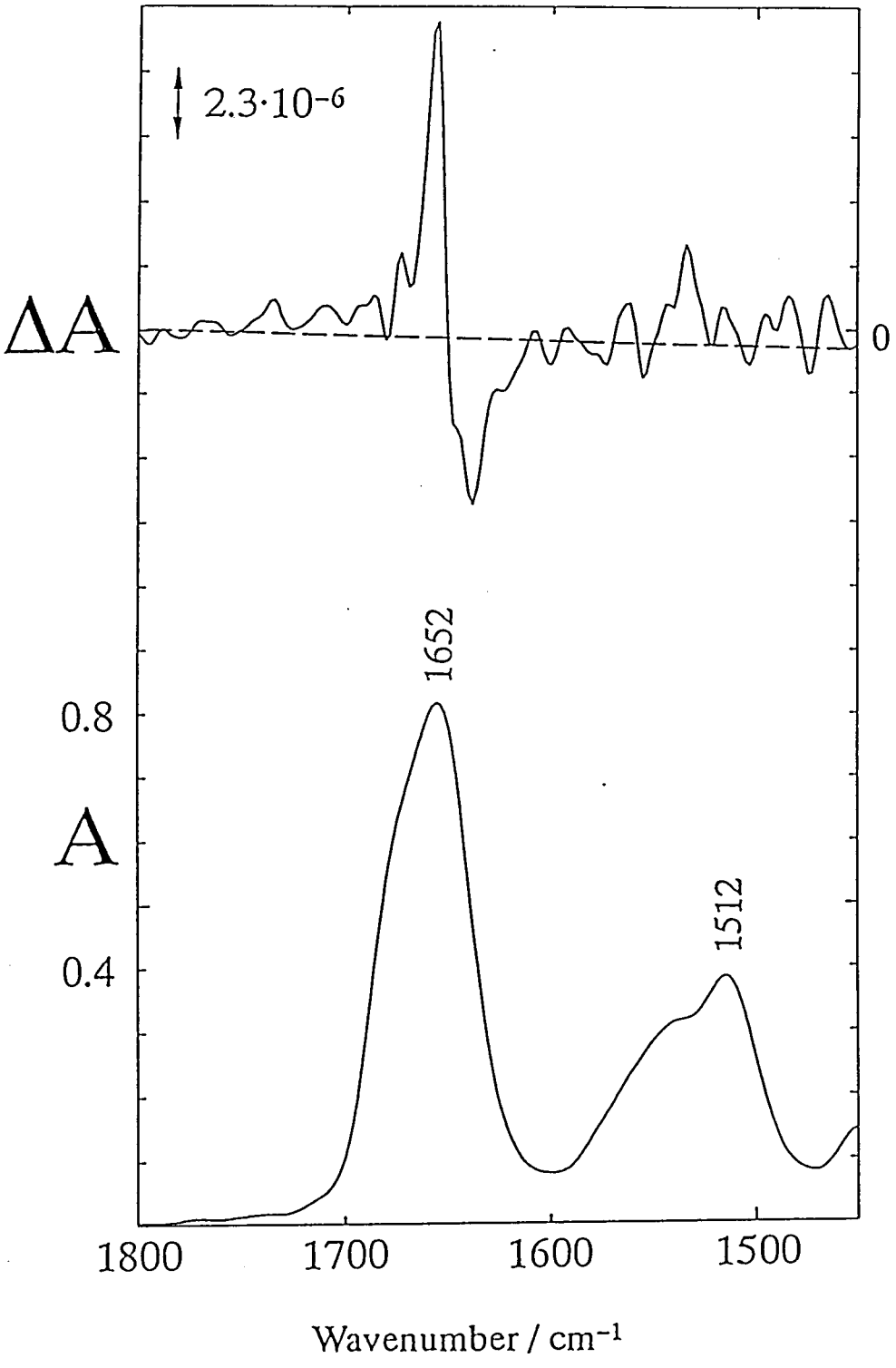


Fig.IV-5 VCD and IR absorption spectra of Ac-Ala-NHMe in 0.2M $CDCl_3$ solution.

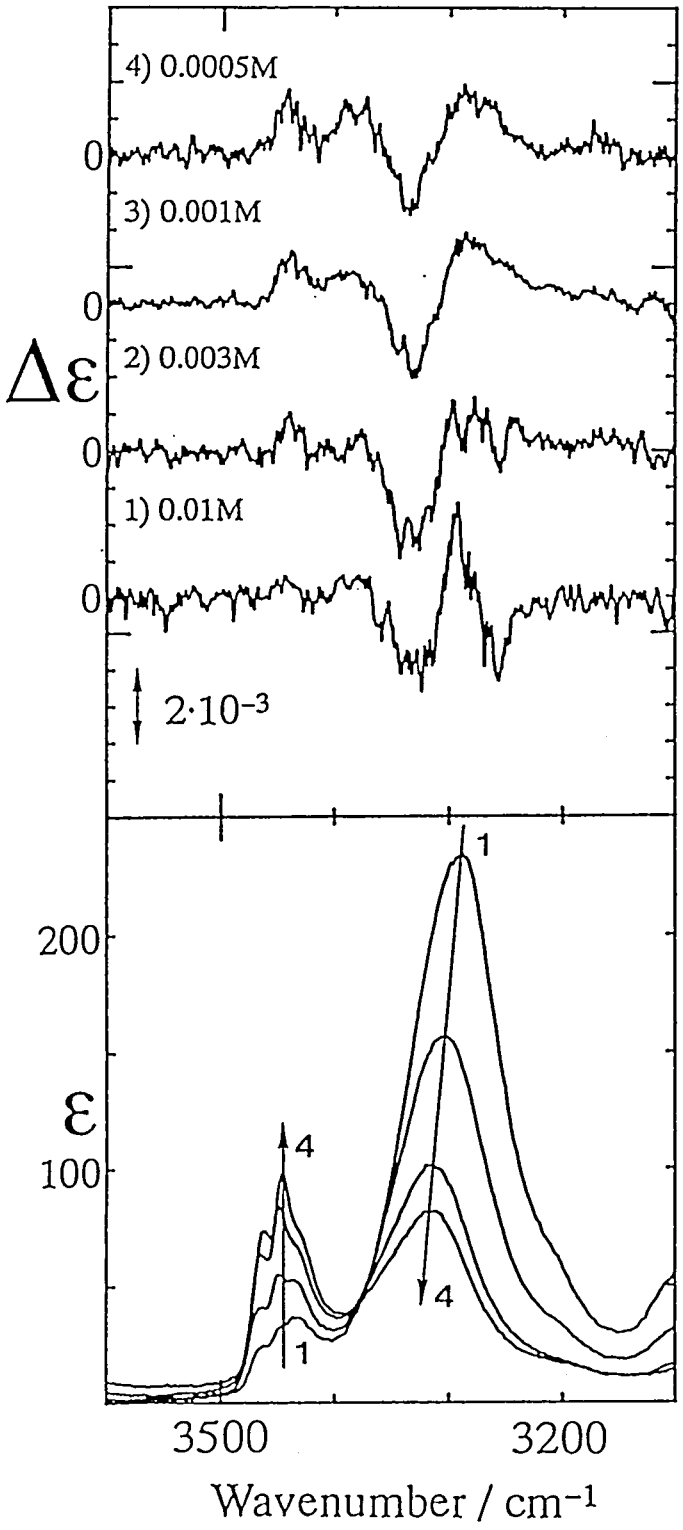


Fig.IV-6(a) VCD and IR absorption spectra of Ac-Leu-NHMe at various concentration in CCl_4 solution.

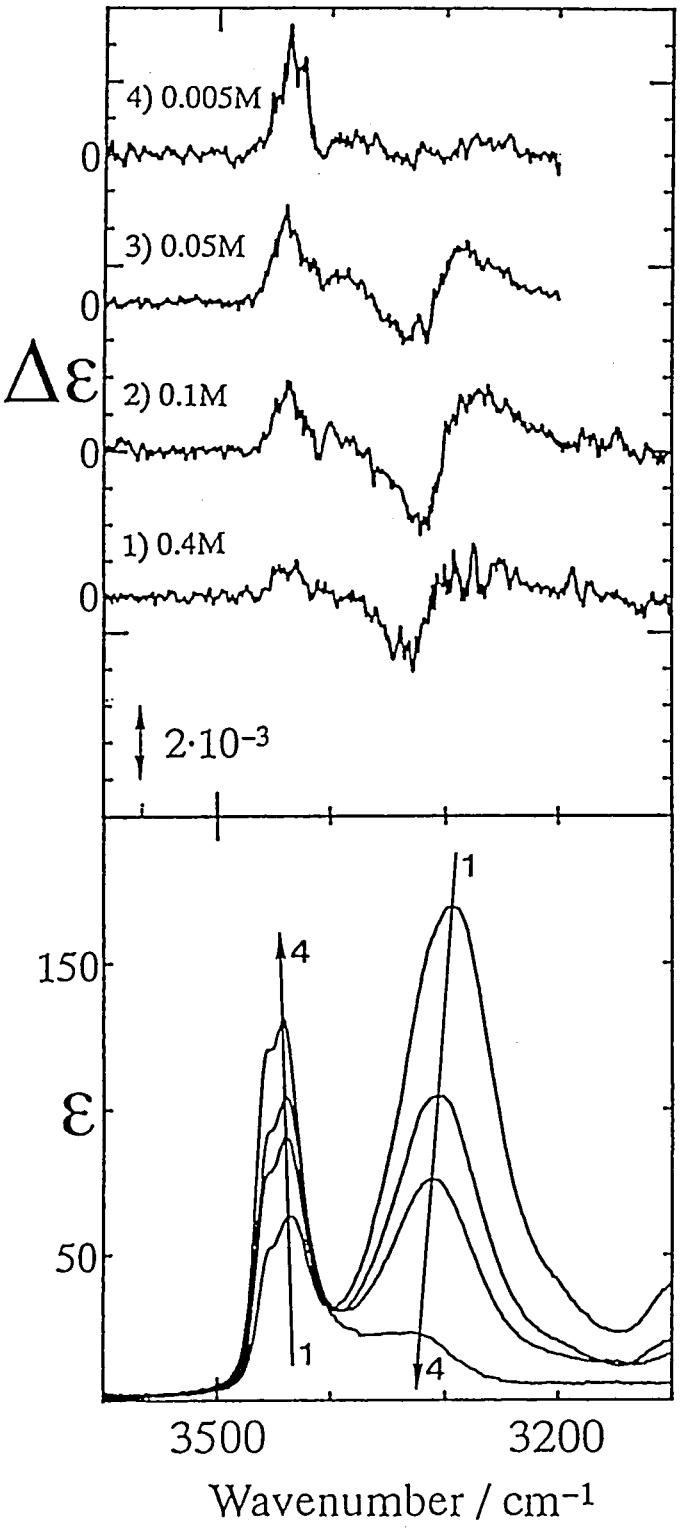


Fig.IV-6(b) (Continuation) $CDCl_3$ solution.

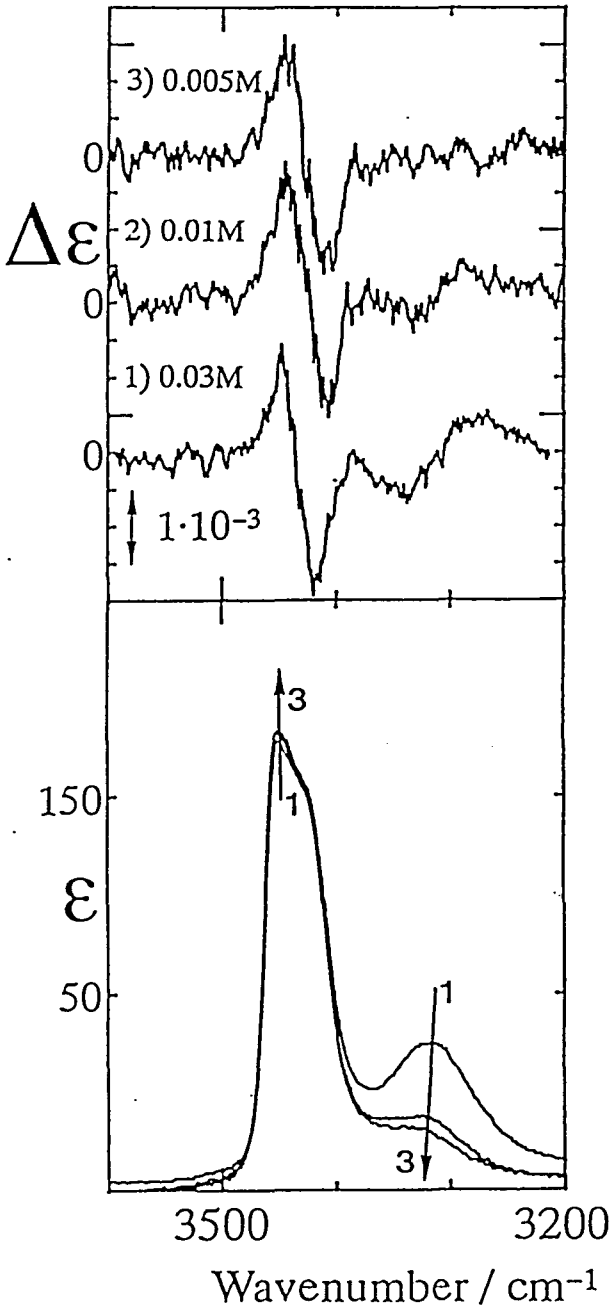


Fig.IV-7 VCD and IR absorption spectra of Ac-Phe-NHMe at various concentration in $CDCl_3$ solution.

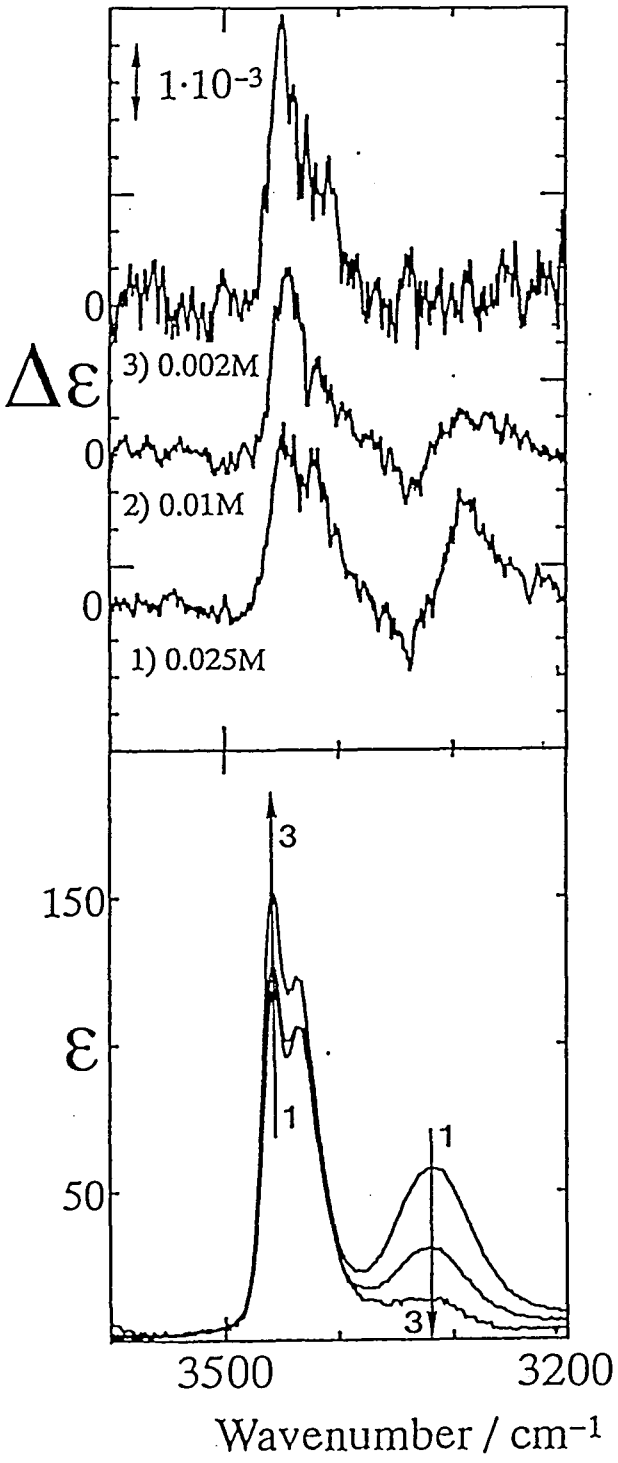
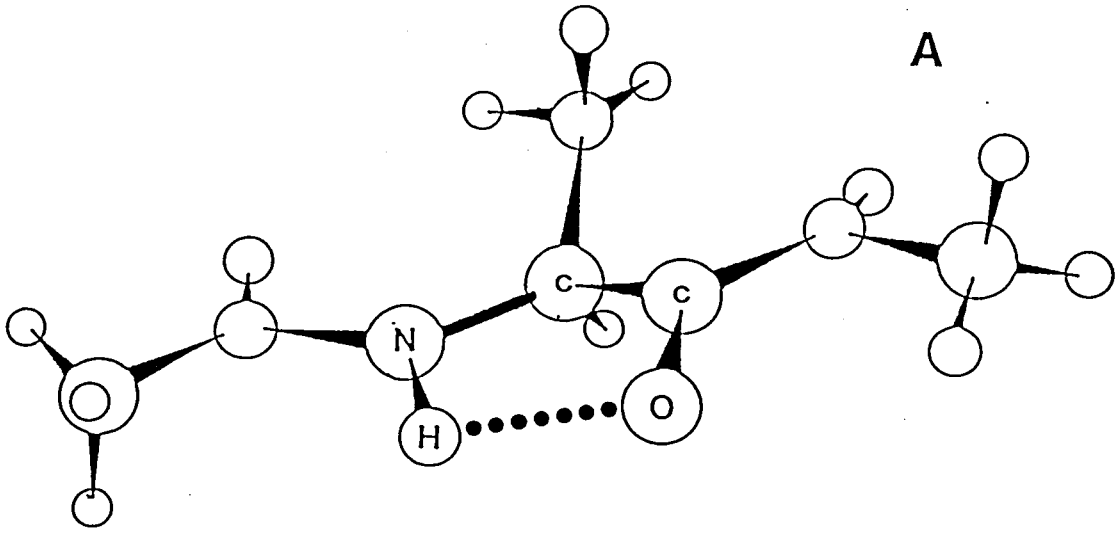


Fig.IV-8 VCD and IR absorption spectra of Ac-Val-NHMe at various concentration in $CDCl_3$ solution.



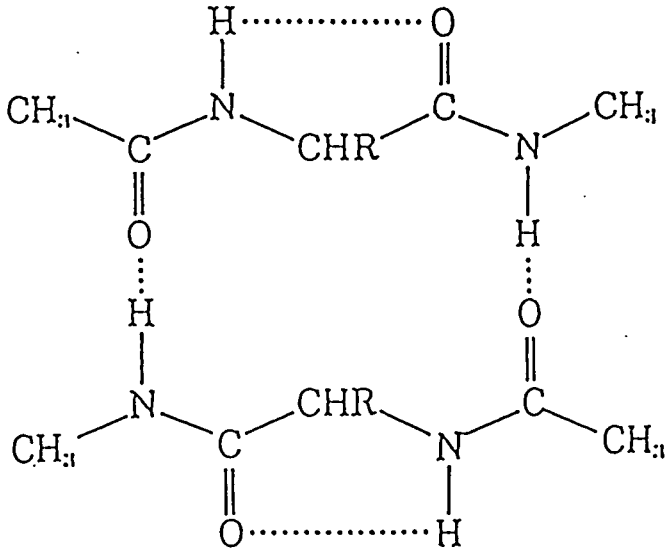


Fig.IV-9 Representation of the C_5 conformation of Ac-Ala-NHMe (A) and the formation of C_5-C_5 dimer aggregation (B). (Hydrogen bond is shown by dotted line.)

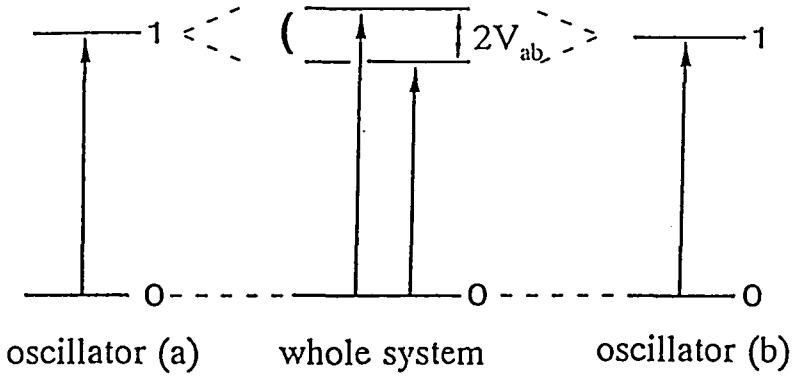


Fig.IV-10 Two split states by the interaction.

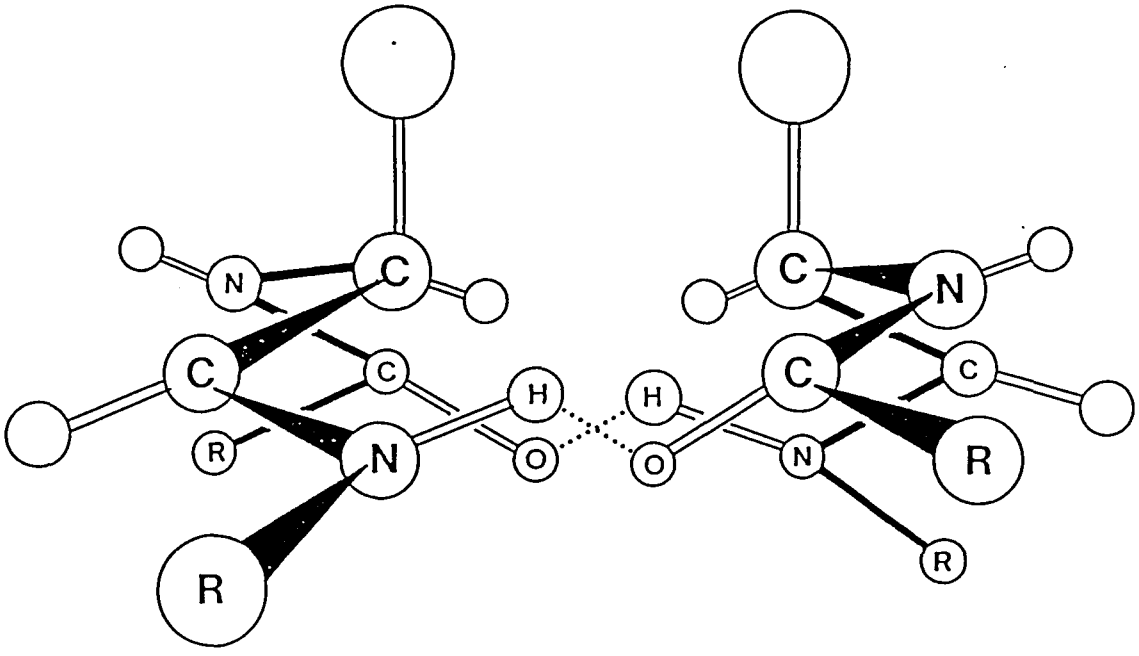


Fig.IV-11 Relative arrangement of the N-H bonds in the C_5-C_5 dimer.

CHAPTER V

VCD of Proline-Containing Dipeptides

(γ-turn Mimetic C_7 Conformation)

INTRODUCTION

Several VCD studies of model polypeptide and oligopeptide systems have been carried out for elucidating the correlation between the VCD for characteristic amide bands and the secondary structure of polypeptide chains[1-8]. However, no VCD study on the local conformation of peptides such as reverse turn structure has been reported.

Proline residue plays a unique role in the formation of backbone structure of peptides and proteins, because the side-chain pyrrolidine ring puts limitation on the torsional angle about the N-C_α bond. Therefore, peptide chains in proteins are often found to reverse their direction at the position of the proline residue. N-Acetyl-proline-N'-methylamide (Ac-Pro-NHMe) is a particularly interesting model peptide because one of its stable conformation has an intramolecular hydrogen bond with seven-membered ring (C_7) called γ-turn (Fig. V-1). Many workers[9-17] reached to a conclusion that a C_7 conformation with an intramolecular hydrogen-bonding between the acetyl C=O and methylamide N-H groups is most stable in dilute apolar solution. In this chapter the concentration dependence of VCD and IR absorption spectra was measured in several solvents and its characteristic VCD spectrum associated with γ-turn was established.

EXPERIMENTAL

The spectra of two model peptides were measured at various concentrations in CCl_4, $CDCl_3$, and DMSO-d_6 solution.

N-acetyl-(L,D)-proline-N'-methylamide : Ac-Pro-NHMe

N-pivaloyl-(L,D)-proline-N'-methylamide : Piv-Pro-NHMe

Both derivatives were gifts from Dr. K.Inoue of Shinshu University. One of the characteristics of the proline residue is that its peptide linkage to the preceding amino acid residue can take the cis conformation more easily than usual peptide bonds. The pivaloyl group ($^{(t)}$Bu-CO-) was introduced in order to prevent cis-trans isomerization of the proline imide link[18] and to obtain a good solubility in CCl_4.

VCD spectra in the amide A region were recorded on a dispersion type VCD spectropolarimeter and those in amide I and II region on Fourier transform VCD spectrometer. In order to remove artifacts and to correct baseline deviation, all spectra were obtained from taking the difference of VCD for two enantiomers, i.e., (L-D)/2.

RESULTS

The IR absorption and VCD spectra of Piv-Pro-NHMe at various concentrations in CCl_4 and $CDCl_3$ are shown in Fig. V-2 and band positions summarized in Table V-1. Two distinct bands are observed at 3440 cm^{-1} and 3330 cm^{-1} over the entire concentration

range in CCl_4. Obviously, the former is due to the free N-H stretching vibration and the latter is due to the hydrogen-bonded N-H stretching vibration. Any change could not be observed in intensity of the two bands in CCl_4 dilute solution below 0.0005 M. The free N-H stretching vibration exhibits positive VCD over the entire concentration range. The intensity of this VCD band grows larger with increase in IR absorption intensity. On the other hand, a drastic variation in VCD of hydrogen-bonded N-H strecthing band with concentration are observed. In high concentration, VCD spectra give rise to a negative-positive couplet from high wavenumber side centered at the absorption maximum. This VCD couplet gradually diminishes upon lowering concentration leaving a positive VCD band at low concentration and a distinct monosignate VCD band at 3330 cm^{-1} is observed finally. Similar spectral change are obtained in $CDCl_3$.

The IR absorption and VCD spectra of Ac-Pro-NHMe are shown in Fig. V-3. The low solubility in CCl_4 prevented measurement in concentrated solution. The absorption spectra show concentration dependence similar to that observed for Piv-Pro-NHMe. VCD corresponding to the hydrogen bonded N-H streching vibration exhibits the same feature as that of Piv-Pro-NHMe; the bisignate couplet in concentrated solution and the monosignate positive VCD in dilute solution. But no distinct VCD was observed for the free N-H stretching vibration.

Fig. V-4 shows the IR absorption and VCD spectra of Piv-Pro-NHMe and Ac-Pro-NHMe in DMSO-d_6 solution. A weak positive VCD is still observed for the N-H stretching vibration of Piv-Pro-

NHMe, but no characteristic VCD is observed for Ac-Pro-NHMe.

The IR absorpotion and VCD spectra in 1800-1500 cm^{-1} region of Ac-Pro-NHMe are presented in Fig. V-5. A positive-negative couplet centered on the absorption band at 1670 cm^{-1} is assigned to the amide C=O stretching band, and negative VCD band at 1626 cm^{-1} is due to the pivaloyl imide C=O stretching band.

DISCUSSION

A number of studies have discussed the conformation of Ac-Pro-NHMe in solution by IR[9-13] or NMR[17]. They concluded that the population of the C_7 conformation is much larger than the total population of the other possible conformers in dilute apolar solvents. Similarly, Rao et al.[20] examined the IR spectra of Piv-Pro-NHMe in CCl_4 and $CDCl_3$ at various concentrations, where C_7 conformer was predominant species in dilute solution.

The concentration dependence observed in absorption spectra (Figs. V-2 and V-3) that the hydrogen bonded N-H stretching band decreases in intensity with dilution down to a certain concentration but remains essentially constant in its intensity at very dilute solution, is interpreted as following. There exists a chemical equilibrium between intermolecular association and intramolecularly hydrogen bonded species. The non-associated species has an intramolecular hydrogen bond which has essentially the same absorption position and band width as the intermolecular hydrogen bonded species.

The hydrogen bonded N-H stretching vibration of Piv- and Ac-peptides in CCl_4 and $CDCl_3$ gives rise to a couplet pattern of VCD with negative-positive lobes from high frequency side in concentrated solution, while a positive monosignate VCD is observed in dilute solution. Absorption spectra show a qualitatively similar feature for dilute and concentrated solution, and thus is difficult to utilize the absorption spectra for monitoring intermolecular association. On the other hand, VCD spectra exhibit a distinctive feature for association. The bisignate couplet originating from the interaction between chromophores in aggregate proves definitely the existence of intermolecular association. The positive monosignate feature of the hydrogen bonded N-H stretching vibration band in dilute solution can be ascribed to the intramolecular hydrogen bond in γ-turn with C_7 conformation.

The free N-H stretching vibration at around 3440 cm^{-1} exhibits a positive VCD band for Piv-Pro-NHMe, but no definite VCD is observed for Ac-Pro-NHMe (Figs. V-2 and V-3). The difference in VCD between Piv-Pro-NHMe and Ac-Pro-NHMe suggests that the structure of hydrogen-bond free form is different for both peptides. The stronger absorption of the hydrogen bond free N-H stretching vibration in $CDCl_3$ compared to that in CCl_4 indicates that hydrogen-bond free conformations are more abundant in $CDCl_3$. An easier cis isomerization[17] of Ac-Pro-NHMe compared to Piv-Pro-NHMe is a possible source for that as well. A positive VCD for amide A of Piv-Pro-NHMe in DMSO-d_6 is ascribed to residual γ-turn structure. No observable VCD for Ac-Pro-NHMe in

DMSO-d_6 originates from the disruption of intramolecularly hydrogen bonded form due to strong interaction of the N-H group with solvent DMSO-d_6.

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Table IV-1. Infrared absorption and VCD bands of Piv-Pro-NHMe and Ac-Pro-NHMe in the amide A region

Coccentration (M)	Free (cm^{-1})		Hydrogen-bonded (cm^{-1})	
	IR	VCD	IR	VCD
Piv-Pro-NHMe				
<CCl_4 soln.>				
1) 0.1	3440	3440	3330	3360,3328
2) 0.01	3440	3442	3330	3350,3330
3) 0.0005	3440	3440	3330	3330
<$CDCl_3$ soln.>				
1) 1.0	3440	3438	3330	3358,3322
2) 0.5	3438	3440	3332	3354,3328
3) 0.01	3440	3440	3330	3328
Ac-Pro-NHMe				
<CCl_4 soln.>				
1) 0.0005	3440		3325	3330
<$CDCl_3$ soln.>				
1) 1.8	3440		3323	3350,3320
2) 0.4	3440		3323	3325
3) 0.01	3440		3325	3325

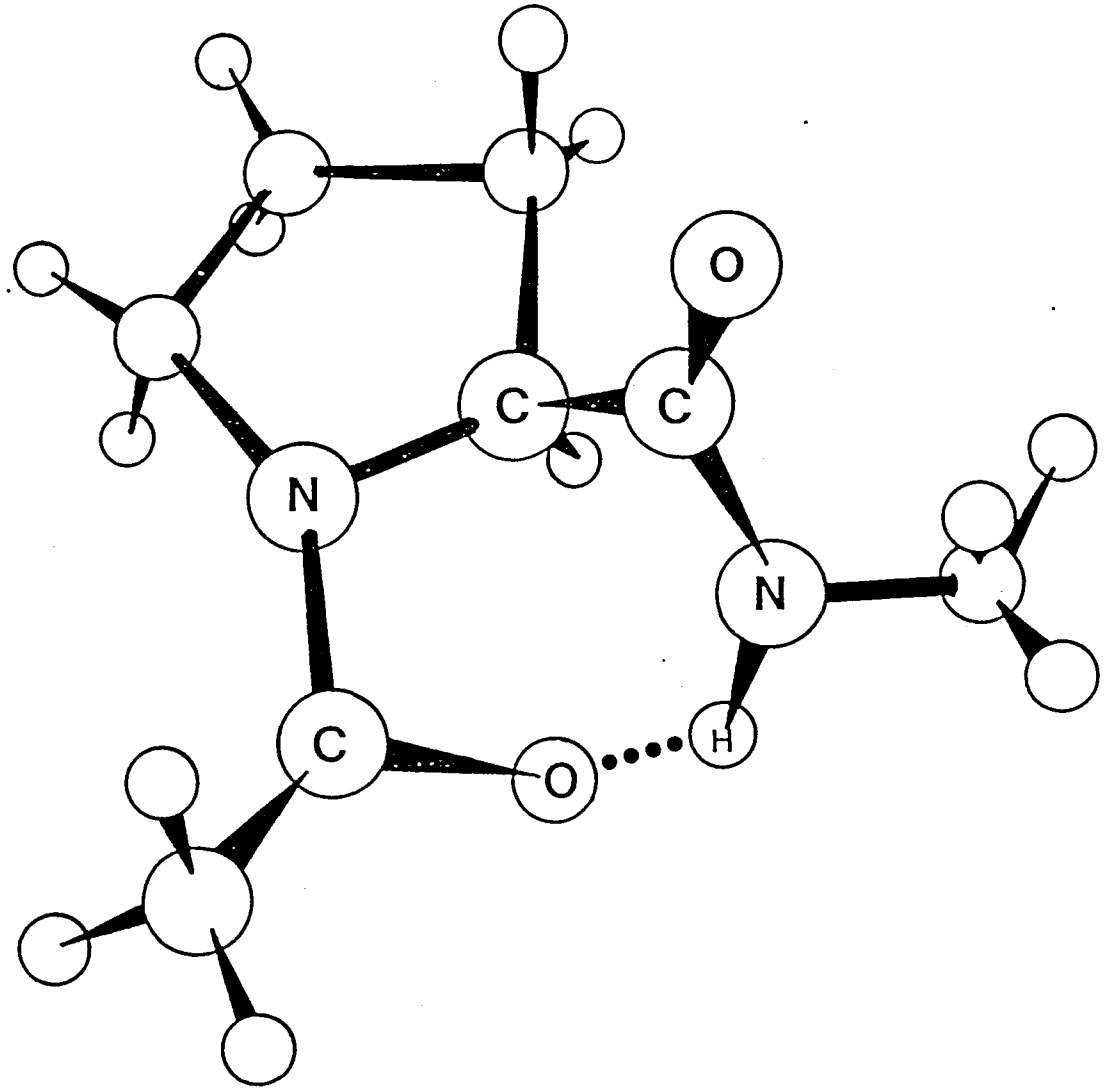


Fig.V-1 Representation of the γ-turn conformation of Ac-Pro-NHMe (Hydrogen bond is shown by dotted line).

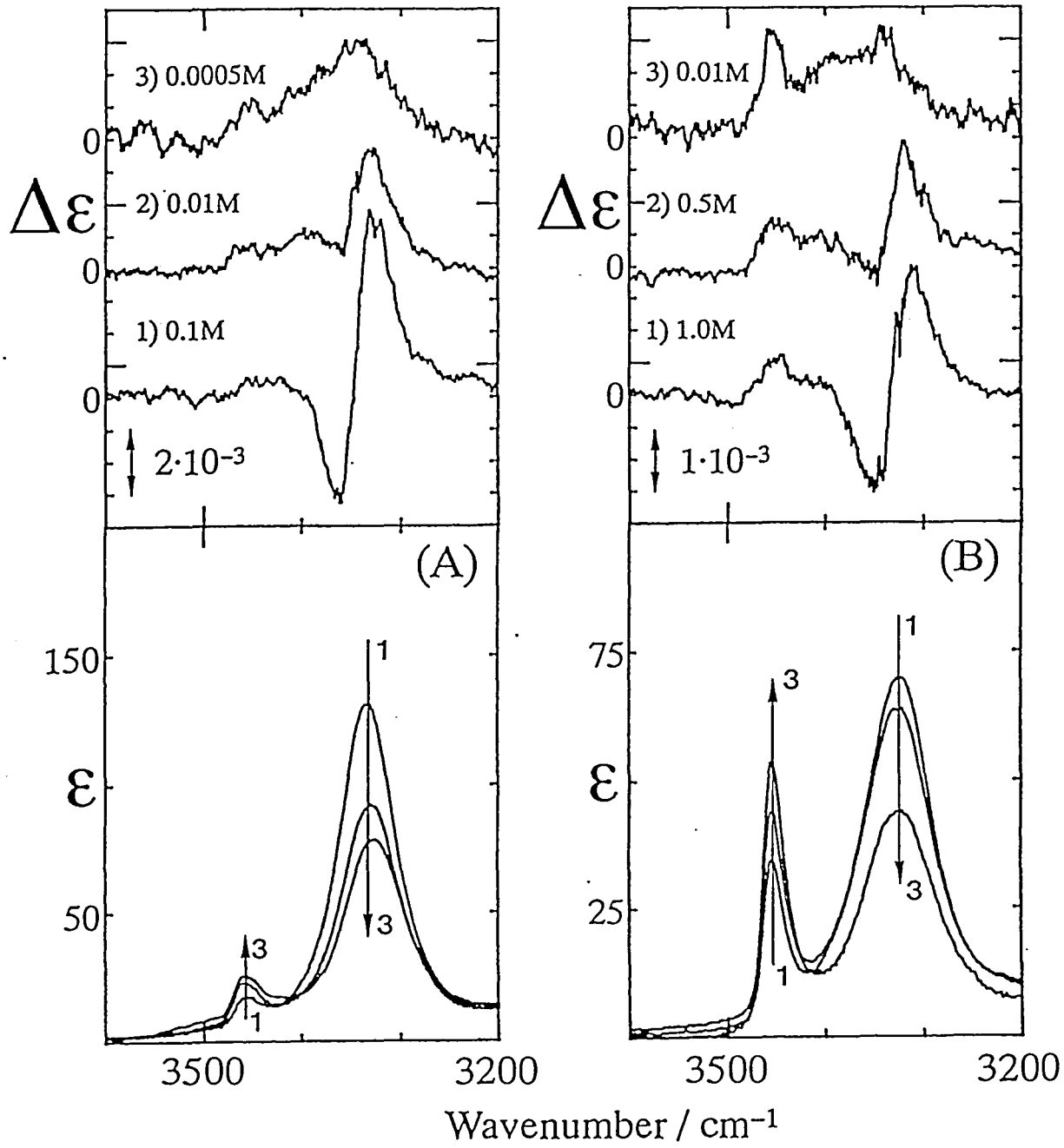


Fig.V-2 VCD and absorption spectra of Piv-Pro-NHMe at various concentration in CCl_4 (A) and $CDCl_3$ (B) solution.

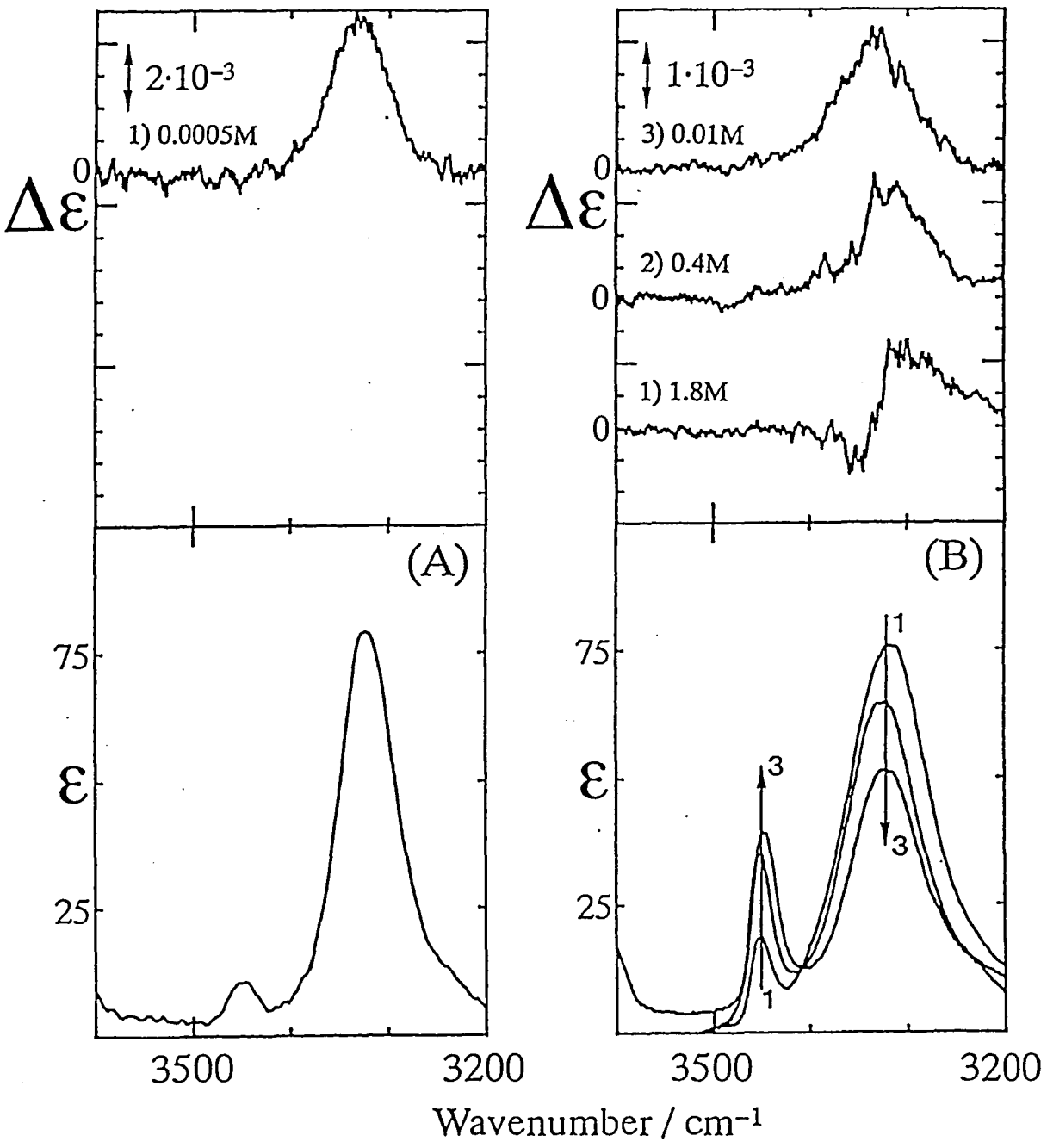


Fig.V-3 VCD and absorption spectra of Ac-Pro-NHMe at various concentration in CCl_4 (A) and $CDCl_3$ (B) solution.

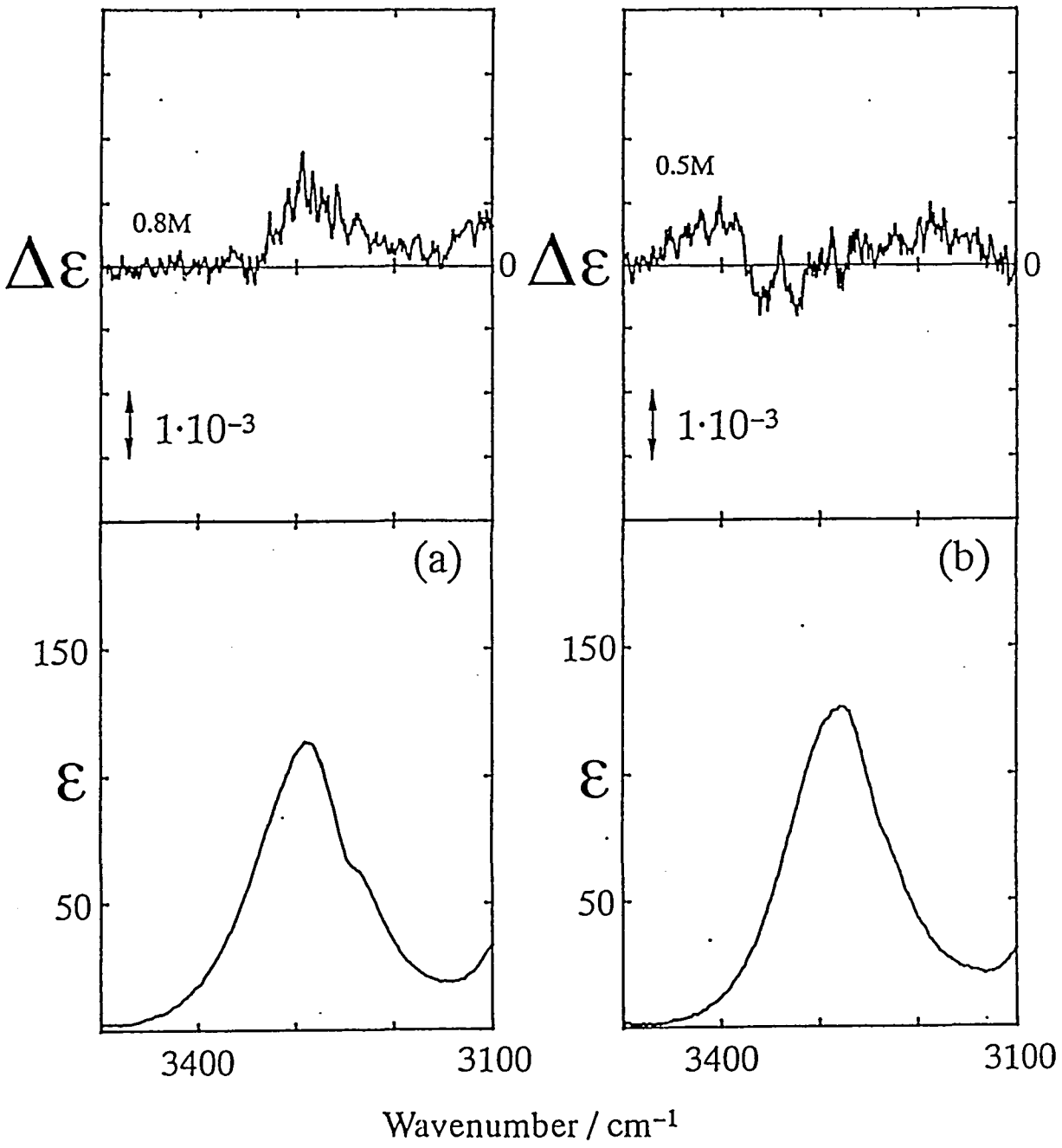


Fig.V-4 VCD and absorption spectra of Piv-Pro-NHMe (a) and Ac-Pro-NHMe (b) in DMSO-d_6 solution.

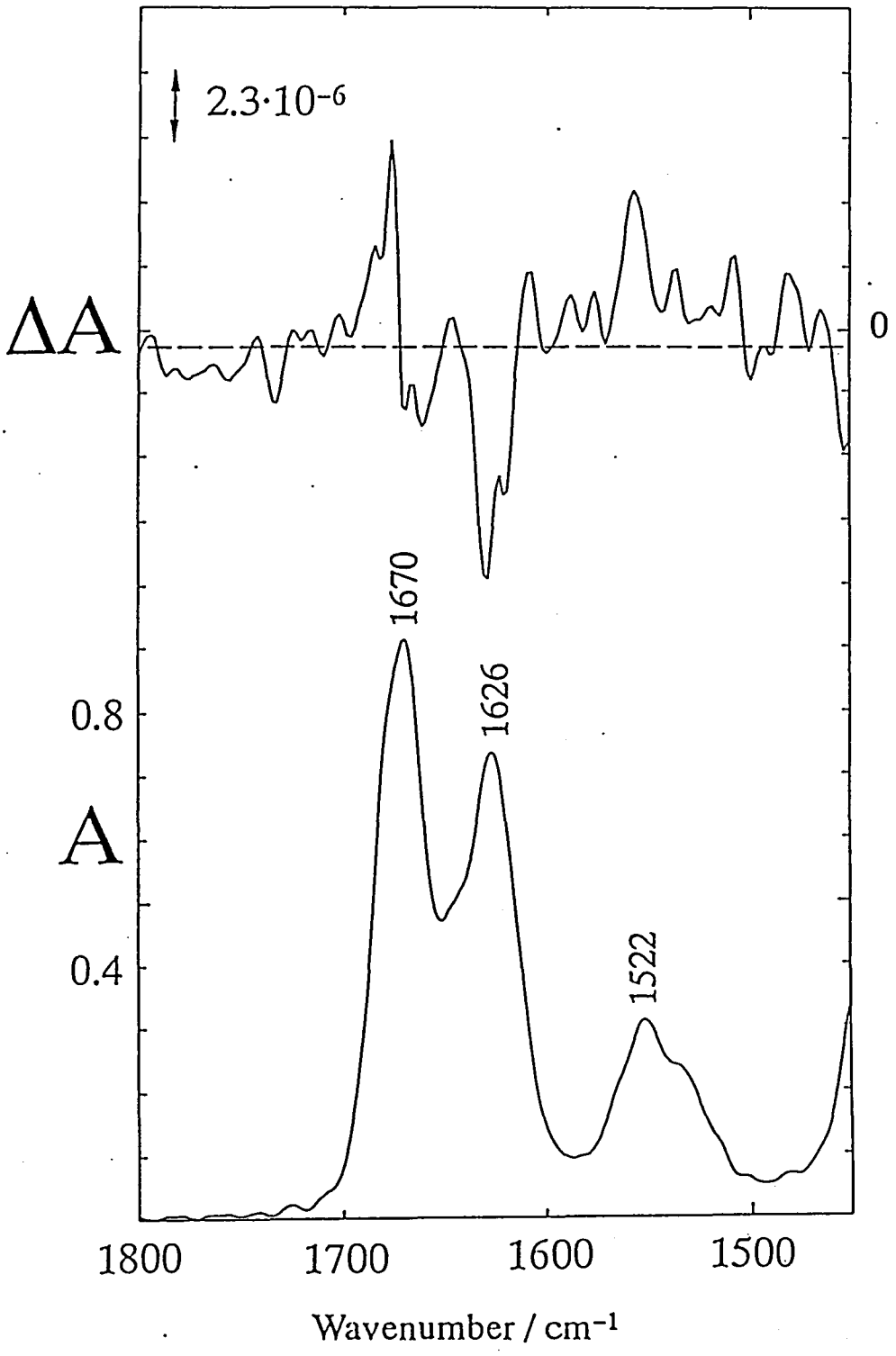


Fig.V-5 VCD and absorption spectra of Ac-Pro-NHMe in 0.2 M $CDCl_3$ solution.

Capter VI

VCD of Proline-Containing Oligopeptides (β-Turn Mimetic C_{10} Conformation and C_7C_5 Conformation)

SUMMARY

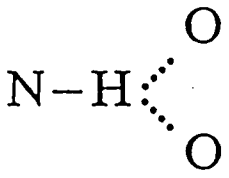
Vibrational circular dichroism (VCD) and IR absorption spectra of the blocked ologopeptides containing prolylglycine (Pro-Gly) dipeptide group; Piv-Pro-Gly-NHMe (PPGA), Piv-Pro-Gly-OMe (PPGE) and Ac-Pro-Gly-OMe (APGE), have been measured in the amide A, I and II regions. In dilute $CDCl_3$ solution absorption spectra of PPGA are fully consistent with the intramolecularly hydrogen-bonded structure, showing a positive VCD at 3345 cm^{-1} and a negative VCD band at 1670 cm^{-1} which are characteristic to type II β-turn. For PPGE and APGE, the concentration dependence of the absorption bands in the amide A region provides evidence for the occurrence of the so-called C_7C_5 conformation stabilized by bifurcated intramolecular hydrogen bond. VCD of the amide A band in dilute solution shows its characteristic positive band centered at the absorption band. A combined analysis of VCD and IR spectra provides a new probe of type II β-turn mimetic C_{10} conformation and C_7C_5 conformation in oligopeptides.

INTRODUCTION

X-ray crystallographic studies on globular proteins have revealed the occurrence of sharp bends involving four consecutive amino acid residues[1-3] usually denoted as β-turns. They possess common structural feature with an intramolecular hydrogen bond between the C=O group of the first residue and the N-H group of the 4th residue (Fig. I-2), and the β-turn involves a ring of 10 atoms (C_{10}) for one turn with one hydrogen bond. On the basis of the resolutions of various crystal structures of cyclic and linear oligopeptides[4], β-turns are classified into several types.[5,6] In particular, types I and II as illustrated in Fig. VI-1 are found most predominantly in proteins. The type II β-turn is related to the type I β-turn by a rotation of the middle peptide unit, in which the oxygen atom is directed to the side chain of the 3rd amino acid residue. Therefore, the 3rd amino acid residue is frequently occupied by glycine residue because steric hindrance between the oxygen atom in the middle peptide unit and the side chain of the 3rd amino acid residue. Proline is also well known to play a key role in β-turn conformations of proteins[6] because the conformational restriction due to the pyrrolidine ring tends to reverse the direction of a peptide chain. On the basis of X-ray crystallographic data on proteins[2], proline is most frequently occurring bend residue in the second position, while glycine, serine, asparagine, aspartic acid and glutamic acid have strong preference at the third

position. Many works on conformation by NMR[7-11], UV·VIS-CD[12-15], IR[11,16-19], and Raman spectroscopies and normal mode calculations[18-23] indicated that (L)Pro-Gly sequence leads to the formation of type II β-turn in solution.[10]

Recently, the occurrence of bifurcated three-center hydrogen bonds are discussed concerning the molecular recognition and protein stability.[24-29] In this characteristic bifurcated hydrogen bonds the donor hydrogen atom (as vertex) interacts with two acceptors as illustrated below,



IR studies[11,30] of Pro-Gly containing peptides suggested that the bifurcated hydrogen bonds were stabilized in dilute solution. In this chapter, analyses of IR absorption and VCD spectra of blocked model peptides containing Pro-Gly were carried out in relation to type II β-turn and bifurcated hydrogen bonds.

EXPERIMENTAL

The blocked tripeptides investigated have the following general formulas:

Piv-(L,D)Pro-Gly-NHMe : PPGA

Piv-(L,D)Pro-Gly-OMe : PPGE

Ac-(L,D)Pro-Gly-OMe : APGE

All derivatives were gifts from Dr. K. Inoue of Shinshu University.

PPGA (pivaloyl prolylglycine methylamide) was selected as the simplest model peptide to form the type II β-turn conformation. The pivaloyl group (Piv : $^{(t)}$Bu-CO-) was used in order to prevent cis-trans isomerization of the proline imide linkage.

On the other hand, PPGE (pivaloyl prolylglycine methylester) was selected as a model peptide favorable to bifurcated hydrogen bonds. APGE (acetyl prolylglycine methylester) was also examined in order to study the influence of the cis-trans isomerization of the imide linkage at proline residue.

RESULTS

< Piv-Pro-Gly-NHMe : PPGA >

In Fig. VI-2 are shown IR absorption and VCD spectra in the amide A region of PPGA in $CDCl_3$ solution. A sharp band at 3446 cm^{-1} is assigned to the free N-H stretching vibration and gives a very weak negative VCD band. A broad band is due to the hydrogen-bonded N-H stretching vibration. The band shows a slightly higher frequency shift (3330 to 3345 cm^{-1}) and becomes narrower with dilution. The corresponding VCD shows a positive band at the absorption peak in dilute solution, and also a positive band at the lower frequency side of the absorption peak in concentrated solution. Similar positive VCD feature was recognized in DMSO-d_6 solution (Fig. VI-3).

To identify the hydrogen bond acceptor sites, the concentration dependences of the absorption spectra in the 1800-1500 cm^{-1} region in $CDCl_3$ solution were examined as shown in Fig. VI-4. The absorption spectra consist of four major bands in this region: a very strong band at 1667 cm^{-1} that accompanies the four shoulders (1702 cm^{-1},1691 cm^{-1}, 1654 cm^{-1}, and 1649 cm^{-1}), a medium feature that is split into peaks at 1609 cm^{-1} and 1596 cm^{-1}, and two weak bands at 1557 cm^{-1} and 1519 cm^{-1}. The bands observed at higher frequency than 1650 cm^{-1} can be assigned to the amide I mode of the 2nd and 3rd peptide bonds.[31-34] The shoulders on the high-frequency side of the main amide I band (1667 cm^{-1}) increase in intensity with dilution and they are assignable to the free amide carbonyls. Inversely, the shoulders on the lower-frequency side due to the hydrogen bonded amide carbonyls diminish. For the imide I band the intensity of the 1609 cm^{-1} band due to the free carbonyl of the pivaloyl group diminish while the lower band at 1596 cm^{-1} assignable to the hydrogen-bonded species increases in intensity when the solution is diluted from 0.2 M to 0.01 M. Amide II bands are observed at 1570-1500 cm^{-1}.

Fig. VI-5 shows the VCD spectrum in this region for 0.1 M $CDCl_3$ solution. The amide I has a negative monosignate VCD band approximately centered on the absorption band. VCD of the imide I band is positive corresponding to the free imide carbonyl stretching vibration and negative band to a hydrogen-bonded imide carbonyl stretching vibration.

< Piv(or Ac)-Pro-Gly-OMe : PPGE , APGE >

IR absorption and VCD spectra in the amide A region of PPGE in CCl_4 solution are shown in Fig. VI-6. Two distinct IR absorption bands are observed over the entire concentration. The weak band at 3436 cm^{-1} assigned to the free N-H stretching vibration gives a very weak positive VCD band. The hydrogen-bonded N-H stretching band showing a slight shift to higher frequency with dilution exhibits a positive VCD in dilute solution and a positively biased (-,+) couplet structure at concentrated solution. The VCD and absorption spectra of PPGE in $CDCl_3$ solution were shown in Fig. VI-7. Similar IR absorption and VCD spectra were observed as seen for CCl_4 solution, but there are two absorption bands around 3450 cm^{-1}. In DMSO-d_6 solution (Fig. VI-8), the N-H group shows a positive VCD band centered at the absorption maximum.

In Fig. VI-9 are shown IR absorption and VCD spectra of APGE in $CDCl_3$ solution. VCD spectrum exhibits a positive band corresponding to the hydrogen-bonded N-H stretching vibration over the entire concentration range. On the other hand, there is no observable VCD in DMSO-d_6 solution (Fig. VI-10).

In the carbonyl streching region, no distinct VCD band above the noise level was measured for PPGE and APGE.

DISCUSSION

In the concentrated solution the hydrogen bonded N-H stretching vibration of PPGE in $CDCl_3$ decreased in intensity with dilution, but any change in intensity in the N-H stretching region of PPGA in $CDCl_3$ could not be observed in dilute solution of 0.015 to 0.003 M. This indicates that the residual hydrogen-bonded N-H stretching at 3345 cm^{-1} is due to intramolecularly hydrogen-bonded species in dilute solution. The increase in the hydrogen-bonded C=O stretching band at 1594 cm^{-1} and the decrease in the hydrogen-bond free C=O band at 1609 cm^{-1} in the imide I region indicate that the pivaloyl C=O group is involved in intramolecular hydrogen bond. The increase in the hydrogen bond free amide C=O stretching vibration at 1691 cm^{-1} and the decrease in the hydrogen bonded C=O band at 1654 cm^{-1} in the amide I region with dilution suggests that the amide C=O is involved in intermolecular hydrogen bonding. Boussard et al.[11] examined the IR absorption and ^{1}H-NHR spectra of some proline-containing tripeptides at low concentration, and suggested that the type II β-turn is the most favorable conformation for PPGA in $CDCl_3$. Furthermore, Rao et al.[12] studied possible conformation of the peptides with general formura Piv-Pro-X-NHMe (X=amino acids) by using nuclear Overhauser effect in NMR study and VIS·UV-CD measurements. They proposed that an intramolecular hydrogen bond is formed between the pivaloyl C=O and the methylamide N-H group to form type II β-turn in the X=Gly peptide in $CDCl_3$ and DMSO-d_6 (Fig. VI-11A). Interpretation of IR absorption spectra in the

present study is consistent with the conclusion in the previous studies. Therefore the positive VCD band at 3345 cm^{-1} in dilute solution is ascribed to the intramolecularly hydrogen bonded N-H stretching band of the methylamide group. Additionally, negative VCD feature at 1670 cm^{-1} corresponding to the amide I band is characteristic for the type II β-turn conformation in apolar solvent.

Dilution of PPGE solution results in a significant shift to lower frequency and reduction in the intensity of the hydrogen-bonded N-H stretching band. The reduction of the intensity may be interpreted as the decrease of intermolecular association with dilution. However, the frequency of the remained N-H stretching band at 3275 cm^{-1} is unusually low for the intramolecularly hydrogen bonded species. It may be interpreted by the formation of bifurcated hydrogen bonds. The conformation in dilute solution can take the C_7C_5 conformation as shown in Fig. VI-11B in which proline and glycine residues accomodate contiguous C_7 and C_5 conformational states, referring to the previous study on the conformation of model peptides, Piv (or Ac)-Pro-NHMe[35,36] and Ac-Gly-NMe_2.[37] Aubry et al.[30] investigated the several blocked di- or tripeptides in dilute CCl_4 or C_2Cl_4 solution, and suggested that a band at 3300 cm^{-1} was assigned to the bifurcated hydrogen bonded N-H stretching vibration. Boussard et al.[11] who also obtained similar spectra for proline-containing tripeptides, ascribed the band at 3270-3300 cm^{-1} to the bifurcated hydrogen bonded species.

Generally, the stronger hydrogen bonding shows the lower

streching frequency. If only C_7 conformation is formed, it should give rise to a sharp absorption band at 3330 cm^{-1} as indicated in Chapter V and when the C_5 conformation predominates, its characteristic absorption band appears at near 3415 cm^{-1} as shown in Chapter IV. The glycyl N-H group in PPGE or APGE can interact with two acceptors at the same time in the C_7C_5 conformation. As a consequence it results in the lower frequency shift of hydrogen-bonded N-H stretching band. Therefore, IR absorption band at 3275-3300 cm^{-1} in dilute solution is ascribed to the bifurcated hydrogen bonded C_7C_5 conformation. In the IR absorption spectrum of PPGE in $CDCl_3$, two bands are observed at 3450 and 3430 cm^{-1}. The former is ascribed to the hydrogen bond free N-H stretching vibration band and the latter to the N-H stretching vibration disturbed by the weak intramolecular hydrogen bond in C_5 form. In $CDCl_3$ compared with CCl_4, PPGE has more chance to take cis form at proline residue. It results in less population of C_7C_5 conformation and more chance for hydrogen bond free form. Positive VCD feature is useful for diagnosis of C_7C_5 conformation. In DMSO-d_6 solution, no VCD feature is observed for APGE. This is due to the breakdown of the intramolecularly hydrogen bonded species owing to strong interaction of the N-H group with solvent DMSO-D_6 molecule.

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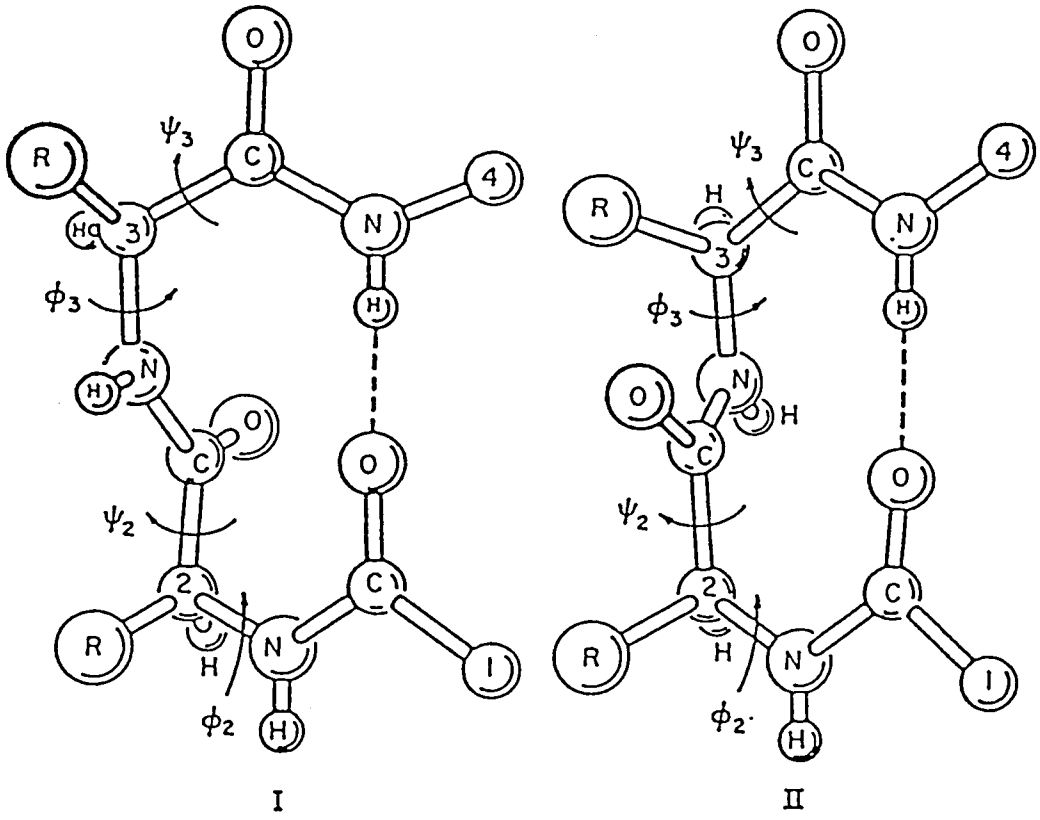


Fig.VI-1 Two types of β-turns of the tetrapeptides.

C_α atoms denoted as 1, 2, 3 and 4 (Hydrogen bond are shown by dotted line).

Bend type I has $(\phi, \psi)_2$=(-60,-30) and $(\phi, \psi)_3$=(-90,0).

Bend type II has $(\phi, \psi)_2$=(-60,120) and $(\phi, \psi)_3$=(80,0).

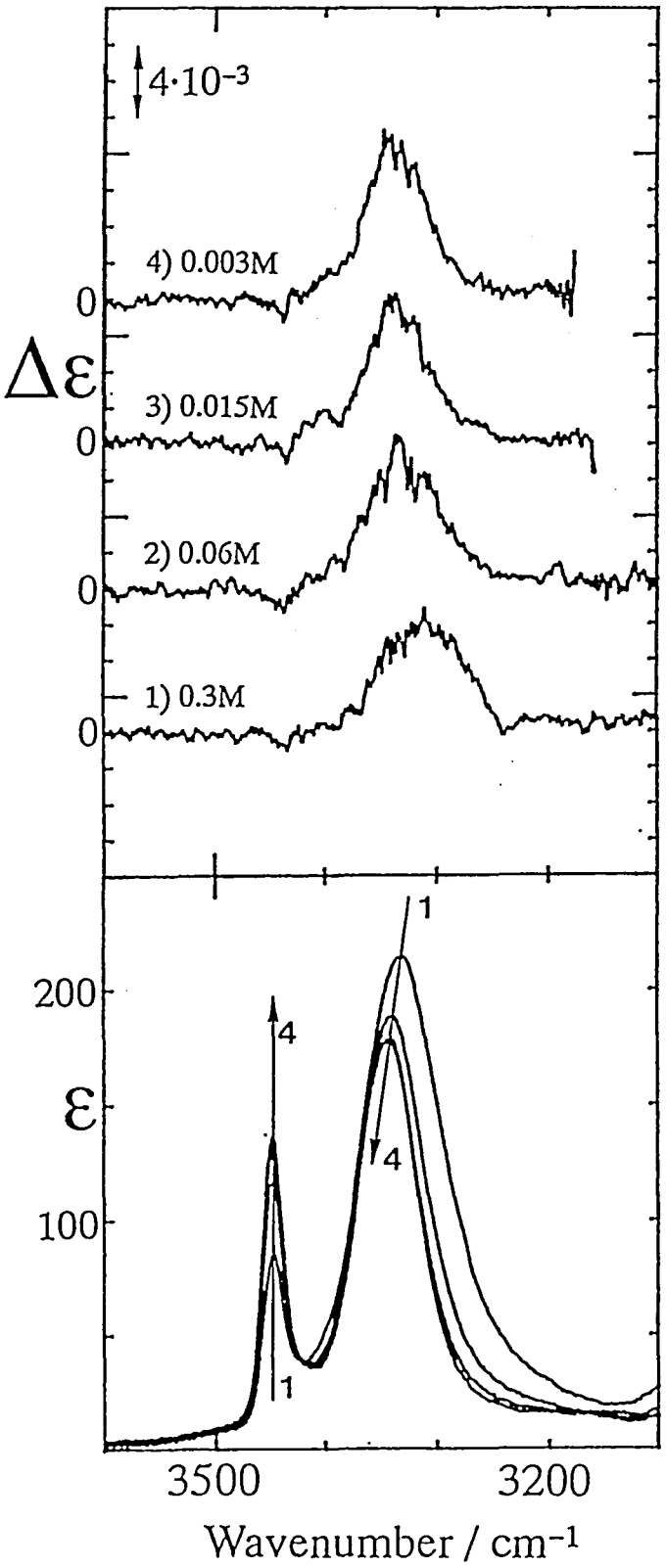


Fig.VI-2 VCD and absorption spectra of Piv-Pro-Gly-NHMe at various concentration in $CDCl_3$ solution.

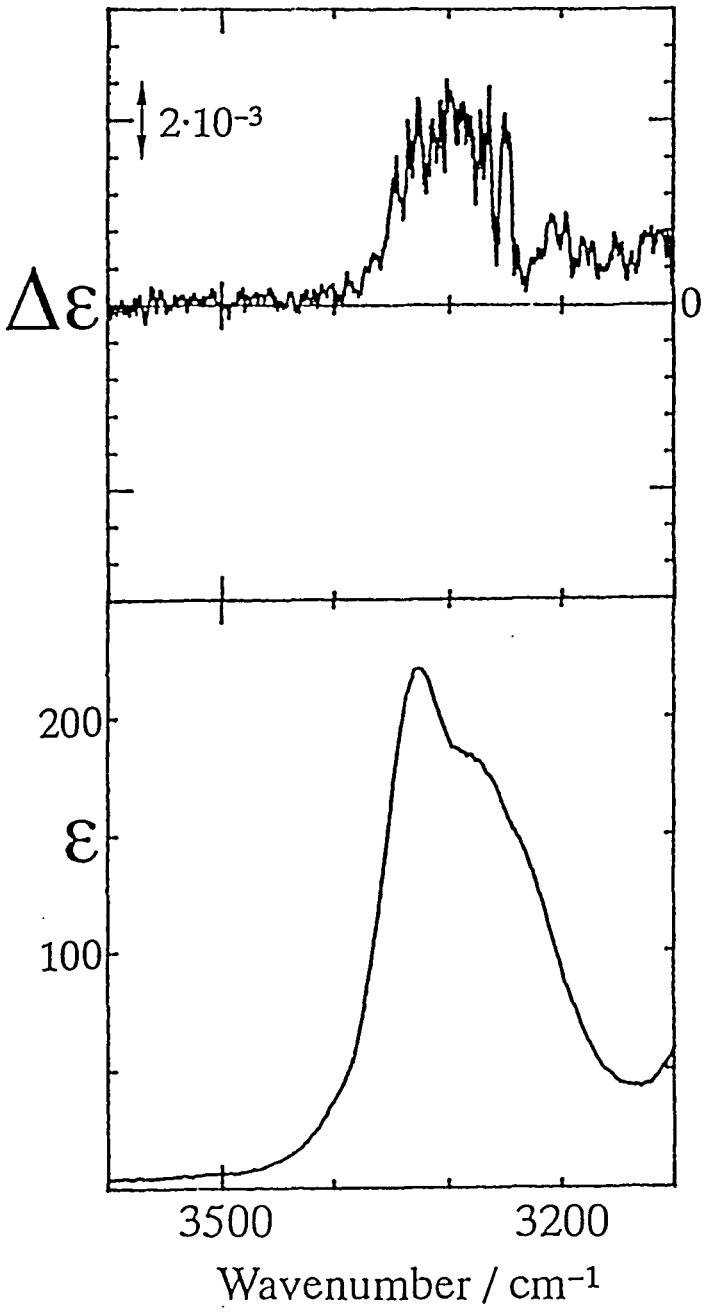


Fig.VI-3 VCD and absorption spectra of Piv-Pro-Gly-NHMe in 0.3M DMSO-d_6 solution.

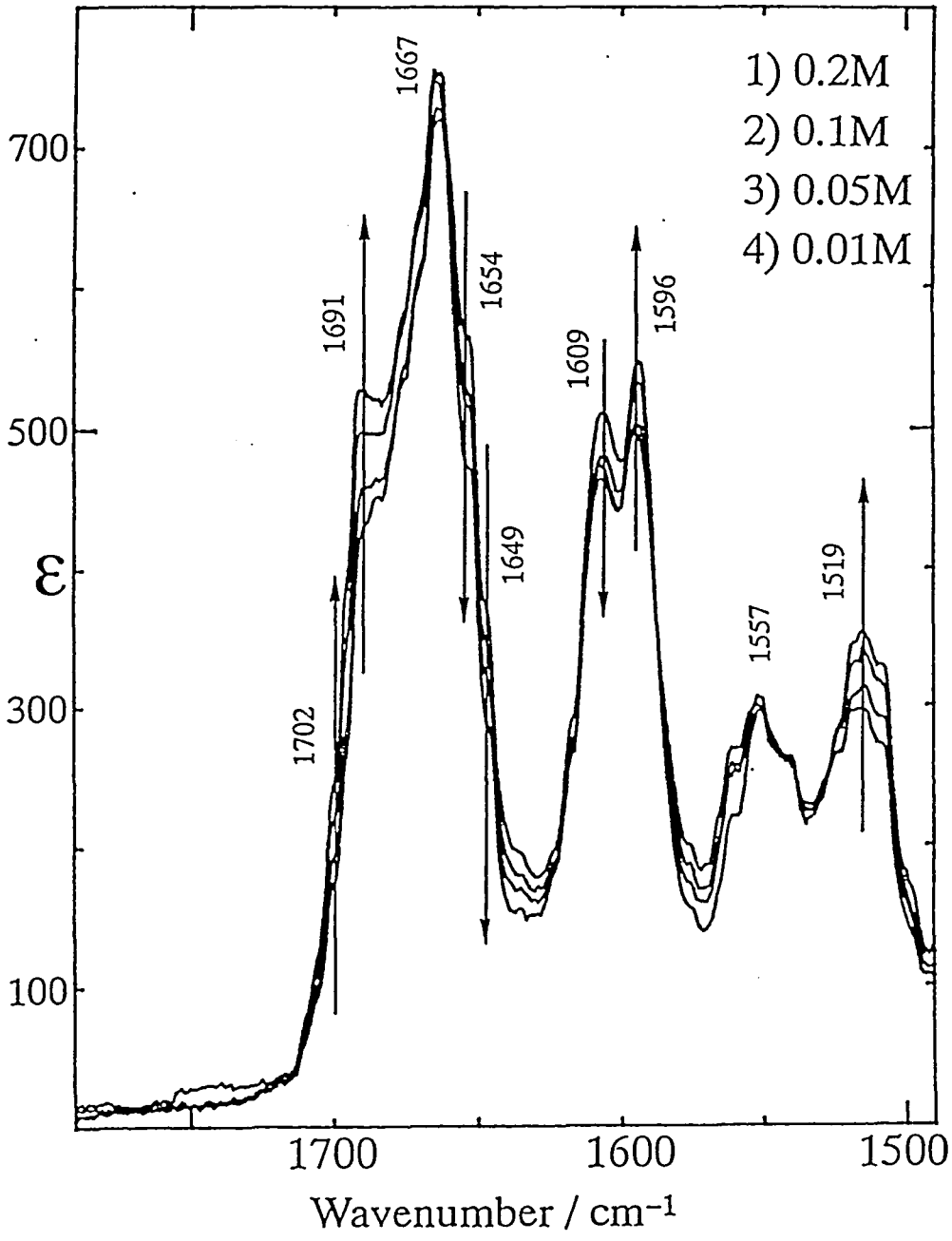


Fig.VI-4 IR absorption spectra of Piv-Pro-Gly-NHMe at various concentration in $CDCl_3$ solution.
(The arrows indicate the direction of intensity variation with dilution.)

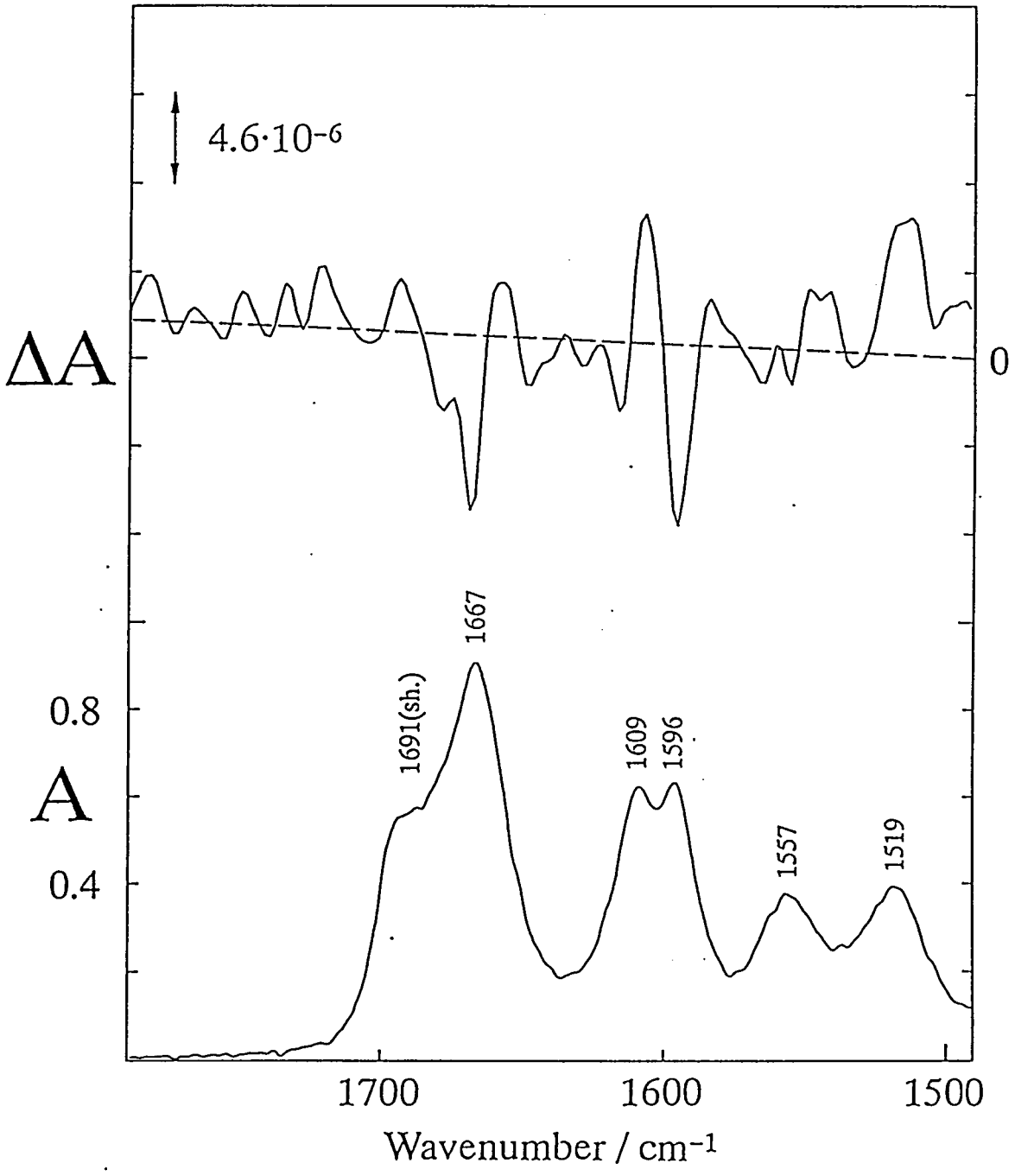


Fig.VI-5 VCD and absorption spectra of Piv-Pro-Gly-NHMe in 0.1M $CDCl_3$ solution.

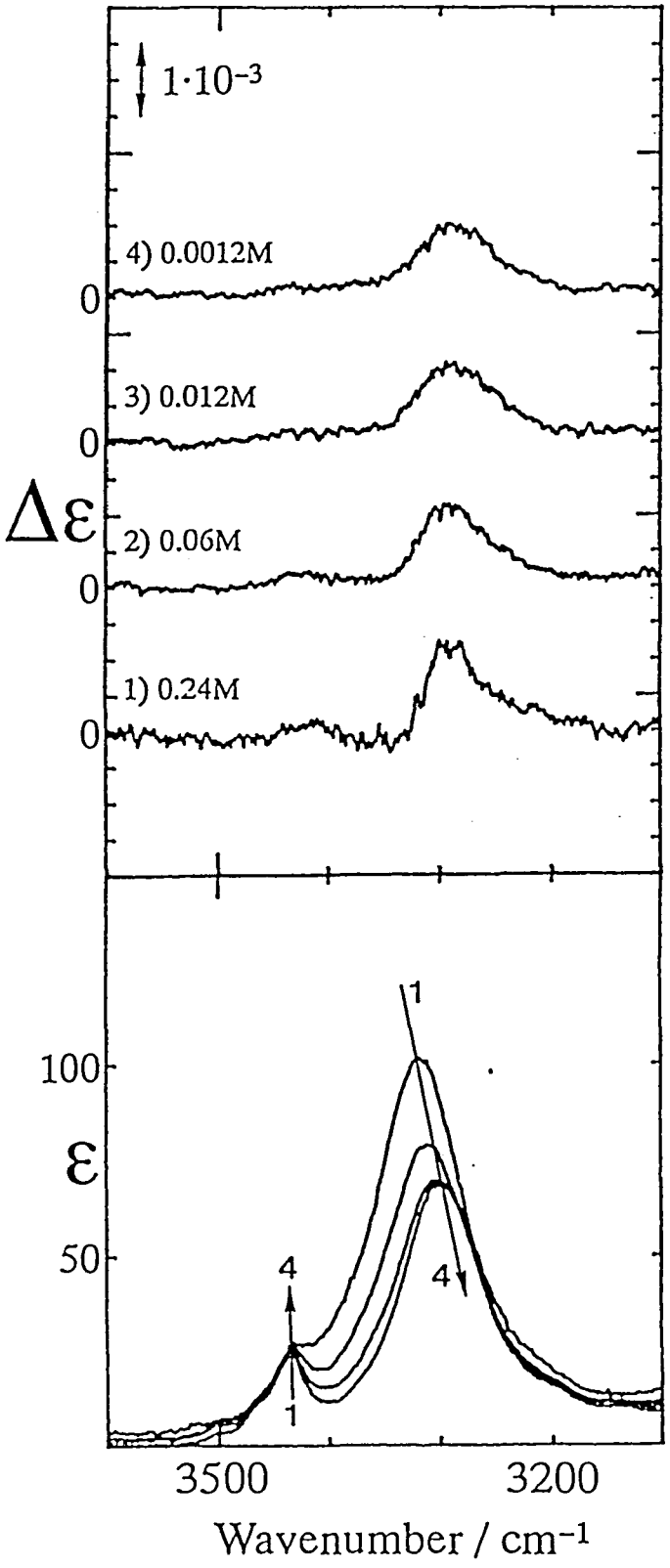


Fig.VI-6 VCD and absorption spectra of Piv-Pro-Gly-OMe at various concentration in CCl_4 solution.

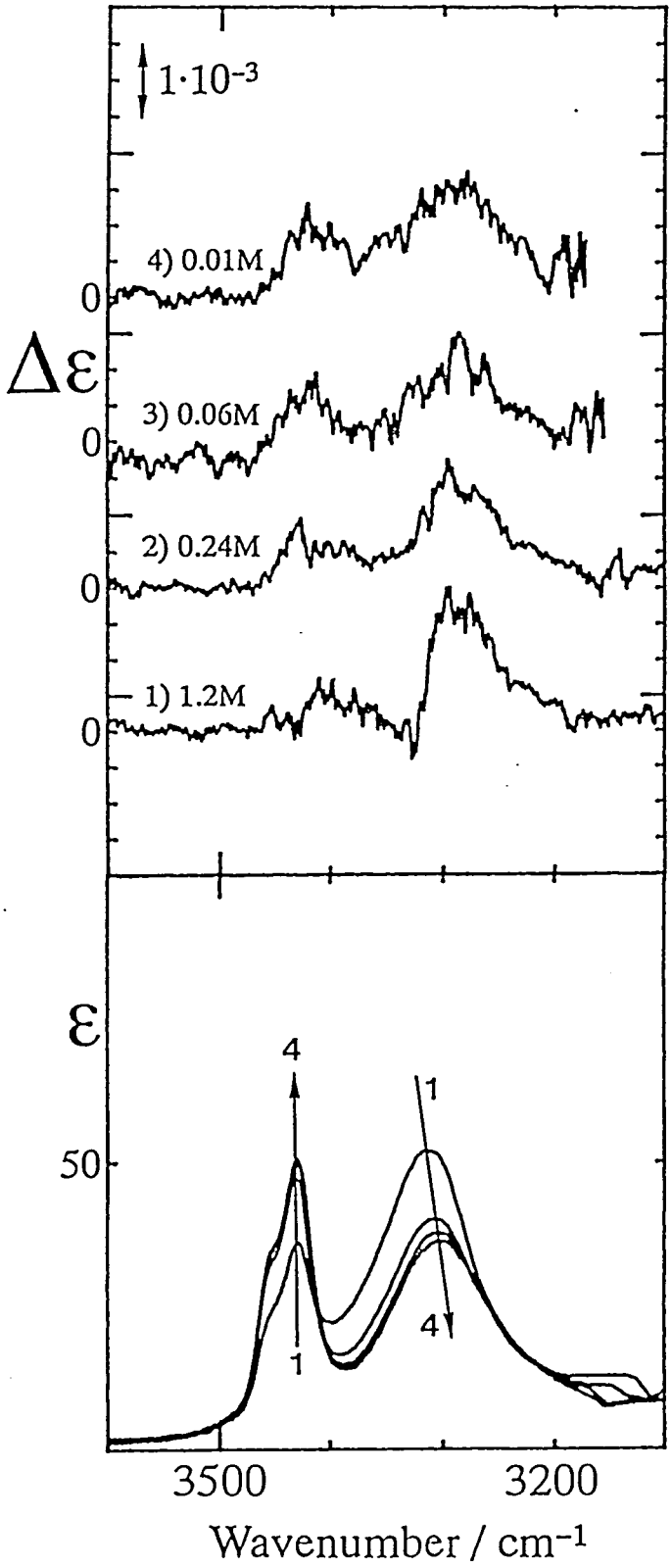


Fig.VI-7 VCD and absorption spectra of Piv-Pro-Gly-OMe at various concentration in $CDCl_3$ solution.

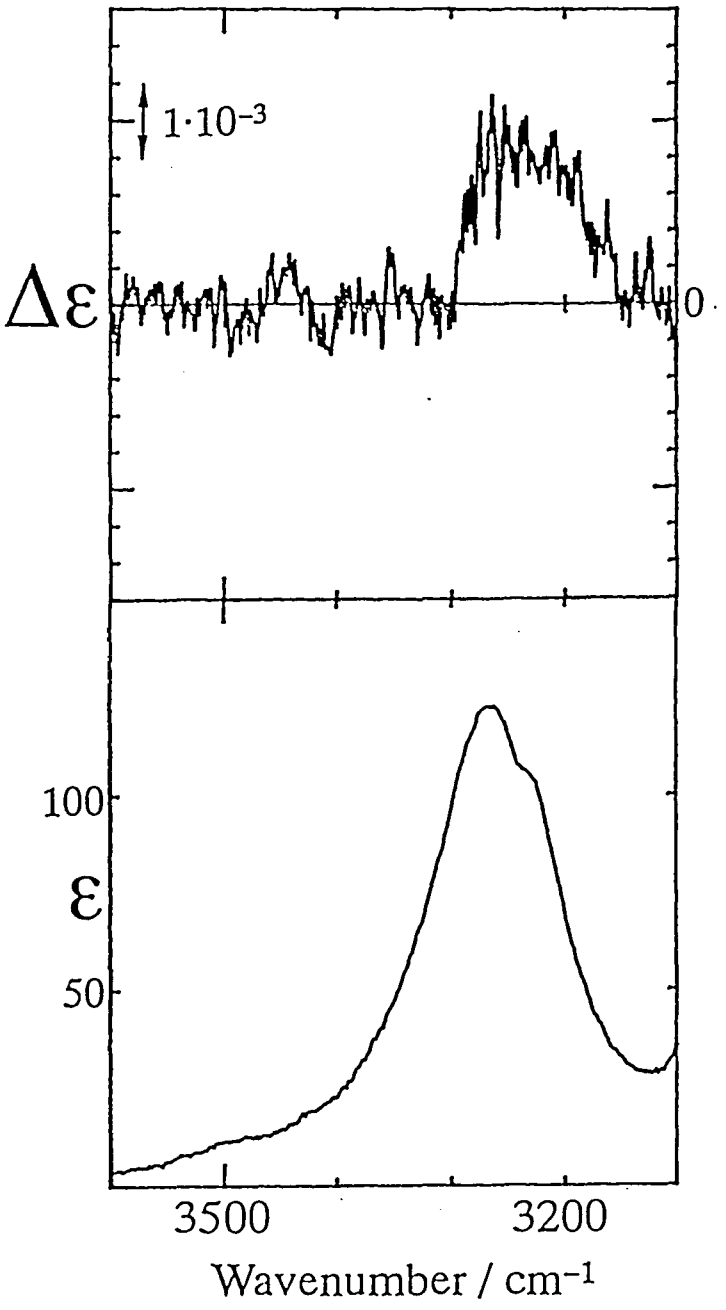


Fig.VI-8 VCD and absorption spectra of Piv-Pro-Gly-OMe in 0.24M DMSO-d_6 solution.

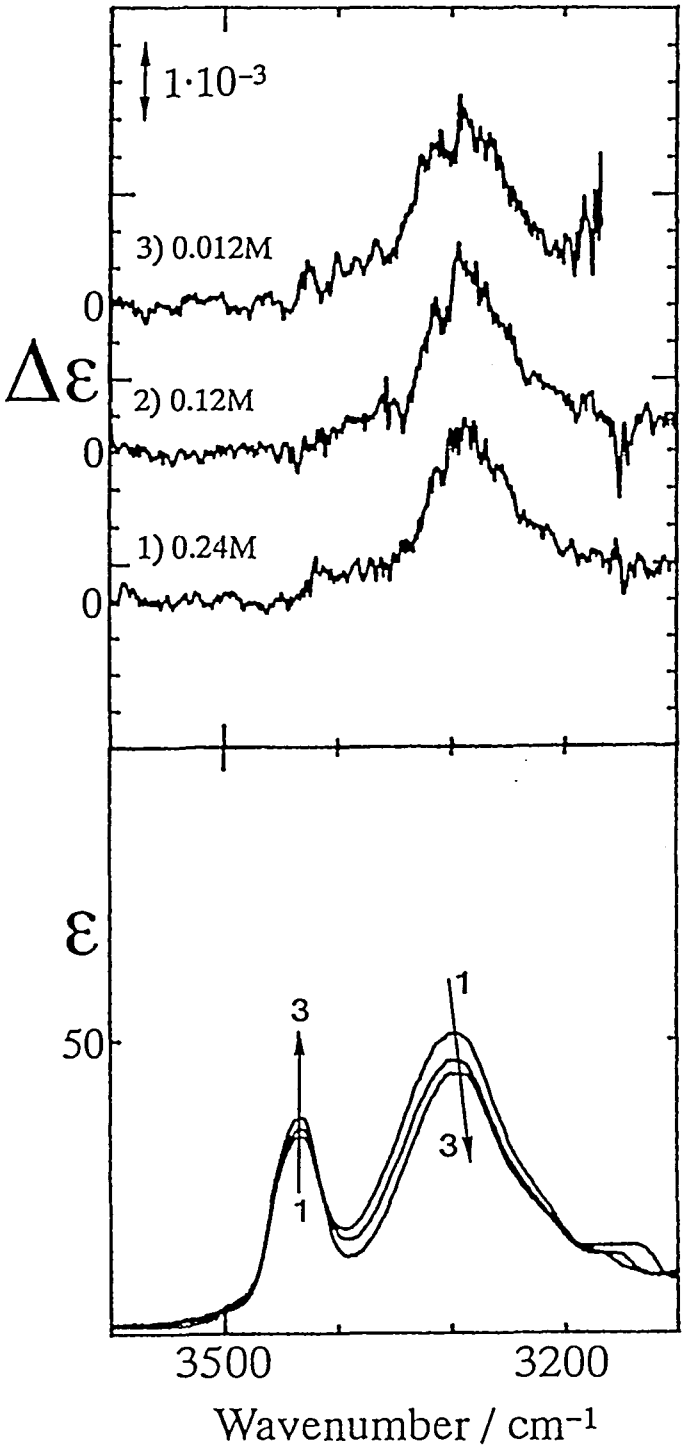


Fig.VI-9 VCD and absorption spectra of Ac-Pro-Gly-OMe at various concentration in $CDCl_3$ solution.

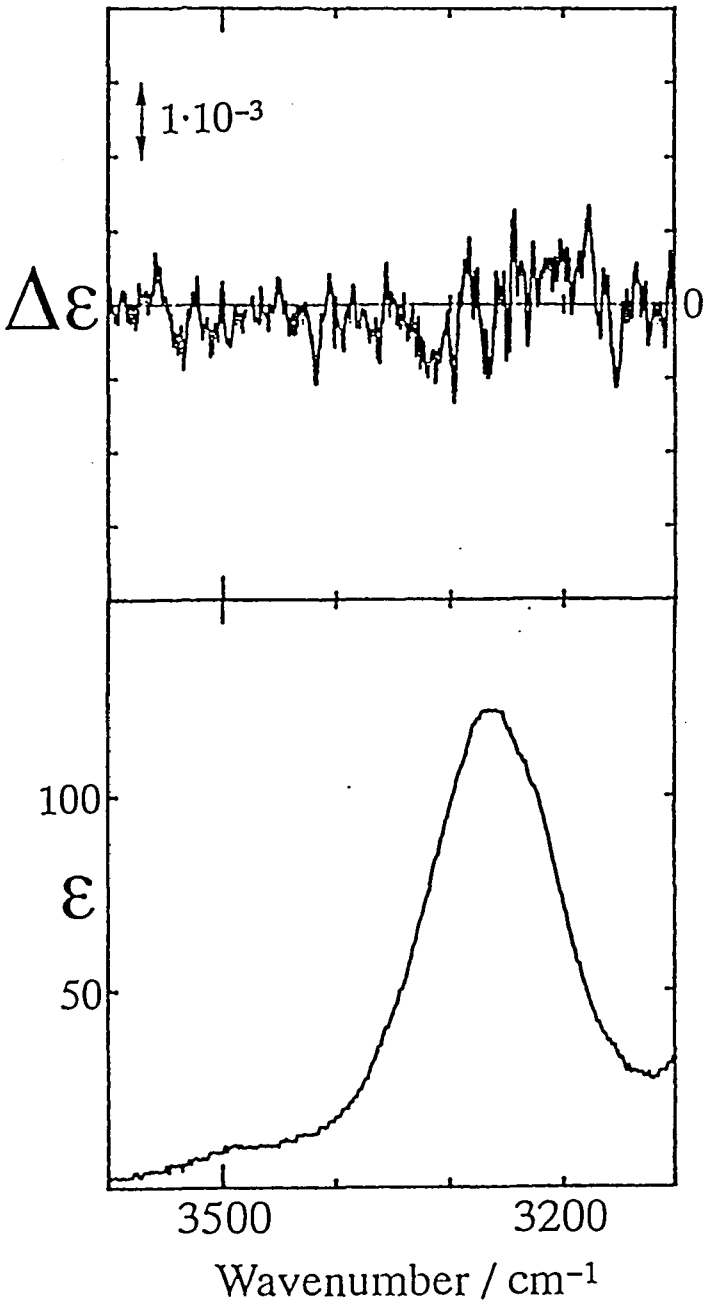


Fig.VI-10 VCD and absorption spectra of Ac-Pro-Gly-OMe in 0.24M DMSO-d_6 solution.

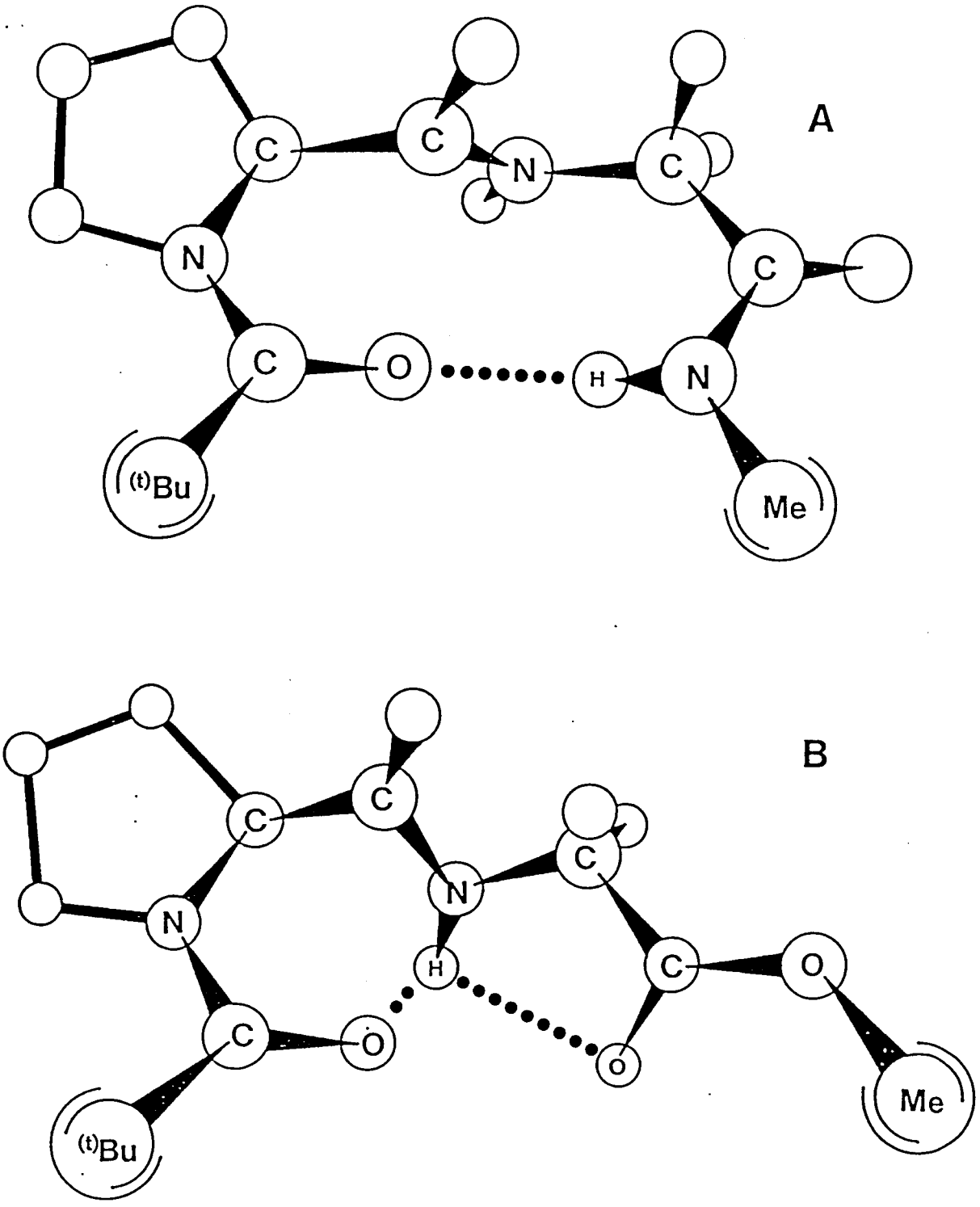


Fig.VI-11 Perspective drawings of hydrogen-bonded conformation.

(A) The type II β-turn of Piv-Pro-Gly-NHMe

(B) The C_7C_5 conformation of Piv-Pro-Gly-OMe

Table VI-1. Infrared absorption and VCD bands of Piv-Pro-Gly-NHMe in the amide A region.

Concentration (M)	Free (cm^{-1})		Hydrogen-bonded (cm^{-1})	
	IR	VCD	IR	VCD
Piv-Pro-Gly-NHMe				
<$CDCl_3$ soln.>				
1) 0.3	3446	3442	3330	3330
2) 0.06	3446	3446	3342	3340
3) 0.015	3446	3442	3345	3345
4) 0.003	3446	3444	3345	3350
<DMSO-d_6 soln.>				
0.3			3325	3300

Table VI-2. Infrared absorption and VCD bands of Piv-Pro-Gly-OMe and Ac-Pro-Gly-OMe in the amide A region.

Concentration (M)	Free (cm^{-1})		Hydrogen-bonded (cm^{-1})	
	IR	VCD	IR	VCD
Piv-Pro-Gly-OMe				
<CCl_4 soln.>				
1) 0.24	3436	3420	3320	3297
2) 0.06	3436	3425	3312	3295
3) 0.012	3436	3430	3305	3290
4) 0.0012	3436	3430	3302	3290
<$CDCl_3$ soln.>				
1) 1.2	3430	3415	3315	3304
2) 0.24	3432	3420	3307	3300
3) 0.06	3432	3420	3302	3285
4) 0.01	3432	3425	3300	3275
<DMSO-d_6 soln.>				
0.24			3265	3265
Ac-Pro-Gly-OMe				
<$CDCl_3$ soln.>				
1) 0.24	3435		3298	3292
2) 0.12	3435		3295	3290
3) 0.012	3435		3293	3290

<DMSO-d_6 soln.>

0.24	3265

Chapter VII

VCD of Biologically Active Cyclic Peptides

SUMMARY

In the preceding chapters the correlation between the VCD spectra and the local structure of oligopeptides has been established based on the analyses of VCD spectra of model peptides. In this chapter VCD and IR absorption spectra of cyclic biologically active oligopeptides, valinomycin, gramicidin S, and N,N'-diacetyl gramicidin S, were measured in the N-H stretching region and discussed by applying the correlation derived in the preceding chapters. The spectral change in VCD observed by the cation complex formation could be explained by the formation of type II' β-turn.

INTRODUCTION

Valinomycin is a cyclic dodecadepsipeptide with the alternating amide and ester bonds of L-valine, D-α-hydroxyisovaleric acid, D-valine, and L-lactic acid. Primary sequence of valinomycin is shown in Fig. VII-1. Valinomycin, so named because of its high valine content, is known to increase the permeability of K^+ across biological membranes, exceeding over Na^+ with a very high selectivity.

The remarkable selectivity for metal cations of ionophores including valinomycin has been studied by many kinds of physicochemical techniques.[1-9] Numerous biophysical studies have been undertaken in attempts to elucidate the molecular mechanism by which valinomycin transports K^+ across membranes. Such studies include crystallographic[10-16] and spectroscopic determination[2-9,17-19] of the structure of non-complexed and cation-complexed valinomycin.

Antibiotic cyclic decapeptide gramicidin S (GrS; cyclo[-(Val-Orn-Leu-(D)Phe-Pro)$_2$-], Figure VII-2) contains, as do a number of other biologically active peptides, cationic amino acid side chains which are essential for activity.

Spectroscopic studies[20-33] revealed that GrS is assumed to take an antiparallel β-sheet structure stabilized by two pairs of transannular hydrogen bonds. Magnetization transfer experiments in NMR study [28] suggest that the (D)Phe-Pro sequences form type II' β-turn.

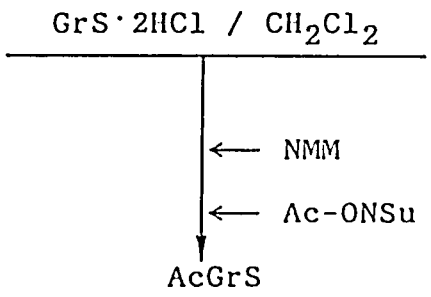
In this chapter, IR absorption and VCD spectra of valinomycin

and GrS were measured and discussed on the basis of the correlation between the VCD and local structure of oligopeptides determined in the preceding chapters.

MATERIALS

Valinomycin was obtained from Sigma Chemical Co. and was used without further purification. Solutions of the alkali metal cation complexes were obtained as follows. To methanol solution of valinomycin a two-fold (molar) excess mount of thiocyanate, NaSCN, KSCN, and CsSCN, was added. After complete dissolution of the salt, methanol was evapolated. The deposition as complex was extracted by chloroform to remove residual metal thiocyanate. The 1:1 alkali cation complex of valinomycin was obtained by evaporating the chloroform solvent.

Gramicidin S was obtained from Sigma Chemical Co. and used without further purification. Additionally, for the purpose of facilitating interpretation of the spectral data, N,N'-diacetyl gramicidin S was synthesized by acetylation of gramicidin S with N-hydroxysuccinimide acetate (Ac-ONSu), and the reaction products were identified by ^{1}H-NMR spectroscopy.



RESULTS

The IR absorption and VCD spectra of valinomycin in various solvents are shown in Fig. VII-3. Two bands are observed at 3392 and 3302 cm^{-1} in CCl_4 solution. Obviously, the former is due to the free N-H stretching vibration and the latter is due to the hydrogen-bonded N-H stretching vibration. Any change in intensity could not be observed below 0.02 M. This shows that the hydrogen-bonded N-H group is due to intramolecularly hydrogen-bonded species. VCD exhibits a very weak negative band corresponding to the free N-H stretching band. The hydrogen-bonded N-H strecthing exhibits a broad positive band with a sharp dip in VCD centered at the absorption maximum.

The titration spectra of valinomycin-cation systems are shown in Fig. VII-4. It is shown that negative VCD lobe around 3310 cm^{-1} grows with the amount of cation, and only negative VCD band remains for 1:1 molar ratio of K^+ cation and valinomycin. The IR absorption and VCD spectra of valinomicin complexes with various metal cations are shown in Fig. VII-5. The free N-H stretching band disappears and the only intramolecularly hydrogen-bonded band is observed, accompanying the negative VCD band.

IR absorption and VCD spectra of gramicidin S in 0.2 M DMSO-d_6 solution are shown in Fig. VII-6. The band at 3280 cm^{-1} can be assigned to the hydrogen-bonded N-H stretching vibration. Any change could not be observed in intensity of this band below 0.2 M (data not shown). This indicates that the hydrogen-bonded N-H group is due to intramolecularly hydrogen-bonded species. The

broad feature at higher frequency (3430-3500 cm^{-1}) is due to water contaminated in the sample. VCD spectra exhibits a negative-positive couplet at the center of the absorption maximum at 3380 cm^{-1}. Measurement in apolar solvent was impossible because of its low solubility. IR absorption and VCD spectra of N,N'-diacetyl gramicidin S are shown in Fig. VII-7. These spectra exhibit drastic solvent dependence.

DISCUSSION

It is recognized that K^+-complexed valinomycin adopts a 'bracelet' conformation (Fig. VII-8) in solution. NMR and IR spectroscopic studies proved the formation of six atom fused β-turns of type II and II' with a central pore in which the cation is coordinated to the six ester groups. This structure has been supported by X-ray diffraction measurements. The metal-free valinomycin is known to adopt 'propeller' conformation with three of the internal hydrogen bonds are of type II β-turn in solvent of medium polarity (for instance CCl_4/DMSO=3/1).

The broad positive VCD feature centered at the hydrogen-bonded absorption peak for uncomplexed valinomycin can be ascribed to the type II β-turn similar to that in Piv-Pro-Gly-NHMe (Fig. VI-2). A sharp dip can be attributed to the contribution of the negative VCD for type II' β-turn structure which is expected to partly exist in the free valinomycin. The hydrogen bond free N-H groups in metal-free valinomycin is involved in the intramo-

lecular hydrogen bond of type II' β-turn. The formation of three of type II' β-turn in the cation-complexed valinomycin is the origin of the negative VCD in the complex.

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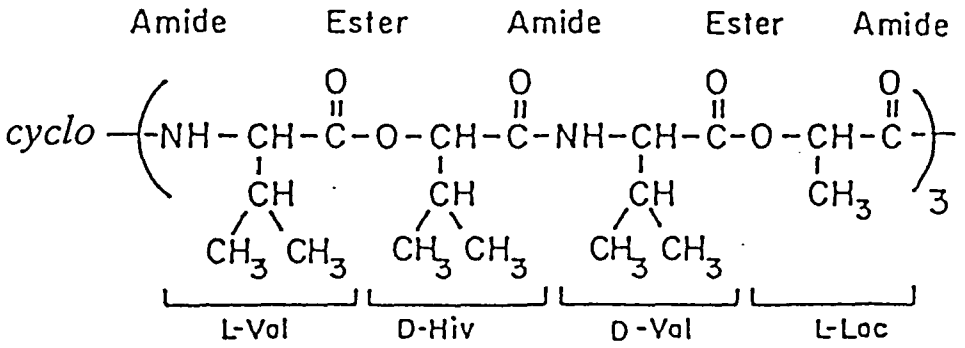


Val, valine

Hiv, α–hydroxyisovaleric acid

Lac, lactic acid

Fig. VII-1. Primary sequence of valinomycin.

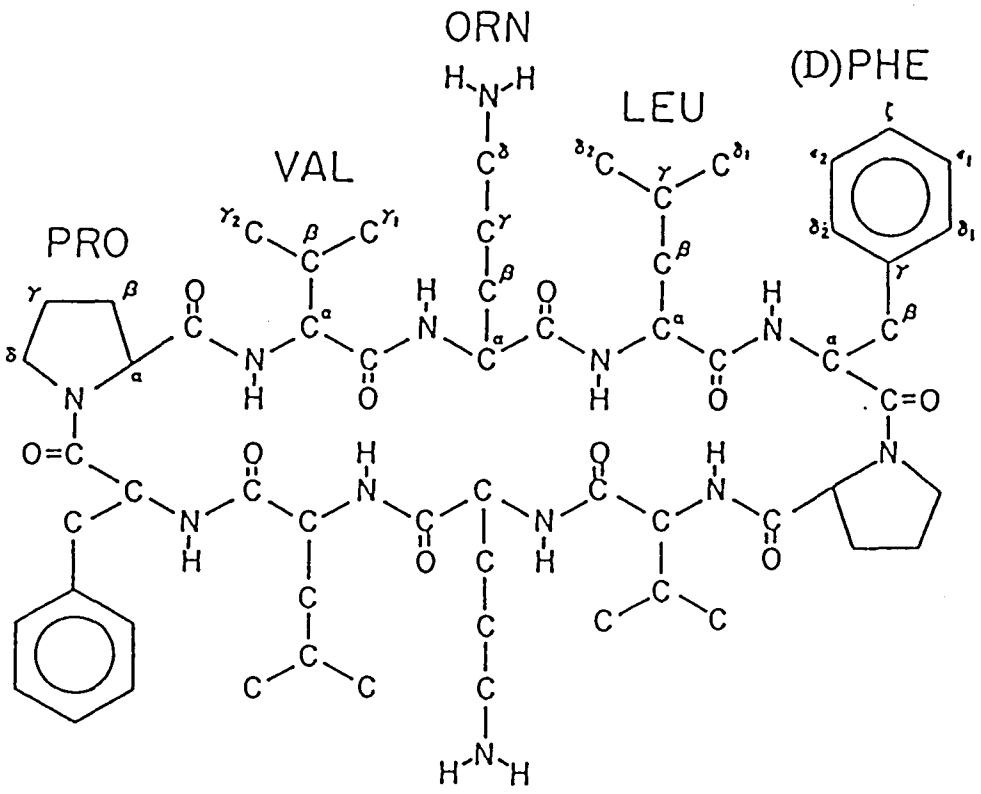


Fig. VII-2. Primary structure of gramicidin S.

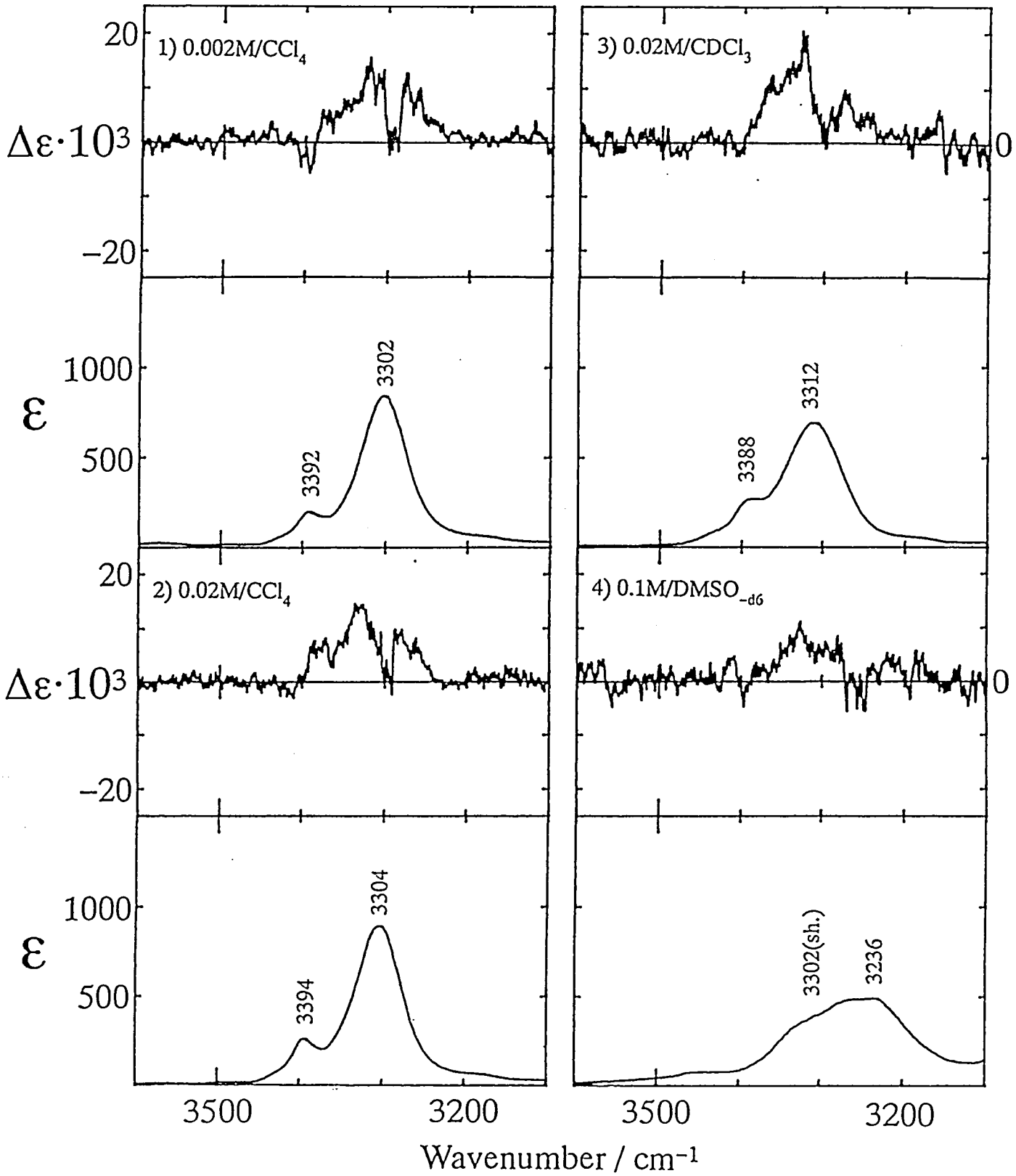


Fig. VII-3. VCD and absorption spectra of valinomycin in various solvent.

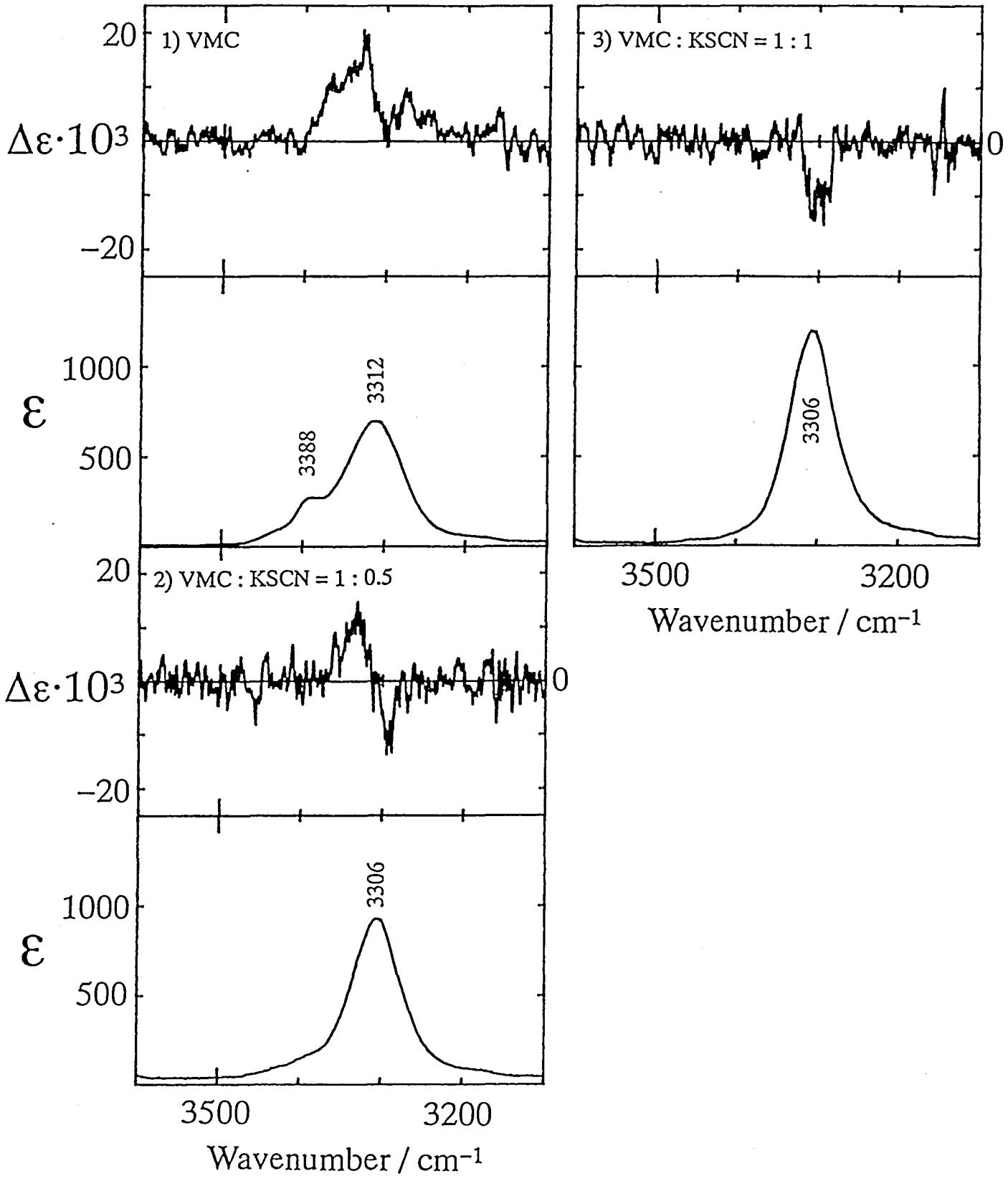


Fig. VII-4. Titration spectra of valinomicin-cation systems.

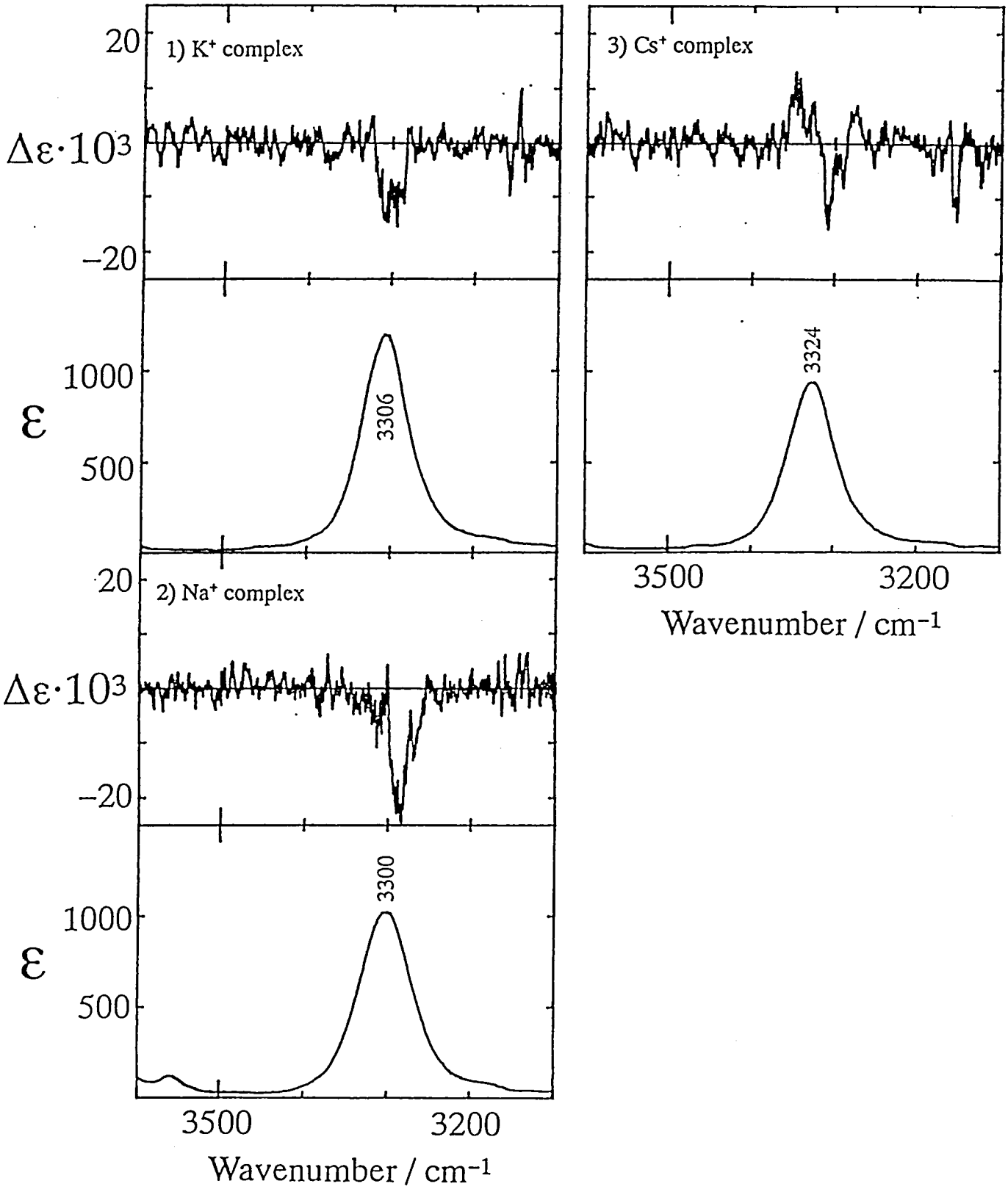


Fig. VII-5. VCD and absorption spectra of 1:1 valinomycin-cation complexes in $CDCl_3$ solution.

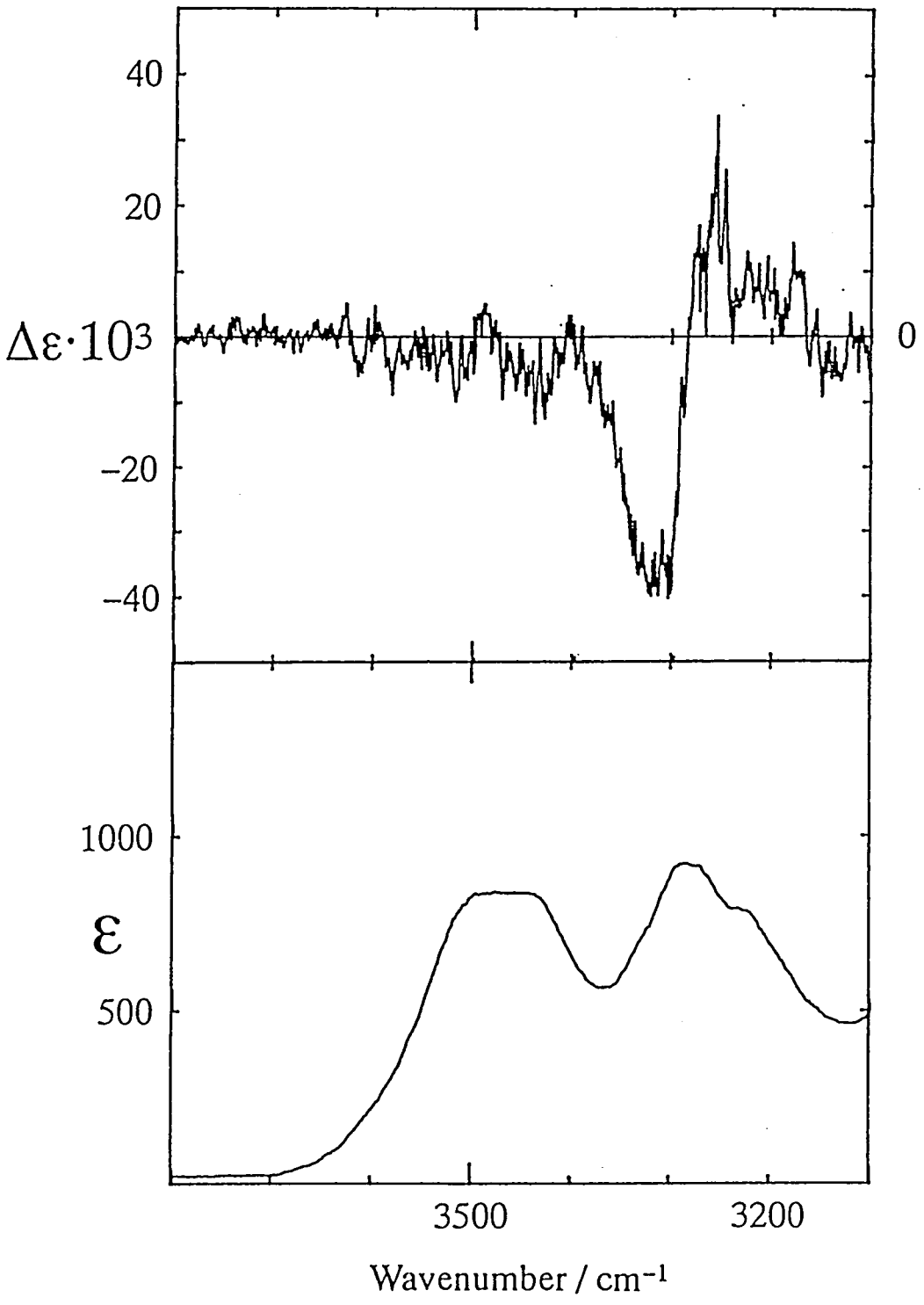


Fig. VII-6. VCD and absorption spectra of gramicidin S in 0.2 M DMSO-d_6 solution.

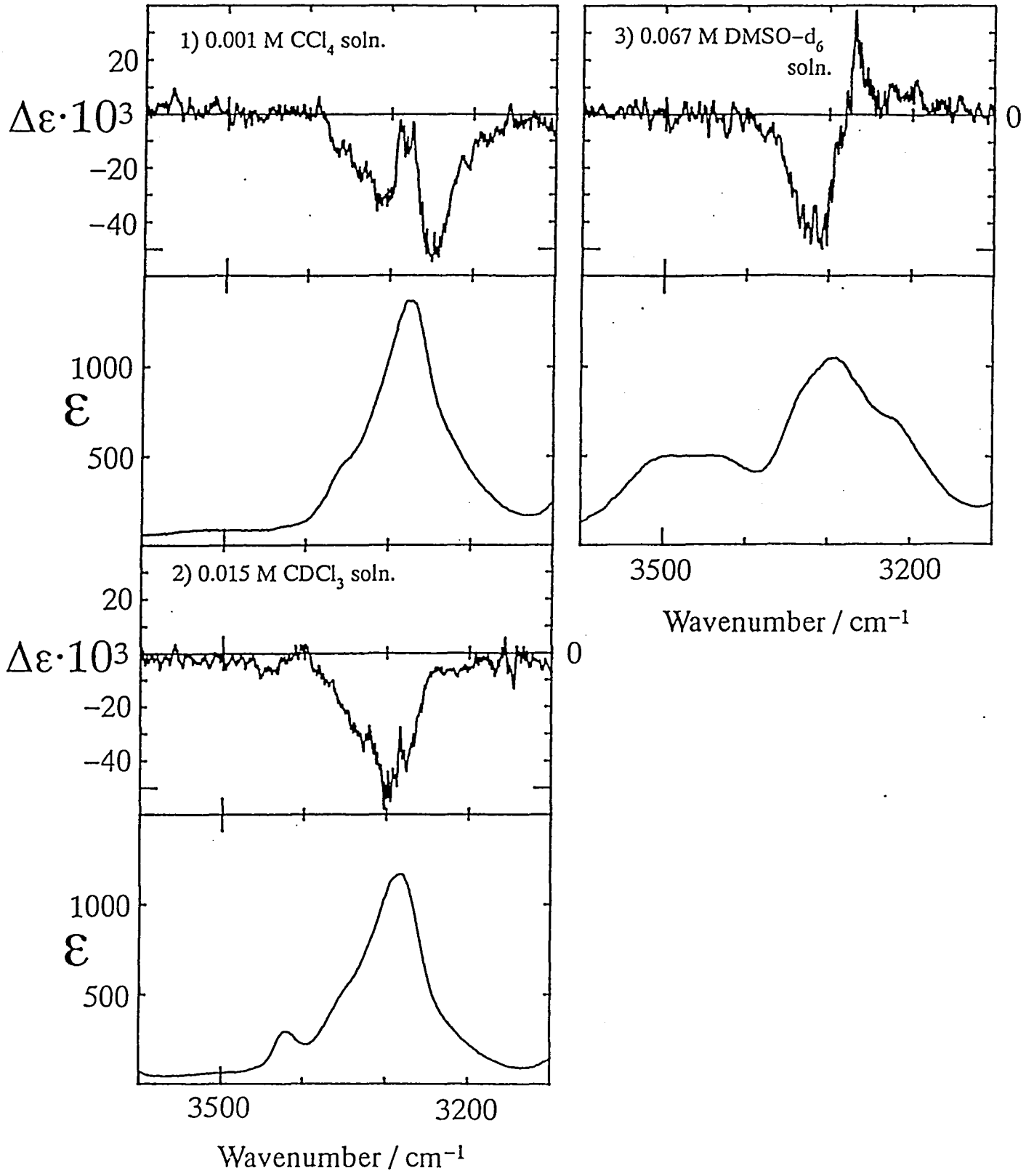
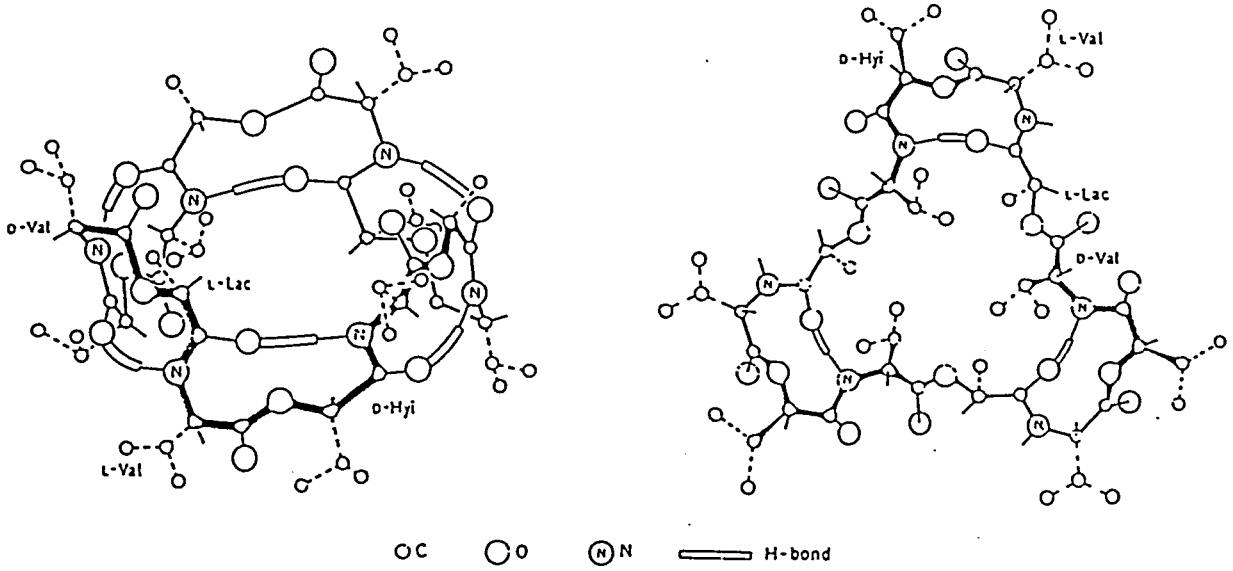


Fig. VII-7. VCD and absorption spectra of N,N'-diacetyl gramicidin S in various solvents.



The predominant conformation of valinomycin in nonpolar solvents

The propeller conformation of valinomycin predominant in solvents of medium polarity

Fig. VII-8. Two types of conformation of valinomycin.

Chapter VIII

Conclusion

In this thesis the study on the local conformation of oligopeptides by use of vibrational circular dichroism (VCD) was undertaken in order to establish the correlation between the VCD feature and the local structure of peptide chains. The correlation between the VCD feature and the local conformation of oligopeptides established in this thesis was summarized in Table VIII-1 and Fig. VIII-1.

The C_5 conformation with the five membered-ring intramolecular hydrogen bond in the same residue exhibits an absorption bands at 3410-3425 cm^{-1} and gives rise to a negative VCD. In concentrated solution the C_5 conformer forms a dimer which gives an absorption band at 3350 cm^{-1} and negative-positive couplet VCD pattern. In much more concentrated solution the oligopeptides give an additional negative VCD band at the lower frequency side of the absorption band.

The γ-turn structure with the C_7 intramolecular hydrogen bond in the proline-containing dipeptides shows a relatively broad absorption band at 3330 cm^{-1} and the corresponding positive VCD band. The frequency and the band width for the absorption band of the C_7 conformer is closely similar to those of the aggregates in concentrated solution. However, the VCD spectrum of the C_7 aggregates is completely different from that of the C_7 monomer and gives a bisignate couplet typical for the aggregate species as shown above. The γ-turn exhibits the characteristic VCD pattern of a negative band for the C=O stretching vibration in the prolyl imide link.

The β-turn often found in folded peptide chains has the C_{10} intramolecular hydrogen bond between the carbonyl group of the first residue and the amino group of the 4th residue. The type II β turn in the Piv-Pro-Gly-NHMe exhibits two absorption bands of the free N-H stretching vibration at 3450 cm^{-1} and of the intramolecularly hydrogen-bonded N-H stretching vibration at 3345 cm^{-1}. The free N-H stretching gives rise to a weak negative band and the hydrogen-bonded N-H stretching gives rise to a positive band in VCD. The VCD in the amide I region exhibits a negative band for the amide I and a negative band for the intramolecularly hydrogen bonded imide I.

In the C_7C_5 conformation found in Piv(or Ac)-Pro-Gly-OMe the N-H group of the Gly residue involves two hydrogen bonds, called as 'bifurcated hydrogen bond', one acceptor site at the pivaloyl (or acetyl) C=O and the other at the C=O in the Gly residue. It displays an interesting behavior that the absorption band for the hydrogen-bonded N-H stretching vibration shifts to lower frequency at 3290 cm^{-1} compared to either that of the C_7 at 3330 cm^{-1} or the C_5 at 3410-3425 cm^{-1}. It gives rise to a positive VCD band.

In conclusion the vibrational circular dichroism of peptides is quite sensitive to the change in local conformation as well as the aggregation mode by intermolecular hydrogen bond. The combined use of VCD technique with the other spectroscopic methods will provide a new insight into the secondary structure of proteins and polypeptides.

Table VIII-1 Correlation between the VCD feature and the local conformation of oligopeptides.

	N-H stretching		C=O stretching	
Conformation	Free	H-bonded	Amide I	Imide I
<Intramolecular>				
C_5	+	-		
C_7 (γ-turn)		+	(+,-)	-
C_{10} (β-turn)	-	+	-	-
C_7C_5		+		
<Intermolecular>				
C_5-C_5 (dimer)		-,+	(+,-)	
H-Bonded Oligomers		-,+,-		

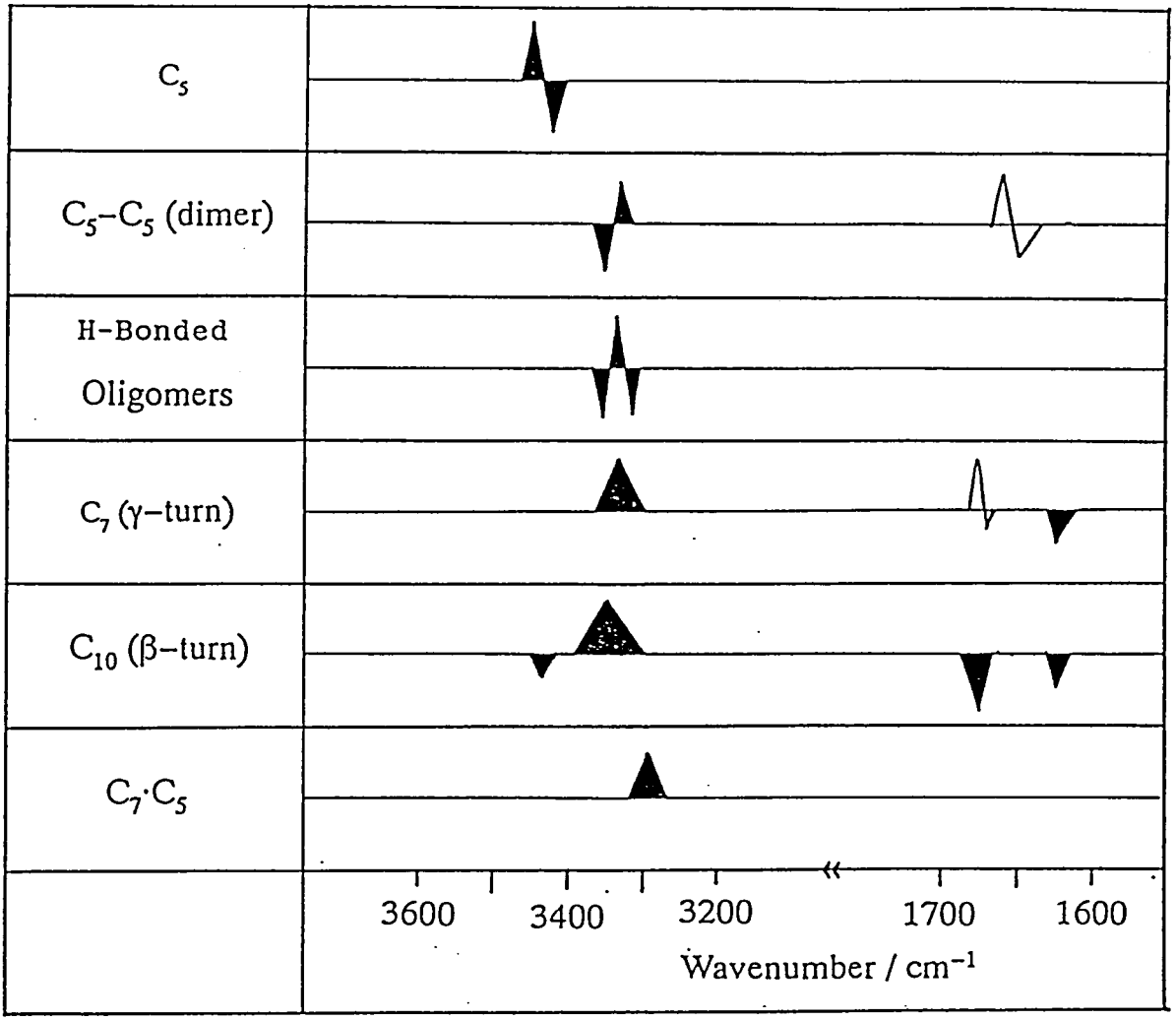


Fig. VIII-1 Correlation between the VCD feature and the local conformation of oligopeptides.

List of Papers

The contents of this thesis will be published in the following papers.

Chapter IV

Vibrational CD Spectra of Oligopeptides

M.Miyazawa, H.Sugeta, and Y.Kyogoku

in preparation.

Chapter V

Vibrational CD Spectra of γ-Turn Conformation in Proline-containing Dipeptides

M.Miyazawa, H.Sugeta, Y.Kyogoku, K.Inoue, and T.Hayakawa

in preparation.

Chapter VI

Vibrational CD Spectra of β-Turn Conformation in Proline-containing Tripeptides

M.Miyazawa, H.Sugeta, Y.Kyogoku, K.Inoue, and T.Hayakawa

in preparation.

Chapter VII

Vibrational CD Spectra of Valinomycin and Its Cation Complex

M.Miyazawa, H.Sugeta, and Y.Kyogoku

in preparation.

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