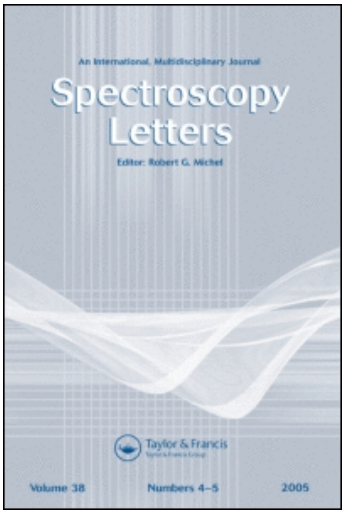


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# Vibrational Spectra of a Linear Alternating L,D Hexapeptide with a Type II β-Turn Structure

Vaman M. Naik[a]
[a] Department of Natural Sciences, The University of Michigan-Dearborn, Dearborn, MI, USA

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# VIBRATIONAL SPECTRA OF A LINEAR ALTERNATING L,D HEXAPEPTIDE WITH A TYPE II $\beta$-TURN STRUCTURE

KEY WORDS: Raman spectra; IR spectra; Boc-(D-aIle-L-Ile)$_3$-OMe; $\beta$-turn $\alpha$-helix; amide modes; vibrational assignment

Vaman M. Naik
Department of Natural Sciences, The University of Michigan-Dearborn
Dearborn, MI 48128 USA



## ABSTRACT

Raman and infrared spectra of Boc-(D-aIle-L-Ile)$_3$-OMe (ILEU-6) and its N-deuterated derivative have been measured. Conformationally sensitive amide vibrations have been identified and compared with the observed and calculated frequencies of Pro-Leu-Gly-$NH_2$ which has a type II $\beta$-turn structure. The observed frequencies of ILEU-6 have also been compared with the normal mode calculations of a standard model type II $\beta$-turn structure and a good agreement has been found in these comparisons. The additional frequencies observed for this particular structure are assigned to the residues that are not involved in the $\beta$-turn.

## INTRODUCTION:

During the past few years the conformational characteristics of polypeptides with a regular sequence of L and D residues along the chain have been investigated. This is mainly due to their analogy with gramicidin A, a natural linear pentadecapeptide that forms an ion conducting channel across natural and synthetic lipid bilayer membranes. These L,D-alternating oligopep-

tides can assume a variety of single and double-helical structures [1-6]. Two octapeptides, Boc-(L-Val-D-Val)$_4$-OMe and Boc-(L-Phe-D-Phe)$_4$-OMe, are shown to exist in a double-stranded antiparallel $\beta$-structure ($\uparrow\downarrow$ $\beta^{5.6}$, where 5.6 denotes the total number of residues per turn of the helix) [7,8].

L,D-peptides with smaller number of residues can take up different conformations, including the $\beta$-turn structures. A theoretical investigation by Chandrasekaran et al. [9] has shown the possibility of existence of a particularly stable conformation for L-D-sequence of three linked peptides having an internal 4→1 type hydrogen bond. Such conformation leads to the reversal of the chain direction and is called "hairpin-bend" or "U-bend" or more commonly $\beta$-turn, as it can occur at the end of two chains hydrogen-bonded in the antiparallel $\beta$-conformation.



In the present work Boc-(D-aIle-L-Ile)$_3$-OMe (ILEU-6) has been studied using infrared (IR) and Raman spectroscopy in the solid state to show that it has amide vibrations that are characteristic of a type II $\beta$-turn structure. X-ray analysis has shown that in the crystalline state this molecule has a $\beta$-turn ($C_{10}$) and an $\alpha$-turn ($C_{13}$) fused together and they are included in a larger 17-membered hydrogen-bonded ring structure ($C_{17}$) as shown in Fig. 1. The observed torsion angles $\phi$ and $\psi$ of the peptide backbone are: $(\phi,\ \psi)_1 = 133°,\ -160°$; $(\phi,\ \psi)_2 = -77°,\ 120°$; $(\phi,\ \psi)_3 = 115°,\ -13°$; $(\phi,\ \psi)_4 = -129°,\ -50°$; $(\phi,\ \psi)_5 = 94°,\ -134°$ and $\phi_6 = -114°$ [10]. The $\beta$-turn observed in this molecule is a type II $\beta$-turn according to Venkatachalam's classification [11]. Krimm and Bandekar [12] have done normal mode vibrational analyses for $\beta$-turns of types I, II, III, I′, II′ and III′. Naik and Krimm [13] have calculated the normal modes of Pro-Leu-Gly-$NH_2$, which has been shown from x-ray crystallographic studies to have a type II $\beta$-turn structure.

## EXPERIMENTAL

Boc-(D-aIle-L-Ile)$_3$-OMe was synthesized and kindly provided by Dr. G. P. Lorenzi. The molecule was N-deuterated by dissolving it in $CH_3OD$ and then adding $D_2O$ to the mixture. Slow evaporation of the solution yields very good single crystals.

IR spectra were recorded in KBr disks using a Bomem DA/3 Fourier Transform IR (FTIR) spectrometer in 4000 - 500 $cm^{-1}$ region with a reso-

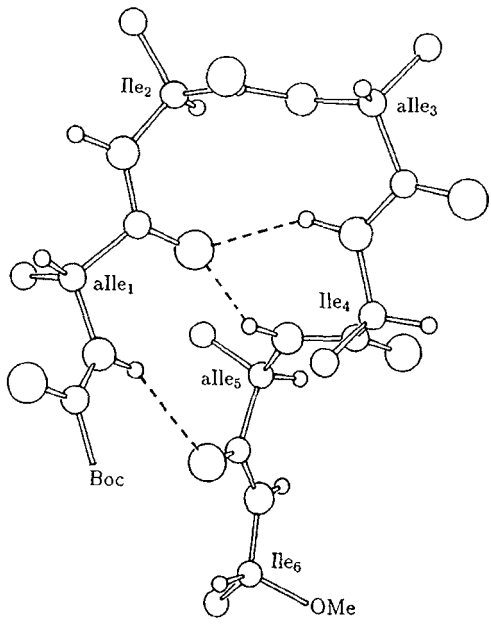


FIG. 1. Structure of Boc-(D-aIle-L-Ile)$_3$-OMe. Only the $C^\beta$ atoms of the side chains are shown. Intramolecular hydrogen bonds are indicated with dashed lines.



lution of 1 cm$^{-1}$. Raman spectra were recorded, using a Spex 1403 double monochromator spectrometer with an excitation line of 514.5 nm from a Spectra-Physics 165 $Ar^+$ laser. An incident laser power of ~150 mw was focused at the samples sealed in glass capillaries. The spectral band pass was ~2 cm$^{-1}$ at 514.5 nm, and the step resolution used was 1 cm$^{-1}$ in all the spectra.

## RESULTS AND DISCUSSION

The observed IR spectra of ILEU-6 and its N-deuterated derivative are shown in Figs. 2-4. The Raman spectra of these molecules are shown in Fig. 5. Table I lists the observed IR and Raman amide A, B, I, II, III and V frequencies. Here, mainly the conformationally sensitive modes, namely amide I, II, III and V will be discussed.

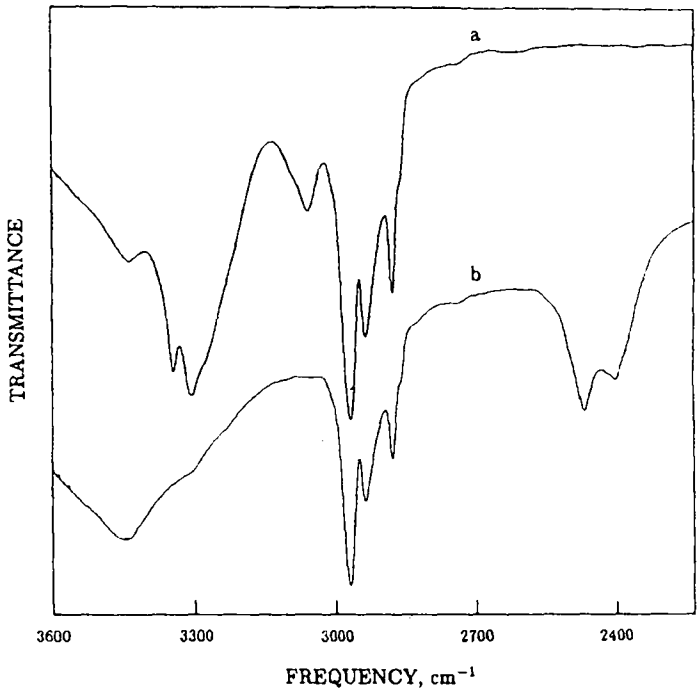


FIG. 2. Infrared spectra in the 3600–2200 cm$^{-1}$ region of (a) Boc-(D-alle-L-Ile)$_3$-OMe and (b) its N-deuterated derivative.



The amide I mode is primarily CO stretch along with some CN stretch and C$^{\alpha}$CN deformation. There are four observed amide I modes in the IR spectrum at 1691, 1683, 1665 and 1639 cm$^{-1}$, and three in the Raman spectrum at 1679, 1663 and 1638 cm$^{-1}$. These modes are similar to those observed in Pro-Leu-Gly-NH$_2$ (PLG) [13] which has a type II $\beta$-turn structure in the solid state. In PLG the lowest IR and Raman frequency is $\sim$ 1650 cm$^{-1}$ which is somewhat higher than the one observed in ILEU-6. This lowest frequency band is outside the range of the amide frequencies (1656-1693 cm$^{-1}$) calculated for a model type II $\beta$-turn [12]. This band may be assigned to the CO groups of Ile$_4$ and alle$_5$, which are involved in an extended hydrogen-bonded structure.

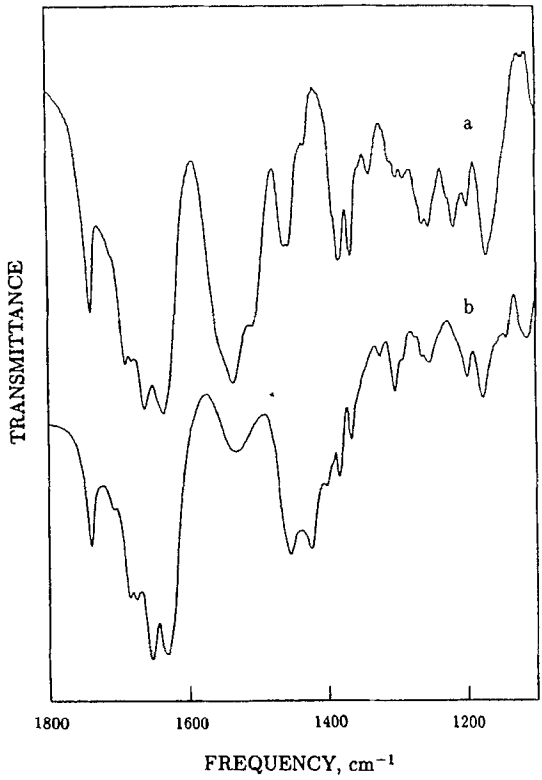


FIG. 3. Infrared spectra in the 1800–1000 cm$^{-1}$ region of (a) Boc-(D-alle-L-Ile)$_3$-OMe and (b) its N-deuterated derivative.

The amide II mode consists mainly NH in-plane motion along with some contribution from CN stretching. This mode is intrinsically weak in the Raman spectrum and often not detectable. In the IR spectrum there are three amide II bands: a shoulder $\sim$ 1550 cm$^{-1}$, a very strong band at 1536 cm$^{-1}$, and a medium strong band at 1509 cm$^{-1}$ all of which loose intensity on N-deuteration (Fig. 3). The 1550 and 1538 cm$^{-1}$ bands are close to the predicted amide II modes of a model type II $\beta$-turn (1540 - 1558 cm$^{-1}$) [12]. The lowest frequency band observed at 1509 cm$^{-1}$ is outside the range of the predicted modes and may be assigned to the NH groups of the residues that

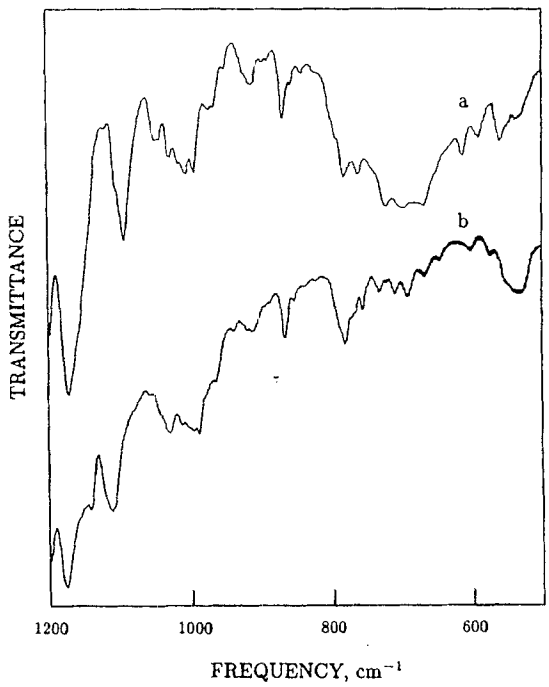


FIG. 4. Infrared spectra in the 1200 – 500 $cm^{-1}$ of (a) Boc-(D-alle-L-Ile)$_3$-OMe and (b) its N-deuterated derivative.



are not involved in the $\beta$-turn or it may be due to the particular structure of this hexapeptide.

The amide III mode consists of NH in-plane motion and CN stretching. There are four bands in the IR spectrum at 1264, 1255, 1227 and 1218 $cm^{-1}$, and five bands in the Raman spectrum at 1270, 1261, 1245, 1229 and 1219 $cm^{-1}$ that are clearly identifiable on N-deuteration (Figs. 3 and 5). PLG shows similar bands in the high frequency region whereas the frequencies observed $\sim$ 1220 $cm^{-1}$ in ILEU-6 are outside the range of frequencies predicted for a model type II $\beta$-turn and the range of amide III frequencies observed in PLG. However, $\beta$-poly-L-alanine shows a strong Raman band at 1222 $cm^{-1}$ and an IR band at 1226 $cm^{-1}$ [14]. These low frequency bands may

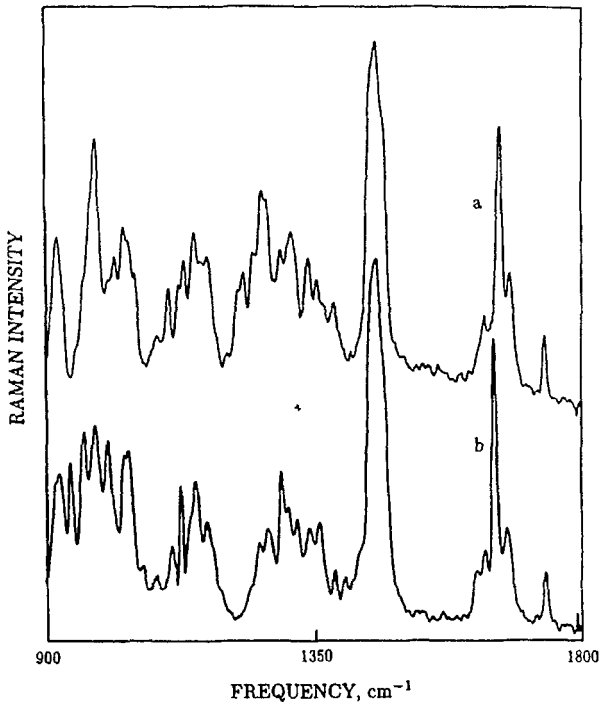


FIG. 5. Raman spectra in the 900–1800 $cm^{-1}$ region of (a) Boc-(D-aIle-L-Ile)$_3$-OMe and (b) its N-deuterated derivative.



be arising from the residues not involved in the formation $\beta$-turn. ILEU-6 being a hexapeptide forms an extended hydrogen bonded structure in addition to a $\beta$-turn structure. It should also be pointed out that amide III modes are affected by the nature of the side chains [15]. Recent vibrational circular dichroism studies have found the existence of coupling between C-H and N-H coordinates for amide III modes [16].

The amide V mode consists of NH out-of-plane bend plus CN torsion. There are four bands in the IR at 720, 696, 672 and 612 $cm^{-1}$ that loose intensity on N-deuteration (Fig. 4). These frequencies agree reasonably well with the calculated (699, 657, 603 and 575 $cm^{-1}$) and observed (699, 647

Table I

Observed amide frequencies ($cm^{-1}$) of ILEU-6 in the crystalline state

| Mode | IR† | Raman† |
|---|---|---|
| Amide A | 3345 vs | 3347 vw |
| | 3307 vs | 3306 vw |
| Amide B | 3085 sh | |
| | 3060 m | |
| Amide I | 1691 s | |
| | 1683 m | 1679 s |
| | 1665 vs | 1663 vs |
| | 1639 vs | 1638 m |
| Amide II | 1550 sh | |
| | 1536 vs | |
| | 1509 m | |
| Amide III | | 1270 m |
| | 1264 w | 1261 s |
| | 1255 w | 1245 m |
| | 1227 sh | 1229 m |
| | 1218 w | 1219 sh |
| Amide V | 720 m | |
| | 696 m | 703 vw |
| | 672 m | |
| | 612 w | 611 vw |

†s=strong, m=medium, v=very, w=weak, and sh=shoulder

and 614 $cm^{-1}$) frequencies of PLG [13]. The amide V mode observed at 720 $cm^{-1}$ may be due to the particular structure of this fused $\beta$-turn and an $\alpha$-turn structure. It should be noted that ILEU-6 has peptides that deviate as much as 18° from planarity [10] and it is possible that the observed high frequency amide V mode is linked to these nonplanar peptide groups.

## CONCLUSIONS

The observed amide I, II, III and V vibrations of ILEU-6 are consistent with the frequencies predicted for a standard type II $\beta$-turn and the observed frequencies of Pro-Leu-Gly-$NH_2$, which has a type II $\beta$-turn structure in the crystalline state. The additional frequencies found in the amide I region $\sim$ 1638 $cm^{-1}$, in the amide II region $\sim$ 1509 $cm^{-1}$, and in the amide III region $\sim$ 1220 $cm^{-1}$ for this hexapeptide are assigned to the extended structure present in this peptide. The additional high frequency amide V band observed at 720 $cm^{-1}$ in the IR spectrum may be associated with the nonplanar peptide groups found in this peptide or it may be a characteristic of this particular structure of ILEU-6, which has a fused $\beta$-turn and an $\alpha$-turn structure.



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