(33) Abragam, A.; Bleaney, B. *Electron Paramagnetic Resonance of Transition Ions*; Oxford University Press: Oxford, 1970; p 541.
(34) Anderson, P. W.; Weiss, P. R. *Rev. Mod. Phys.* **1953**, *25*, 269–276.
(35) Damoder, R.; More, K. M.; Eaton, G. R.; Eaton, S. S. *Inorg. Chem.* **1984**, *23*, 1320–1326.
(36) Morvan, C.; Demarty, M.; Thellier, M. *Plant Physiol.* **1979**, *63*, 1117–1122.
(37) Gorter, C. J.; VanVleck, J. H. *Phys. Rev.* **1947**, *72*, 1128–1129.
(38) Fishman, M. L.; Pfeffer, P. E.; Barford, R. A.; Doner, L. W. *J. Agric. Food Chem.* **1984**, *32*, 372–378.
(39) Davis, M. A. F.; Gidley, M. J.; Morris, E. R.; Powell, D. A.; Rees, D. A. *Int. J. Biol. Macromol.* **1980**, *2*, 330–332.
(40) Ebel, C.; Ingold, K. U.; Michon, J.; Rassat, A. *Nouv. J. Chim.* **1985**, *9*, 479–485.
(41) Keana, J. F.; VanNice, F. L. *Physiol. Chem. Phys. Med. NMR* **1984**, *16*, 477–480.
(42) Eaton, S. S.; Eaton, G. R. *Coord. Chem. Rev.* **1978**, *26*, 207–262.
(43) Hyde, J. S.; Rao, K. V. S. *J. Magn. Reson.* **1978**, *29*, 509–516.
(44) Darvil, A.; McNeil, M.; Albersheim, P.; Delmer, D. *The Biochemistry of Plants*; Academic: New York, 1980; Vol. I, pp 91–162.
(45) Huber, D. J. *J. Food Sci.* **1984**, *49*, 1310–1315.
(46) Irwin, P. L.; Gerasimowicz, W. V.; Pfeffer, P. E.; Fishman, M. *J. Agric. Food Chem.* **1985**, *33*, 1197–1201.
(47) Irwin, P.; Pfeffer, P.; Gerasimowicz, W.; Pressey, R.; Sams, C. *Phytochemistry* **1984**, *23*, 2239–2242.

# $^1$H NMR Study of Helical Structures Initiated by an $\alpha$-Aminoisobutyric Acid Residue in Oligoleucines[1]

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*Received July 22, 1986*

ABSTRACT: The helical structure of oligoleucines containing an Aib residue was investigated by $^1$H NMR spectroscopy using $Me_2SO$-$d_6$ and $CDCl_3$ as solvents. From the temperature dependence of the NH chemical shifts of the peptides, the conformations of a series of the oligoleucines in $Me_2SO$-$d_6$ have been shown to be as follows: The peptides Boc-Aib-Leu$_n$–OX (X = Bzl and H, $n$ = 4 and 5) have an incipient $3_{10}$-helical structure, while Boc-Leu$_n$-Aib-OH ($n$ = 3 and 4) has a randomly coiled structure. The other peptides having the Aib residue in the internal sequence appear to have a $3_{10}$- or an $\alpha$-helical structure. From titration curves of the amide NH chemical shifts and those of the coupling constants $J_{NH-C^{\alpha}H}$ of the Leu residues using the $CDCl_3$–$Me_2SO$-$d_6$ solvent system, the conformations of a series of the oligoleucines in the solvent system have been shown to be as follows: Boc-Aib-Leu$_3$–OH (**3′**) has a type III $\beta$-turn structure, Boc-Aib-Leu$_5$–OH (**5′**) has a $3_{10}$-helical structure, and Boc-Leu$_2$-Aib-Leu$_4$–OH (**8′**) has an $\alpha$-helical structure. The peptide Boc-Leu$_4$-Aib-Leu$_4$–OH (**9′**) has an $\alpha$-helical structure over the peptide sequence for high concentrations of $CDCl_3$ in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system, but in high $Me_2SO$-$d_6$ concentration, the $\alpha$-helical structure is limited in the peptide sequence from the Leu(2) CO to the C terminus. It has also been shown that the helical structures are stable at the peptide sequences of the C-terminal side of the Aib residue and are loosened toward the C terminus along the peptide sequences. The loosening of the helical structures also occurs from the C terminus with increasing the concentration of $Me_2SO$-$d_6$. From the above results, it was concluded that the Aib residue inserted into oligoleucines initiated $3_{10}$- or $\alpha$-helical folding by the hydrogen bonds of two carbonyl groups on both sides of the Aib residue with Leu NH protons included in the peptide sequence of the C-terminal side of the Aib residue.

## Introduction

The conformational space of an Aib residue in peptides is always severely restricted by steric hindrance, and the backbone dihedral angles ($\phi = \pm 60 \pm 20°$, $\psi = \pm 30 \pm 20°$) of the Aib residue are mainly found in the region of the conformational map that includes both the $\alpha$-helix (right-handed $\alpha$-helix: $\phi = -57°$, $\psi = -47°$) and the $3_{10}$ helix (right-handed $3_{10}$ helix: $\phi = -60°$, $\psi = -30°$).[2] In fact, Aib-rich peptide fragments often found in membrane-channel-forming polypeptides[3] are well recognized to have $3_{10}$ and $\alpha$-helixes, and the conformational preference of linear Aib-rich peptides has been attributed to the restriction of the backbone dihedral angles $\phi$ and $\psi$ of the Aib residues. On the other hand, it has been reported that oligoleucines Boc–Leu$_n$–OBzl ($n$ = 6 and 9) have a $\beta$-sheet structure in the solid state and are scarcely soluble or absolutely insoluble in most organic solvents.[4] It has also been reported that Boc–Leu$_7$–OMe has a $\beta$-sheet structure in polar solvents.[5,6] In our recent papers concerning the strategy for solubility improvement of peptides,[7-11] it was demonstrated that by replacement of an Ala residue in oligopeptides with an Aib residue the solubility of the peptides was remarkably increased due to a $\beta$-sheet → helix conformational transformation and that these peptides became readily soluble in the usual organic solvents. The great ability of the Aib residue to promote helical structures was further demonstrated by IR spectral conformational analysis in dichloromethane of oligoleucines containing only one Aib residue. From these results, the restriction of the values of the backbone dihedral angles $\phi$ and $\psi$ of an amino acid residue to those of the helical regions was suggested to be one of the important initiation mechanisms of helical folding in natural proteins.[8] We also expected that this novel strategy for solubility improvement give a breakthrough in creating proteins with novel properties that could not be achieved by genetic engineering technology.[7,8] Therefore, investigation of the helix-promoting properties of the Aib residue is very important.

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0024-9297/87/2220-1227$01.50/0 

This report studies the conformation of oligoleucines containing one Aib residue by $^1$H NMR. The peptides used are as follows: Boc-Leu$_4$–OBzl (**1**), Boc–Leu$_5$–OBzl (**2**), Boc-Aib-Leu$_3$–OBzl (**3**), Boc-Aib-Leu$_3$–OH (**3′**), Boc-Aib-Leu$_4$–OBzl (**4**), Boc-Aib-Leu$_4$–OH (**4′**), Boc-Aib-Leu$_5$–OBzl (**5**), Boc-Aib-Leu$_5$–OH (**5′**), Boc-Leu-Aib-Leu$_2$–OBzl (**6**), Boc-Leu-Aib-Leu$_4$–OH (**7′**), Boc-Leu$_2$-Aib-Leu$_4$–OBzl (**8**), Boc-Leu$_2$-Aib-Leu$_4$–OH (**8′**), Boc-Leu$_4$-Aib-Leu$_4$–OH (**9′**), Boc-Leu$_3$-Aib–OH (**10′**), and Boc-Leu$_4$-Aib–OH (**11′**). At first, the occurrence of incipient helical structures in a highly polar solvent, $Me_2SO$-$d_6$, is investigated from the temperature dependence of the NH chemical shifts of these peptides. The investigation of the incipient helical structures by $^1$H NMR would give useful information for the initiation mechanism of helical structures promoted by the Aib residue and elucidates the effect of the position of an Aib residue in a peptide sequence on the ability to promote helical folding. In solvents with lower polarity, the incipient helical structure is expected to be more stable than in a highly polar solvent such as $Me_2SO$. Then, titration curves of the NH chemical shifts and those of the coupling constants $J_{NH-C^\alpha H}$ of the oligoleucines **3′**, **5′**, **8′**, and **9′** are obtained by using the $CDCl_3$–$Me_2SO$-$d_6$ solvent system to elucidate the degree of shielding of the amide NH protons of these peptides from solvation and to get information about the stability of the helical structures of the peptides. The type of helical structure, a $3_{10}$ or an $\alpha$-helix, is also discussed from the $^1$H NMR data.

## Experimental Section

**Materials.** The syntheses and physical properties of the peptides **1–5**, **3′**, **4′**, and **9′–11′** have been reported previously.[7,8,10] The peptides **6–8** were obtained by the method described previously,[7,12] from Boc-Leu$_n$-Aib–OH ($n$ = 1 or 2) and HCl·Leu$_n$–OBzl ($n$ = 2 or 4) using *N*-methylmorpholine as an amine and dicyclohexylcarbodiimide and 1-hydroxy-1*H*-benzotriazole as coupling reagents. Found for **6**: C, 64.86; H, 8.97; N, 8.90. Anal. Calcd for $C_{34}H_{56}O_7N_4$: C, 64.53; H, 8.92; N, 8.85. Amino acid analysis: Aib, 1.00; Leu, 2.93. Found for **7**; C, 63.51; H, 9.12; N, 9.55. Anal. Calcd for $C_{46}H_{78}O_9N_6 \cdot {}^1/_2H_2O$: C, 63.61; H, 9.17; N, 9.68. Amino acid analysis: Aib, 0.97; Leu 5.00. Amino acid analysis of **8**: Aib, 0.87; Leu, 6.00.

The peptides **5′**, **7′** and **8′** were obtained from the peptides **5**, **7**, and **8**, respectively, by hydrogenolysis using Pd/C as a catalyst. Homogeneity of these peptides was ascertained by the fact that each peptide had a single peak in HPLC. Found for **8**: C, 60.23; H, 9.48; N, 11.03. Anal. Calcd for $C_{45}H_{83}O_{10}N_7 \cdot H_2O$: C, 60.04; H, 9.52; N, 10.89.

The amino acid residues of the peptides were numbered from the N terminus of the peptide chain so that, for example, the amide proton of an N-terminal Leu residue is labeled Leu(1) NH or merely NH 1 and the C$^\alpha$ proton of the N-terminal Leu residue Leu(1) C$^\alpha$H or merely C$^\alpha$H 1. Tetramethylsilane ($Me_4Si$) from Merck Co. and $Me_2SO$-$d_6$ (99.8%) and $CDCl_3$ (99.8%) from CEA were used for $^1$H NMR measurements.

**Measurements.** $^1$H NMR spectra were obtained on a JEOL FX 200 NMR spectrometer. Approximately 0.03 M solutions were prepared for NMR measurements except for the experiments on the concentration dependence of the NH chemical shifts. All the variations of the NH chemical shifts with temperature change were linear. Chemical shifts were recorded with $Me_4Si$ as an internal reference.

## Results and Discussion

**$^1$H NMR Spectra of the Peptides.** The $^1$H NMR spectra in the amide NH region of the peptides at 60 °C are shown in Figure 1. The singlet peaks were assigned to the NH peaks of the Aib residues, and the highest field resonances were assigned to the NH protons of the N-terminal amino acid residues blocked by the Boc group.[13,14] The peaks of the amide NH and C$^\alpha$H protons were assigned from both comparison of their chemical shifts among these peptides and decoupling experiments as described below.

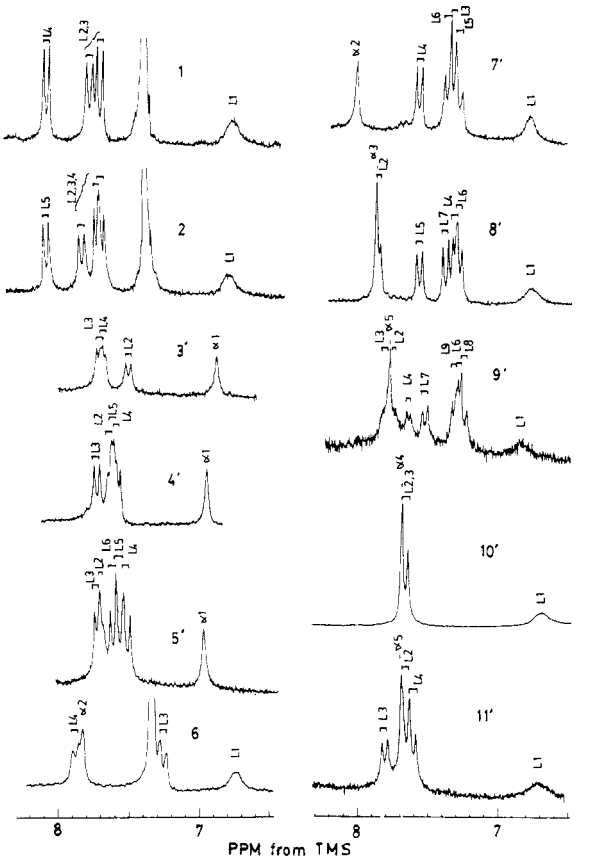


**Figure 1.** Partial $^1$H NMR spectra of the peptides **1–11′** in $Me_2SO$-$d_6$ at 60 °C together with their assignments (L, Leu; $\alpha$, Aib).

The C-terminal Leu NH peaks of **3–5**, **3′–5′**, **8**, and **8′** were assigned from the comparison of the NH chemical shifts and their temperature dependences between the C-terminal free and C-terminal blocked peptides having the same peptide sequence. Namely, except for one peak, the NH chemical shifts and their temperature dependences scarcely changed regardless of the presence of the C-terminal blocking benzyl group. The one remaining peak of each C-terminal free peptide resonated at a lower field by about 0.2 ppm than that of the corresponding peak of its C-terminal blocked peptide. These pairs of peaks were also shown to have similar temperature dependences. Thus, the pairs of peaks were assigned to the NH protons of the C-terminal Leu residues which would resonate at different fields according to the type of C-terminal group. The NH chemical shifts of the other amino acid residues and their temperature dependences of the peptides with the same peptide sequence were similar between corresponding residues, suggesting that the peptides have essentially the same conformation despite the C-terminal blocking group in $Me_2SO$.

The other NH peaks of the peptides were assigned from decoupling experiments of the C$^\alpha$H peaks which, in turn, were assigned as described below. The C$^\alpha$H peaks of the Leu residues following an Aib residue were assigned by use of the spectrum of **6**. The peak centered at 3.81 ppm (at 30 °C) in the spectrum of **6** was assigned to the Leu(1) C$^\alpha$H from the Leu(1) NH chemical shift and the decoupling experiment. The peak centered at 4.30 ppm in the spectrum of **6** was assigned to the Leu(4) C$^\alpha$H from comparison of the C$^\alpha$H chemical shift with those of the C-terminal Leu

**Table I**
**Temperature Dependence of NH Chemical Shifts ($\times 10^3$ ppm/°C)**

| peptide | NH1 | NH2 | NH3 | NH4 | NH5 | NH6 | NH7 | NH8 | NH9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 7.4 | (3.5 | 4.4)[a] | 6.0 | | | | | |
| 2 | 6.8 | (4.0 | 4.0 | 5.1)[b] | 6.1 | | | | |
| 3 | *7.4*[c] | 5.1 | 3.1 | 5.1 | | | | | |
| 3′ | *7.2* | 5.2 | 2.5 | 6.6 | | | | | |
| 4 | *7.1* | 5.3 | 2.2 | 3.9 | 4.8 | | | | |
| 4′ | *6.9* | 5.5 | 2.4 | 3.5 | 4.6 | | | | |
| 5 | *7.0* | 5.6 | 2.9 | 3.4 | 3.8 | 5.2 | | | |
| 5′ | *7.0* | 6.0 | 3.2 | 3.8 | 3.3 | 5.3 | | | |
| 6 | 7.5 | *5.9* | 3.0 | 3.4 | | | | | |
| 7′ | 8.1 | *6.8* | 3.5 | 2.6 | 1.7 | 3.6 | | | |
| 8 | 7.2 | 5.2 | *4.7* | 3.1 | 2.4 | 1.9 | 3.7 | | |
| 8′ | 6.8 | 4.2 | *4.1* | 3.0 | 2.3 | 2.5 | 3.4 | | |
| 9′ | 6.7 | 4.2 | 5.0 | 4.3 | *4.8* | 3.6 | 2.5 | 2.6 | 3.5 |
| 10′ | 7.2 | 4.0 | 3.4 | *6.0* | | | | | |
| 11′ | 7.2 | 4.2 | 4.9 | 3.9 | *6.2* | | | | |

[a] Values for the NH2 or NH3 peaks. [b] Values for NH2, NH3, or NH4. [c] Values in italics are those for the Aib NH peaks.

peaks of **3**–**5** and **8** (4.30–4.37 ppm). The remaining peak at 4.24 ppm was assigned to the C$^\alpha$H of Leu(3) following the Aib residue. Thus, the C$^\alpha$H of a Leu residue following an Aib residue was revealed to resonate slightly upfield of the C$^\alpha$H of a Leu residue in the internal of the oligoleucine sequences which resonated at 4.27–4.33 ppm. Then, in the spectra of **3**–**5** and **3′**–**5′**, the peaks at the highest fields in the C$^\alpha$H region were assigned to the Leu(2) C$^\alpha$H. In addition, the C$^\alpha$H peaks of Leu(3)–Leu(5) of **3**–**5** and **3′**–**5′**, except for the C-terminal Leu residues, were assigned by assuming that the C$^\alpha$H of a Leu residue nearer to the Aib residue in the peptide sequence resonated at the higher field.

The peaks of Leu C$^\alpha$H of **7′**, **8**, and **8′** were assigned from comparison of their chemical shifts and the method described above. The peaks of the C$^\alpha$H of the Leu(2) residues preceding the Aib residue of **8** and **8′** were shown to resonate at a higher field in the Leu C$^\alpha$H region, similar to the case of the C$^\alpha$H peak of the Leu residue following an Aib residue. It was also observed that the C$^\alpha$H protons of the N-terminal Leu(1) residues preceding the Aib residue of **6** and **7′** resonated at a higher field by about 0.1 ppm than that of the C$^\alpha$H peaks of the N-terminal Leu(1) residues preceding a Leu residue. Then, in the spectra of **10′** and **11′**, the peaks at the highest fields in the Leu C$^\alpha$H region, except for the N-terminal Leu C$^\alpha$H peaks, were assigned to the C$^\alpha$H protons of Leu(3) and Leu(4) preceding the Aib residue, respectively. The C$^\alpha$H peaks of Leu(2) and Leu(3) of **11′** were assigned by assuming that the C$^\alpha$H of a Leu residue nearer to the Aib residue in the peptide sequence resonates at a higher field.

In the spectrum of **9′**, the NH and C$^\alpha$H peaks of Leu(2)–Leu(4) were assigned from comparison of their chemical shifts with those of the Leu NH and C$^\alpha$H peaks of **11′**, and the NH and C$^\alpha$H peaks of Leu(6)–Leu(9) were assigned from comparison of their chemical shifts with those of the Leu NH and C$^\alpha$H peaks of **7′** and **8′**.

**Conformation of the Peptides in $Me_2SO$.** The NH chemical shifts of **3** were shown to exhibit no concentration dependence by spectrum measurements in 0.006 M solution, indicating that the NH protons of **3** are free from intermolecular hydrogen bonding in the concentration range 0.006–0.03 M.[14] Considering the high polarity of the solvent, the other peptides are also expected to be free from intermolecular hydrogen bonding.

The temperature dependences of the NH chemical shifts are summarized in Table I. It has been well recognized that, in highly polar solvents such as $Me_2SO$-$d_6$, the temperature dependence of NH chemical shifts is small when NH protons are shielded from the solvent ($2 \times 10^{-3}$ ppm/°C) but becomes large when NH protons are exposed to the solvent ($6 \times 10^{-3}$ ppm/°C).[14-17] Our previous study of the oligoleucines **1** and **2** has shown that they have a randomly coiled structure in $Me_2SO$.[4] As shown in Table I, the NH chemical shifts of the N- and C-terminal Leu residues of **1** and **2** exhibit a large temperature dependence and those of the Leu residues in the internal sequences also exhibit a relatively large temperature dependence, which indicates that these NH protons are almost fully solvated in $Me_2SO$-$d_6$.

On the other hand, the NH chemical shifts of Leu(3) of **3** and **3′** exhibit a small temperature dependence, in contrast to the fact that the NH chemical shifts of the other amino acid residues of these peptides exhibit a large temperature dependence. This result suggests contribution of a $\beta$-turn structure formed by the hydrogen bonding of the Leu(3) NH with the Boc group CO. The carbonyl oxygen is on the Boc–Aib bond with a fixed dihedral angle of the Aib residue, permitting the type I and type III $\beta$-turn structures when the Aib residue is at the $i + 1$ position.[18]

The NH chemical shifts of Leu(3) of **4**, **4′**, **5**, and **5′** exhibit a small temperature dependence and Leu(4) and Leu(5) of **4** and **4′**, and Leu(4)–Leu(6) of **5** and **5′** also exhibit a relatively small temperature dependence compared to that of the corresponding Leu residues of **1** and **2**. This result indicates the occurrence of successive intramolecular hydrogen bonds of the NH protons from Leu(3) through the C-terminal Leu(5) or Leu(6). The presence of two free amide NH protons of the N-terminal dipeptide sequences of these peptides and the presence of successive intramolecular hydrogen bonds clearly suggest contribution of successive $i \rightarrow i - 4$ hydrogen bonds forming a $3_{10}$-helical structure.[17,19,20] It is also suggested that the $3_{10}$-helical structure of the peptides Boc-Aib-Leu$_n$–OX (X = Bzl and H, $n$ = 4 and 5) is initiated by the intramolecular hydrogen bonding of the Leu(3) NH with the Boc group CO which makes the type III $\beta$-turn structure, analogously in the case of **3** and **3′**. The gradual increase in the temperature dependence of the NH chemical shifts from Leu(3) to the C-terminal Leu indicates that the $3_{10}$-helical structure of **4**, **4′**, **5**, and **5′** is loosened gradually toward the C-terminal direction in $Me_2SO$-$d_6$. The conformational study of more tightly hydrogen bonded species of the peptides **3′** and **5′** will be described later.

Although the NH chemical shift of Leu(3) of **7′** exhibits a slightly larger temperature dependence compared to that of the Leu(3) NH chemical shifts of **4**, **4′**, **5**, and **5′**, the NH chemical shifts of Leu(4)–Leu(6) in the C-terminal side sequence from Leu(3) of **7′** exhibit a smaller temperature dependence compared to that of the corresponding Leu

of 4, 4′, 5, and 5′. This result indicates that the presence of the Leu(1) residue preceding the Aib residue promotes stabilization of the helical conformation. The presence of two solvated amide NH protons of the N-terminal dipeptide sequence of 7′ and the presence of successively shielded NH protons of Leu(3)–Leu(6) suggest the contribution of a $3_{10}$-helical structure. However, a slightly larger temperature dependence of Leu(3) and the smallest temperature dependence of Leu(5) of 7′ also suggest the contribution of successive $i \rightarrow i-5$ hydrogen bonds forming an $\alpha$-helical structure in which the Leu(5) NH is intramolecularly hydrogen bonded with the Leu(1) CO on the Leu–Aib peptide bond with a fixed dihedral angle of the Aib residue permitting an $\alpha$-helical structure.

The NH chemical shifts of the Aib(3) residues of 8 and 8′ exhibit a relatively large temperature dependence, and the NH chemical shifts of Leu(4)–Leu(7) exhibit a temperature dependence comparable to that of the NH chemical shifts of the corresponding Leu(3)–Leu(6) of 7′, respectively. The presence of three free amide NH protons of the N-terminal tripeptide sequences of 8 and 8′ and the presence of successively shielded NH protons of Leu(4)–Leu(7) suggest contribution of an $\alpha$-helical structure, that is, the successive $i \rightarrow i-5$ hydrogen bonds beginning with the Boc group CO with the Leu(4) NH through the C terminus. However, the contribution of a $3_{10}$-helical structure containing intramolecular hydrogen bonds beginning with the Leu(1) CO with the Leu(4) NH through the C-terminal region is not neglected from these temperature dependence data. The type of helical structure will be investigated from titration curves of the NH chemical shifts using the $CDCl_3$–$Me_2SO$-$d_6$ solvent system as described later.

The NH chemical shifts of Leu(1)–Aib(5) of 9′ exhibit a comparable temperature dependence to that of the NH chemical shifts of Leu(1)–Leu(4) of 2, while the NH chemical shifts of Leu(6)–Leu(9) of 9′ exhibit a comparable temperature dependence to that of the NH chemical shifts of the corresponding Leu of 7′, 8, and 8′, indicating that only the peptide sequence of the C-terminal side of the Aib residue has a helical structure. The NH chemical shifts of the amino acid residues of 10′ and 11′ also exhibit a temperature dependence comparable to that of the NH chemical shifts of the corresponding Leu residues of 1 and 2, respectively, showing that the peptides 10′ and 11′ have a randomly coiled structure in $Me_2SO$-$d_6$. These results indicate that the Aib residue introduced alone in oligoleucines has no helix-promoting ability toward the N-terminal direction in contrast to the helix-promoting ability toward the C-terminal direction.

The NH chemical shifts at 30 °C of all the amino acid residues of the peptides used here except for 3′–5′ and 8′ are plotted against their temperature coefficients in Figure 2. Those of only the C-terminal amino acid residues of 3′–5′ and 8′ are also plotted. All the amino acid NH peaks are classified into four groups. The NH protons free from intramolecular hydrogen bonding were revealed to resonate at low field (7.64–8.29 ppm) in the amide NH region, although the N-terminal NH protons blocked with Boc groups resonate at high field (6.90–7.18 ppm), as has been well recognized.[13,14] The low-field positions of the NH protons free from intramolecular hydrogen bonding are probably due to the strong intermolecular hydrogen bonding of the NH protons with $Me_2SO$-$d_6$. On the other hand, the NH protons contained in the helical structure were found to resonate at a relatively high field (7.34–7.62 ppm) except for several weakly hydrogen-bonded NH protons of the C-terminal Leu and the NH protons of the

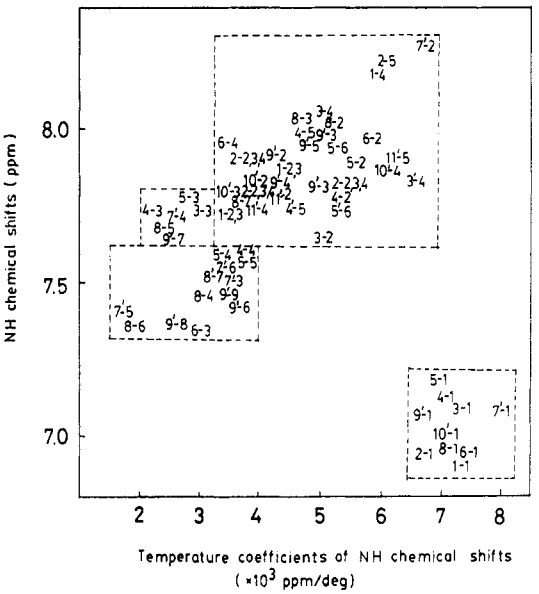


**Figure 2.** Plots of the NH chemical shifts of the peptides 1–11′ against their temperature coefficients in $Me_2SO$-$d_6$. The numbers attached by a hyphen represent the peptide and residue numbers, respectively.

Leu residues following the Aib–Leu sequence such as Leu(3) of 3–5. The NH protons of the Leu residues following the Aib–Leu sequence, except for the C-terminal Leu(4) of 6, were shown to resonate in a relatively narrow range of low field (7.65–7.79 ppm). The characteristic NH chemical shifts of these Leu residues could be attributed to the conformation of the peptide sequence in which the Leu NH protons are at the N-terminal region of the helical structure. But it is more probable that the characteristic NH chemical shifts are due to the position in the peptide sequence of the Leu residue. The relation of the NH chemical shifts to their temperature coefficients as mentioned above is very interesting in relation to the availability of the NH chemical shifts for assignments of peaks and preliminary prediction of the peptide conformation.

**Conformation of the Peptides 3′, 5′, 8′, and 9′ in $CDCl_3$ and in the $CDCl_3$–$Me_2SO$-$d_6$ Solvent System.** The conformation of the peptides 3′, 5′, 8′, and 9′ was investigated in $CDCl_3$ and in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system and revealed as follows:

**Boc-Aib-Leu$_3$–OH (3′).** Figure 3a shows the titration curves of the NH chemical shifts of 3′ in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system. The NH peaks of Aib(1) and Leu(2) show large downfield shifts with increasing concentration of $Me_2SO$-$d_6$, especially up to about 50 vol %, indicating that in $CDCl_3$ and in the $CDCl_3$–$Me_2SO$-$d_6$ solvent systems the Aib(1) and Leu(2) NH protons are in a conformation easily solvated by the hydrogen bond forming solvent, $Me_2SO$-$d_6$. They are fully solvated in $Me_2SO$-$d_6$ as described above. The amide NH proton, in a conformation susceptible to solvation, is well recognized to show a large downfield shift in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system with increasing concentration of $Me_2SO$-$d_6$, especially up to about 50 vol %.[17,19-24] On the other hand, relatively small dependences of the NH chemical shifts on the concentration of $Me_2SO$-$d_6$ are observed for the Leu(3) and Leu(4) NH protons. The Leu(3) NH peak shows a small downfield shift with increasing concentration of $Me_2SO$-$d_6$ up to about 20 vol %, and, upon further increase in the concentration of $Me_2SO$-$d_6$, the Leu(3) NH chemical shift scarcely changes. The Leu(4) NH peak shifts gradually downfield with increasing concentration of $Me_2SO$-$d_6$ from 10 to 100 vol %. The small dependence of the Leu(3)

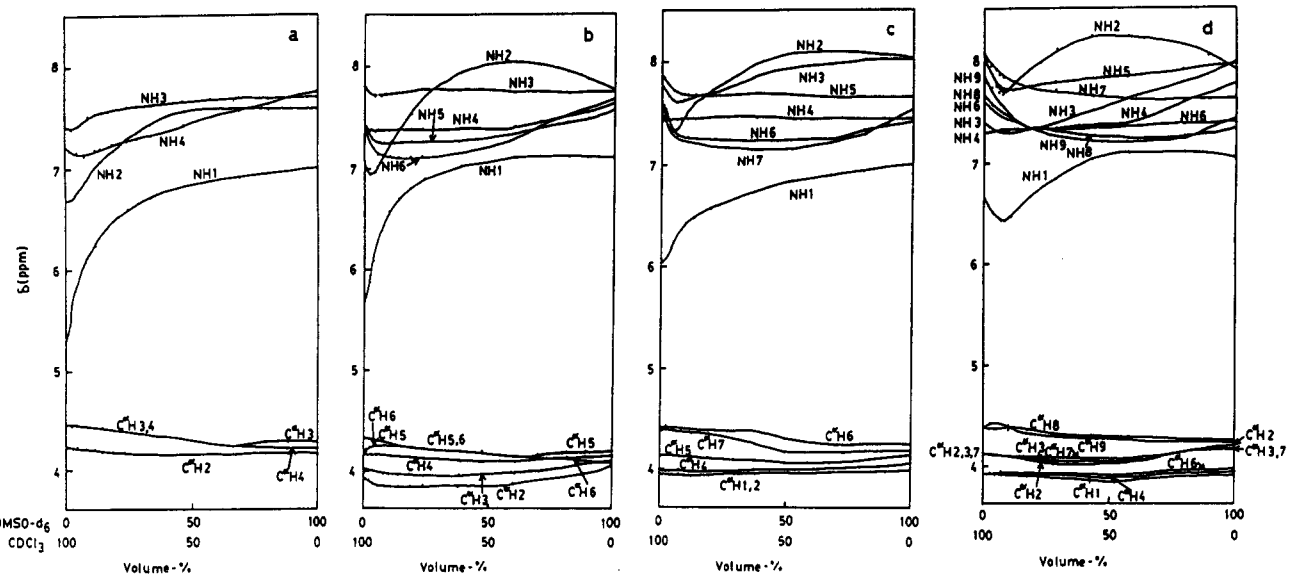


**Figure 3.** Titration curves of the NH and $C^{\alpha}H$ chemical shifts in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system: (a) the peptide 3′; (b) the peptide 5′; (c) the peptide 8′; (d) the peptide 9′.

NH chemical shift on the concentration of $Me_2SO$-$d_6$ indicates that the Leu(3) NH proton is almost shielded from the solvent due to intramolecular hydrogen bonding,[17,19-24] while the gradual downfield shift of the Leu(4) NH chemical shift indicates that the NH proton is gradually solvated in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system (Figure 3a).

The dihedral angles $\phi$ and $\psi$ of the $i + 1$ residue in the type I ($\phi = -60°$, $\psi = -30°$) and type III ($\phi = -60°$, $\psi = -30°$) $\beta$-turn structure[18] lie in the range of permitted dihedral angles $\phi$ and $\psi$ of the Aib residue ($\phi = \pm 60 \pm 20°$, $\psi = \pm 30 \pm 20°$).[19] The coupling constant $J_{NH-C^{\alpha}H}$ of the amino acid residue at the $i + 2$ position of the type III $\beta$-turn structure is expected to be about 3 Hz from the dihedral angle $\phi = -60°$.[25] On the other hand, in a randomly coiled structure, a large contribution of an extended structure of peptide sequence with a dihedral angle $\phi$ about $-120°$ would give a relatively large coupling constant of approximately 10 Hz.[25] As shown in Figure 4a, the coupling constant $J_{NH-C^{\alpha}H}$ of Leu(2) is 5–6 Hz in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system, indicating the contribution of the type III $\beta$-turn structure to the conformation of the Boc-Aib-$Leu_2$ sequence with Aib(1)-Leu(2) sequence at the corner of the $\beta$-turn. From the coupling constant $J_{NH-C^{\alpha}H}$ of Leu(3) of about 7 Hz in $CDCl_3$, it is suggested that the contribution of the type III $\beta$-turn structure with the Leu(2)–Leu(3) sequence at the corner of the $\beta$-turn is negligible in $CDCl_3$.

**Boc-Aib-$Leu_5$–OH (5′).** Figure 3b shows the titration curves of the NH chemical shifts of 5′ in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system. The NH chemical shifts of Aib(1) and Leu(2) show strong dependences on the concentration of $Me_2SO$-$d_6$, indicating that the Aib(1) and Leu(2) protons are in a conformation easily solvated by $Me_2SO$-$d_6$ both in $CDCl_3$ and in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system, as in the case of 3′. On the other hand, relatively small dependences of the NH chemical shifts on the concentration of $Me_2SO$-$d_6$ are observed for the Leu(3)–Leu(6) NH protons. Especially, the Leu(3) NH peak has a constant chemical shift except in the range of $Me_2SO$-$d_6$ concentration from 0 to 5 vol %. With regard to the Leu(4)–Leu(6) NH peaks, it is shown that over the range of $Me_2SO$-$d_6$ concentration from 10 to 50 vol % the NH chemical shifts are constant, but these peaks show downfield shifts in the range of concentration from 50 to

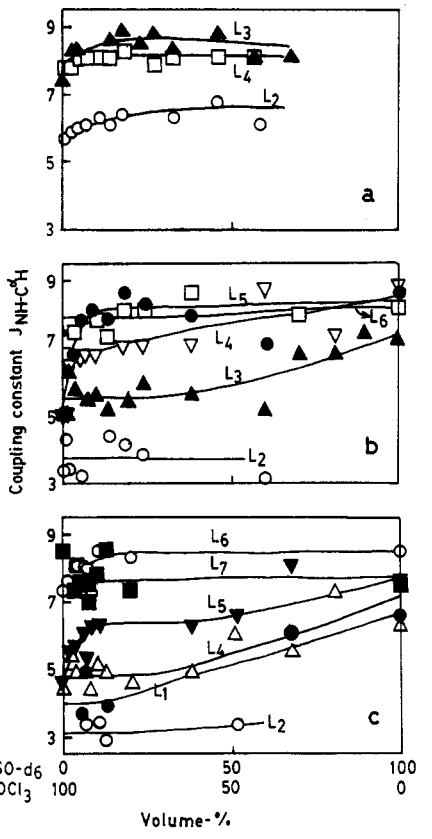


**Figure 4.** Titration curves of the coupling constants $J_{NH-C^{\alpha}H}$ of the Leu residues in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system (L, Leu): (a) the peptide 3′ (○) L2, (▲) L3, (□) L4; (b) the peptide 5′ (○) L2, (▲) L3, (▽) L4, (●) L5, (□) L6; (c) the peptide 8′ (●) L1, (○) L2, (△) L4, (▼) L5, (○) L6, (■) L7.

100 vol %, and that for Leu residues closer to the C terminus in the peptide sequence the downfield shift of the Leu NH peak is larger. These results indicate that in $CDCl_3$ only or in the $CDCl_3$-rich solvents in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system the Leu(3)–Leu(6) NH protons are shielded from the solvent due to successive intramolecular hydrogen bonds. The presence of the two easily solvated amide NH protons of the N-terminal dipeptide sequence and the successive intramolecular hydrogen bonds of the Leu(3)–Leu(6) NH protons indicate that the

peptide 5′ has a $3_{10}$-helical structure in these solvents.[17,19,20] It is also indicated that solvation occurs gradually from the C-terminal Leu NH toward the N-terminal direction along the peptide sequence with increasing concentration of $Me_2SO$-$d_6$ from 50 to 100 vol %, although the Leu(3) NH is almost fully shielded from the solvent.

The titration curves of the coupling constants $J_{NH-C^{\alpha}H}$ of the Leu residues of 5′ are shown in Figure 4b. The fact that the coupling constant $J_{NH-C^{\alpha}H}$ of Leu(2) in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system is as small as 3–4 Hz suggests a large contribution of the type III $\beta$-turn structure with the Aib(1)-Leu(2) sequence at the corner of the $\beta$-turn. The coupling constant $J_{NH-C^{\alpha}H}$ of the Leu(3) residue is also relatively small (5–5.5 Hz) in the range of concentrations of $Me_2SO$-$d_6$ from 0 to 50 vol %, suggesting a contribution of the type III $\beta$-turn structure with the Leu(2)-Leu(3) sequence at the corner of the $\beta$-turn. Successive type III $\beta$-turn structures construct a $3_{10}$-helical structure,[18,19] and the results support the suggestion from the titration curves of the NH chemical shifts that the peptide 5′ has a $3_{10}$-helical structure. The coupling constants of the Leu(4) and Leu(5) are relatively large (5–6.5 and 6–8 Hz, respectively) even in solvents with a large concentration of $CDCl_3$($CDCl_3$; 100–60 vol %), suggesting a little participation of the Leu(5) and Leu(6) NH protons to the $3_{10}$-helical structure in these solvents. These results indicate that even in the $CDCl_3$-rich solvents the $3_{10}$-helical folding is loosened from the C terminus.

**Boc-Leu$_2$-Aib-Leu$_4$–OH (8′).** Figure 3c shows the titration curves of the NH chemical shifts of 8′. The Leu(1) and Leu(2) NH chemical shifts show strong dependence on the concentration of $Me_2SO$-$d_6$, but the Aib(3) NH chemical shift shows a relatively weak dependence on the concentration although the titration curve is analogous to that of the Leu(2) NH. These results indicate that the Leu(1), Leu(2), and Aib(3) NH protons are in a conformation easily solvated by $Me_2SO$-$d_6$ in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system. On the other hand, the Leu(4) and Leu(5) NH protons have constant chemical shifts over the concentration range of $Me_2SO$-$d_6$ more than 5 vol %, indicating these NH protons are almost fully shielded in the solvent. The Leu(6) and Leu(7) NH chemical shifts show analogous titration curves to those of the Leu(4)–Leu(6) residues of 5′. This indicates a similar tendency of these protons against the solvent composition, although the influence of the solvent composition on the conformation of 8′ is small compared to its influence on that of 5′. These results indicate that in $CDCl_3$ or in the high-$CDCl_3$ concentration of the $CDCl_3$–$Me_2SO$-$d_6$ solvent system the Leu(4)–Leu(7) NH are shielded from the solvent due to successive intramolecular hydrogen bonds. The presence of the easily solvated three amide NH protons in the N-terminal tripeptide sequence and the successive intramolecular hydrogen bonds of Leu(4)–Leu(7) indicate that the peptide 8′ has an $\alpha$-helical structure. It also indicates that the $\alpha$-helical structure is loosened from the C terminus with increasing concentration of $Me_2SO$-$d_6$ in the solvent system.

The titration curves of the coupling constants $J_{NH-C^{\alpha}H}$ of the Leu residues of 8′ are shown in Figure 4c. The dihedral angle $\phi = -57°$ of the $\alpha$-helix gives about 3 Hz for the coupling constant $J_{NH-C^{\alpha}H}$.[25] As shown in Figure 4c, in high $CDCl_3$ concentrations (100–50 vol %), the coupling constants of Leu(1), Leu(2), and Leu(4) are ~4, 3–3.5, and ~5 Hz, respectively. This result supports the $\alpha$-helical structure suggested from the titration curves of the NH chemical shifts, beginning with the hydrogen bond of the Boc group CO with the Leu(4) NH. With increase of the $Me_2SO$-$d_6$ concentration from 50 to 100 vol %, the coupling constants of Leu(1) and Leu(4) increase to 6–7 Hz, indicating the helical structure is loosened in the high-$Me_2SO$-$d_6$ concentration. The coupling constant of Leu(5) increases from 5 to 6.5 Hz with increasing concentration of $Me_2SO$-$d_6$ from 0 to 10 vol %, indicating that in $CDCl_3$ the Leu(6) NH participates in the $\alpha$-helical structure and the contribution of the Leu(6) NH to the helical structure quickly becomes small with increasing concentration of $Me_2SO$-$d_6$ from 0 to 10 vol %. The coupling constant of Leu(6) is large (7.5–8.5 Hz) even in the solvents with a high proportion of $CDCl_3$, indicating little participation of the Leu(7) NH in the helical structure in these solvents.

**Boc-Leu$_4$-Aib-Leu$_4$–OH (9′).** As shown in Figure 3d, the Leu(1), Leu(2), and Leu(3) NH chemical shifts show strong dependences on the concentration of $Me_2SO$-$d_6$, indicating these NH protons are in a conformation easily solvated by $Me_2SO$-$d_6$ in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system. On the other hand, the Leu(4) NH peak has a constant chemical shift in the range of $Me_2SO$-$d_6$ concentration from 0 to 50 vol %. The Leu(6)–Leu(9) NH peaks also have an almost constant chemical shift except in the ranges of $Me_2SO$-$d_6$ concentration from 0 to 20 vol %, where the NH peaks show upfield shifts, and from 80 to 100 vol % for Leu(8) and Leu(9), where the Leu(8) and Leu(9) NH peaks show small downfield shifts. These results indicate that in the solvents with high $CDCl_3$ concentration the peptide 9′ has an $\alpha$-helical structure beginning with the hydrogen bonds of the Boc group CO with the Leu(4) NH through the C terminus as in the case of 8′. The Leu(4) NH peak shows a large downfield shift with increasing concentration of $Me_2SO$-$d_6$ from 50 to 100 vol %. Thus, it is clear that in solvents of high $Me_2SO$-$d_6$ content all the amide NH protons included in the peptide sequence of the N-terminal side of the Aib residue are solvated and that the peptide 9′ has an $\alpha$-helical structure in the range of the peptide sequence from the Leu(2) CO through the C terminus. This result corresponds well with the result of temperature dependence of the NH chemical shifts in $Me_2SO$-$d_6$, and suggests that the Aib residue inserted into oligoleucines initiates $3_{10}$- or $\alpha$-helical folding by the hydrogen bonds of the two carbonyl groups both sides of the Aib residue, with Leu NH protons included in the peptide sequence of the C-terminal side of the Aib residue. The small downfield shifts of the Leu(8) and Leu(9) NH peaks with increasing concentration of $Me_2SO$-$d_6$ from 80 to 100 vol % indicate that the helical structure of the C-terminal sequence of 9′ is loosened in solvents with high $Me_2SO$-$d_6$ content, as well as in the case of 5′ and 8′, but the degree of loosening of the helical structure is very small compared to 5′ and 8′. This fact indicates that with regard to the peptides examined here an increase in the peptide chain length stabilizes the helical structure.

Broad NH peaks and overlaps of several NH peaks made it difficult to obtain most of the coupling constants $J_{NH-C^{\alpha}H}$ of the Leu residues of 9′. The coupling constant $J_{NH-C^{\alpha}H}$ of Leu(2) was about 4 Hz in the $CDCl_3$-rich solvents ($CDCl_3$, 100–70%) and the coupling constants $J_{NH-C^{\alpha}H}$ of Leu(7) and Leu(9) were 4.9–6.3 and 7 Hz in the $CDCl_3$-rich solvents ($CDCl_3$, 100–60%), respectively. The small coupling constants of Leu(2) in solvents with high $CDCl_3$ concentration indicate that the Leu(1) CO participates in the helical structure in these solvents, supporting the $\alpha$-helical structure over the whole range of the peptide suggested from the NH chemical sifts titration curves.

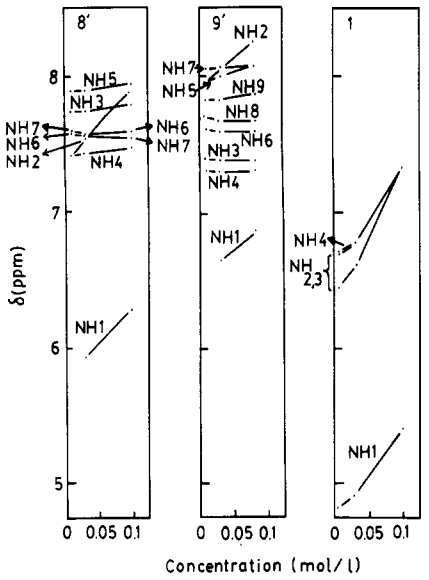


**Figure 5.** Concentration dependences of the NH chemical shifts of the peptides 8', 9', and 1 in $CDCl_3$.

It is inferred from the above results that oligoleucines containing an Aib residue at the N terminus of the peptide sequences have a tendency to assume a $3_{10}$-helical structure and that oligoleucines containing an Aib residue at the third position from the N terminus or at a more internal position have a tendency to assume an $\alpha$-helical structure.

As shown in Figure 3, in the range of $Me_2SO$-$d_6$ concentration from 0 to 10 or 20% only the NH peaks of the fourth amino acid residues from the N terminus of 5', 8', and 9' have a constant chemical shift. The other NH peaks participating in intramolecular hydrogen bonding shift upfield in the same range of $Me_2SO$-$d_6$ concentration increasingly as the Leu residue departs from the fourth amino acid in the peptide sequence. The coupling constants $J_{NH\text{-}C^{\alpha}H}$ of Leu(4) and Leu(5) of 5', those of Leu(5) and Leu(6) of 8', and that of Leu(7) of 9' also show sharp increases with increasing concentration of $Me_2SO$-$d_6$ from 0 to 10%. In most cases for NH protons participating in intramolecular hydrogen bonds forming helical structures the magnitude of the upfield shifts of a peptide implies relative weakness of the intramolecular hydrogen bonds of the peptide. This fact suggests that the helical structures in $CDCl_3$ solution are partially loosened in the range of $Me_2SO$-$d_6$ concentration from 0 to 10 or 20%. Most of the NH peaks in the N-terminal region of the peptide sequence also shift upfield in the range of $Me_2SO$-$d_6$ concentration from 0 to 10 or 20%. The intermolecular hydrogen bonds of the amide NH protons in the N-terminal dipeptide sequences, as described below, would also be disrupted in the same range of $Me_2SO$-$d_6$ concentration. A similar upfield shift has been observed for the initially intermolecularly hydrogen bonded amide NHMe proton of Ac-Ala-NHMe when the intermolecular hydrogen bond is disrupted with increasing concentration of $CF_3COOH$ in the titration experiment using the $CDCl_3$–$CF_3COOH$ solvent system.[26] The reason for the upfield shifts is not clear as yet but this behavior may aid in peak assignment of peptides in helical structures.

It was revealed that in the $CDCl_3$–$Me_2SO$-$d_6$ solvent system as well as in $Me_2SO$-$d_6$ the NH peaks of the Leu residues following an Aib-Leu sequence resonated at a relatively low field compared to the other peaks of the NH protons participating in intramolecular hydrogen bonding, as shown in Figure 3. This result supports the suggestion that the lower field shift of the NH peaks of the Leu residues following an Aib-Leu sequence is due to the position in the peptide sequence of the Leu residues. It was shown that the NH peaks of the two amino acid residues of the N-terminal dipeptide sequences of 3', 5', 8', and 9' showed large downfield shifts in $CDCl_3$ with increasing peptide chain length and stability of the helical structure. It was also revealed from the titration curves of the $C^{\alpha}H$ chemical shifts (Figure 3) that the $C^{\alpha}H$ peaks of the C-terminal Leu showed a relatively large upfield shift with increasing concentration of $Me_2SO$-$d_6$ compared to the other $C^{\alpha}H$ peaks. In addition, except for the C-terminal $C^{\alpha}H$ Leu peaks, the relative positions of the $C^{\alpha}H$ peaks are almost unchanged with change of solvent from $CDCl_3$ to $Me_2SO$-$d_6$. This fact is convenient for assignment of the $C^{\alpha}H$ peaks in one solvent or the other.

The concentration dependences of the NH chemical shifts of 8' and 9' in $CDCl_3$ are shown in Figure 5 along with those of the Leu NH chemical shifts of the oligoleucine 1 in this solvent. All the NH peaks of 1 show large downfield shifts with increasing peptide concentration, indicating that all the NH protons of 1 are intermolecularly hydrogen bonded at these concentrations.[14] On the other hand, only the NH peaks of two Leu residues in the N-terminal dipeptide sequences of 8' and 9' show large downfield shifts with increasing concentration from 0.01 to 0.1 or 0.08 M, respectively. Other peaks of the peptides show almost constant chemical shifts in these concentrations. This result indicates that only the two NH protons of the Leu residues in the N-terminal dipeptide sequences participate in intermolecular hydrogen bond formation and the other NH protons are intramolecularly hydrogen bonded or fully solvated.[14] The almost constant chemical shifts of the Aib(3) NH peak of 8' and the Leu(3) NH peak of 9' at these concentrations indicate that they are not involved in intermolecular hydrogen bonding. Examination with CPK models indicates that these NH protons cannot readily form intermolecular hydrogen bonds when 8' and 9' are assumed to adopt an $\alpha$-helical structure.

In $CDCl_3$, the Aib(1) and Leu(2) NH peaks of 5' also showed downfield shifts with increasing peptide concentration from 0.01 to 0.03 M. This is in contrast to the almost constant chemical shifts observed for the other NH peaks of 5' at these concentrations. The result indicates that only the Aib(1) and Leu(2) NH peaks of 5' are intermolecularly hydrogen bonded. It is demonstrated from CPK models that intermolecular hydrogen bonding of the Aib(1) and Leu(2) NH protons is permitted when 5' is assumed to adopt a $3_{10}$-helical structure.

**Registry No.** 1, 92782-17-7; 2, 92782-18-8; 3, 100642-89-5; 3', 100643-01-4; 4, 100642-90-8; 4', 100643-02-5; 5, 100642-91-9; 5', 107658-07-1; 6, 107658-04-8; 7, 107658-05-9; 7', 107658-08-2; 8, 107658-06-0; 8', 107658-09-3; 9', 100643-04-7; 10', 99593-07-4; 11', 99593-08-5; BOC-Leu-Aib-OH, 72485-34-8; BOC-$Leu_2$-Aib-OH, 107658-10-6; H-$Leu_2$-OBzl·HCl, 71145-33-0; H-$Leu_4$-OBzl·HCl, 107658-11-7.

## References and Notes

(1) Part 5 of "Peptides and Proteins Containing $\alpha,\alpha$-Disubstituted $\alpha$-Amino Acid Residues". Part IV: Narita, M.; Ishikawa, K.; Kudo, K.; Endo, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 3125. Abbreviations used are the following: Aib, $\alpha$-aminoisobutyric acid residue; Leu, L-leucine residue; Boc, *tert*-butoxycarbonyl; OBzl, benzyl ester.

(2) Paterson, Y.; Rumsey, S. M.; Benedetti, E.; Nemethy, G.; Scheraga, H. A. *J. Am. Chem. Soc.* **1981**, *103*, 2947 and references cited therein.

(3) Toniolo, C.; Bonora, G. M.; Bavaso, A.; Benedetti, E.; Blasio, B. D; Pavone, V.; Pedone, C. *Biopolymers* **1983**, *22*, 205 and references cited therein.

(4) Narita, M.; Ishikawa, K.; Nakano, H.; Isokawa, S. *Int. J. Peptide Protein Res.* **1984**, *24*, 14.

(5) Toniolo, C.; Bonora, G. M.; Palumbo, M.; Peggion, E. *Biopolymers* **1978**, *17*, 1713.
(6) Bonora, G. M.; Maglione A.; Toniolo, C. *Polymer* **1974**, *15*, 767.
(7) Narita, M.; Doi, M.; Sugasawa, H.; Ishikawa, K. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1473.
(8) Narita, M.; Ishikawa, K.; Sugasawa, H.; Doi, M. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1731.
(9) Narita, M.; Chen, J. Y.; Sato, H.; Kim, Y. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 2494.
(10) Narita, M.; Fukunaga, T.; Wakabayashi, A.; Ishikawa, K.; Nakano, H. *Int. J. Peptide Protein Res.* **1984**, *23*, 306.
(11) Narita, M.; Ishikawa, K.; Chen, J. Y.; Kim, Y. *Int. J. Peptide Protein Res.* **1984**, *24*, 580.
(12) Narita, M.; Ishikawa, K.; Kudo, K.; Endo, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 3125.
(13) Asakura, T. *Makromol. Chem., Rapid Commun.* **1980**, *1*, 227.
(14) Pysh E. S.; Toniolo, C. *J. Am. Chem. Soc.* **1977**, *99*, 6211.
(15) Stimson, E. R.; Zimmerman, S. S.; Scheraga, H. A. *Macromolecules* **1977**, *10*, 1049.
(16) Urry, D. W.; Cunningham, W. D.; Ohnishi, T. *Biochemistry* **1974**, *13*, 609.
(17) Iqbal, M.; Balaram, P. *J. Am. Chem. Soc.* **1981**, *103*, 5548.
(18) Zimmerman, S. S.; Scheraga, H. A. *Biopolymers* **1977**, *16*, 811.
(19) Vijayakumar, E. K. S.; Balaram, P. *Biopolymers* **1983**, *22*, 2133.
(20) Vijayakumar, E. K. S.; Balaram, P. *Tetrahedron* **1983**, *39*, 2725.
(21) Nagaraj, R.; Shamala, N.; Balaram, P. *J. Am. Chem. Soc.* **1979**, *101*, 16.
(22) Balaram, H.; Prasad B. V. V.; Balaram, P. *J. Am. Chem. Soc.* **1983**, *105*, 4065.
(23) Toniolo, C.; Bonora, G. M.; Barone, V.; Bavoso, A.; Benedetti, E.; Blasio, B. D.; Grimaldi, P.; Lelj, F.; Pavone, V.; Pedone, C. *Macromolecules* **1985**, *18*, 895.
(24) Raj, P. A.; Balaram, P. *Biopolymers* **1985**, *24*, 1131.
(25) Bystrov, V. F.; Ivanov, V. T.; Portnova, S. L.; Balashova, T. A.; Ovchinnikov, Y. A. *Tetrahedron* **1973**, *29*, 873.
(26) Asakura, T. *Makromol. Chem.* **1981**, *182*, 1135.

# Heteronuclear *J*-Resolved Solid-State NMR of Filled Natural Rubber

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ABSTRACT: Heteronuclear *J*-resolved $^{13}C$ NMR spectra of solids can be obtained by the combined use of magic angle spinning and proton multiple-pulse decoupling. In a cured, carbon black filled, natural rubber, MAS alone appears to be sufficient to reveal scalar $^{13}C$–$^{1}H$ couplings, meaning that there must be extensive molecular motions. The nature of these motions are discussed by making a comparison to the motions in the plastic crystal adamantane. It is concluded that susceptibility effects due to the presence of the filler play an important role in the $^{13}C$ line width.

## Introduction

It is known that *J*-resolved $^{13}C$ NMR spectra of solids can be obtained by the combined use of magic angle spinning (MAS) and proton multiple-pulse decoupling.[1] The effect of multiple-pulse decoupling is to average the proton homonuclear dipolar interaction $H_{D,II}$[3] and thus render the $^{13}C$–$^{1}H$ heteronuclear dipolar interaction $H_{D,IS}$ inhomogeneous so that it can be averaged by magic angle spinning.[4] Then, in contrast with standard heteronuclear decoupling, the isotropic carbon–proton coupling $H_{J,IS}$ remains present. It is scaled however by multiple-pulse decoupling.[3]

Until now most applications of this technique were on spherical organic molecules, like camphor and adamantane,[1a,b,c,2] which, at room temperature, are in a "plastic" crystalline state where the molecules reorient rapidly around their symmetry axes and even diffuse through the lattice. This results in considerable averaging of the dipolar interactions. The remaining part of the homonuclear dipolar interactions can then be averaged by proton multiple-pulse decoupling. It must be noted, however, that Miura et al.[1d] recently succeeded in resolving *J* multiplets in rigid solids using a double-bearing probe with an accurate setting of the rotation axis and a short multiple-pulse cycle (i.e., short 90° pulses).

Early proton NMR studies of rubber samples by Gutowsky et al.[5] show very narrow lines at room-temperature, indicating that dipolar interactions are averaged by $CH_3$ rotation and segmental motions. Duch and Grant[6] succeeded in getting direct $^{13}C$ spectra of natural rubber using conventional high-resolution techniques. Thus natural rubber seems a good candidate for *J*-resolved spectroscopy in the solid state. It appears that even for cured, carbon black filled rubber MAS alone is sufficient to obtain heteronuclear *J*-resolved spectra, showing that there are extensive molecular motions present. The nature of these motions are discussed by comparing a series of spectra, obtained with and without MAS and dipolar decoupling, to those of the well-studied plastic crystal adamantane.

## Experimental Section

**Materials.** Adamantane (Gold Label) was obtained from Aldrich Chemical Co. The rubber samples were prepared from Standard Malaysian rubber and contained 50 parts per hundred HAF carbon black. The samples were cured with 2.5 parts per hundred sulfur. The average molecular weight between cross-links is of the order of 10 000.

**Measurements.** Spectra were recorded on a Bruker CXP 300 (carbon frequency, 75.4 MHz) and on a home-built 180-MHz spectrometer (carbon frequency, 45.3 MHz). On the CXP 300 spectra were obtained in a double-bearing CP–MAS probe operating with 4.5-$\mu$s 90° pulses. The MAS experiments were carried out with spinning speeds between 2 and 3 kHz.

## Results and Discussion

Figure 1 shows the heteronuclear 2D *J* spectrum of cured, carbon black filled, natural rubber at room temperature obtained with the proton-flip experiment.[7] The

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