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NMR Study of Amino Acids and Peptides; Glycine and Valine Peptides

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The PMR spectra of 22 amino acids and peptides were measured in a D_2O solution. From the change in the NMR parameters in amino acids and their di- and tripeptides, it has been concluded that a tripeptide is a good model for polypeptides. The chemical shift of the α-proton of an amino-acid residue in tripeptide can be obtained from those of amino acid and dipeptide. The spin-spin coupling constant of an amino acid grows larger, and the population of a *trans* rotamer becomes larger, as its amino (or carboxyl) group forms a peptide bond. The PMR spectra of two diasteroisomers of valyl-valyl-valine were also measured, and the influences of the configuration of an amino-acid residue on the NMR parameters of its peptides were investigated.

The high-resolution NMR technique has been applied to the studies of oligopeptides and proteins. The PMR spectra of simple amino acids and dipeptides have been measured in an aqueous medium by Takeda and Jardetzky,[1] while Bovey and Tiers,[2] employing trifluoroacetic acid as the solvent, have investigated the relations between the NMR parameters and the peptide structures.

Several works[3-5] have also appeared trying to investigate the structures of proteins on the basis of the PMR spectra of amino acids and peptides. Spectrometers operating at 220 MHz have recently become available and have been used for this purpose.[4-5]

Sheinblatt[6] proposed a method for determing the sequence of amino acid residues in di- and tripeptides; he also measured[7] the rate of the exchange of the peptide hydrogen of glycylglycine. Morlino and Martin[8] reported glycylmethylene splitting due to a chemical-shift nonequivalence in amino acid-glycyl dipeptides. Kim and Martell[9] studied glycine dipeptides and their complexes with copper and nickel in an aqueous solution. Systematic analyses of the chemical shifts of the oligopeptides have also been reported by some workers. Gorkom[10] tried to distinguish a long-range shielding effect on the chemical shifts. For the chemical-shift calculation of an amino acid in a given polypeptide, a simple additive law has been found to hold by Nakamura and Jardetzky.[11-12] Beecham and Ham[13] reported the chemical-shift change of glycine and L-leucine residues in seversl peptides.

In this investigation, we have systematically investigated the changes in the NMR parameters (chemical shifts and spin-spin coupling constants) in amino acids and their di- and tri-peptides; we will here report a rather large difference in the vicinal coupling constants of amino-acid residues. From these changes, we deduced the structures of the peptides (the conformations of the side chains); we found that a tripeptide is a good model for polypeptides from the point of view of NMR parameters.

Experimental

The common amino acids and peptides were purchased

1) M. Takeda and O. Jardetzky, *J. Chem. Phys.*, **26**, 1346 (1957).
2) F. A. Bovey and G. V. D. Tiers, *J. Amer. Chem. Soc.*, **81**, 2870 (1958).
3) M. Mandel, *J. Biol. Chem.*, **240**, 1586 (1965).
4) B. Bak, C. Dambmann, F. Nicloaisen, E. J. Pedersen and N. S. Bhaccha, *J. Mol. Spect.*, **26**, 78 (1968).
5) C. C. McDonald and W. D. Phillips, *J. Amer. Chem. Soc.*, **91**, 1513 (1969).
6) M. Sheinblatt, *ibid.*, **88**, 2845 (1966).
7) M. Sheinblatt, *ibid.*, **87**, 572 (1965).
8) V. J. Morlino and R. B. Martin, *ibid.*, **89**, 3107 (1967).
9) M. K. Kim and A. E. Martell, *ibid.*, **91**, 872 (1969).
10) M. van Gorkom, *Tetrahedron Lett.*, **1966**, 5433.
11) A. Nakamura and O. Jardetzky, *Proc. Natl. Acad. Sci. U.S.*, **58**, 2212 (1967).
12) A. Nakamura and O. Jardetzky, *Biochem.*, **7**, 1226 (1968).
13) A. F. Beecham and N. S. Ham, *Tetrahedron*, **24**, 2773 (1968).

from Wako Pure Chemical Ind., Ltd. (Special Grade), or Tokyo Kasei Ind., Ltd. (GR). The L-valyl-L-valine, L-valyl-glycyl-glycine, L-valyl-L-valyl-D-valine, L-valyl-L-glutamic acid, L-valyl-L-valyl-L-glutamic acid, L-alanyl-glycine, and L-alanyl-glycyl-glycine were from the Ajinomoto Co., Ltd., while the glycyl-L-valyl-glycine, L-valyl-glycine, and L-valyl-L-valyl-L-valine were from the Institute for Protein Research, Osaka University; the D_2O was from E. Merck A.G. Darmstadt, "Solvent for NMR Spectroscopy" (99.75D%).

All the chemicals employed were PMR spectroscopically pure and were used without further purification.

The NMR spectra were obtained with samples prepared by dissolving 25 mg of the amino acids or peptides in 0.5 m*l* of D_2O, with 5 mg of sodium 2,2-dimethyl-2-silapentane 5-sulfonate(DSS) as a reference substance.

However, L-valyl-L-valine, L-valyl-L-valyl-L-valine, L-valyl-L-valyl-D-valine, L-valyl-L-valyl-L-glutamic acid, glycyl-glycine, and glycyl-glycyl-glycine were not soluble to that extent, and so about 2% D_2O solutions were prepared with these peptides. All the peptides are considered to have zwitter-ion forms in these solutions.

The NMR spectra were measured with a 60 MHz or a 100 MHz NMR spectrometer (JNM 3H-60 and JNM 4H-100) at a probe temperature of 29 ± 1°C. The chemical shift values were read from the frequency counter (Universal Counter MF-47 A, Anritsu Electric Co., Ltd.); the accuracy of the measurements is estimated to be within ±0.05 Hz. All the chemical shifts were

TABLE 1. NMR PARAMETERS FOR AMINO ACIDS AND PEPTIDES MEASURED IN D_2O WITH DSS (60 MHz)

	J_{vic} (Hz)	δ_{CH_α} (Hz from DSS)	δ_{CH_3} (Hz from DSS)	δ_{CH_2} (Hz from DSS)
Glycine	—	—	—	213.6
Glycyl-glycine	—	—	—	(1) 231.4
(1) (3)	—	—	—	(3) 228.9
Glycyl-glycyl-glycine	—	—	—	(1) 235.4
(1) (2) (3)	—	—	—	(2) 243.7
	—	—	—	(3) 228.2
L-Alanine	7.2	227.8	88.9	—
Glycyl-L-alanine	7.2	251.3	81.9	230.6
L-Alanyl-glycine	7.2	248.5	93.6	227.5(q)
DL-Alanyl-DL-alanine	(1) 7.2	246.7	92.6	—
(1) (3)	(3) 7.2	250.8	82.8	—
DL-Analyl-glycyl-glycine	7.2	250.0	93.8	(2) 241.4
(2) (3)				(3) 226.8
L-Valine	4.3	215.8	57.7:60.9	—
Glycyl-L-valine	5.5	245.4	52.4:54.7	232.2
L-Valyl-glycine	6.1	228.1	61.2:62.4	228.0(q)
L-Valyl-L-valine	(1) 6.0	233.0	—	—
(1) (3)	(3) 6.5	244.5	—	—
L-Valyl-L-glutamic acid	5.8	230.6	—	—
Glycyl-L-valyl-glycine	6.6	254.6	55.3:56.4	(1) 234.2
(1) (3)				(3) 226.4
L-Valyl-glycyl-glycine	6.0	232.3	62.2	(2) 242.6(q)
(2) (3)				(3) 227.0
L-Valyl-L-valyl-L-valine	(1) 5.8	232.2	—	—
(1) (2) (3)	(2) 8.6	252.5	—	—
	(3) 6.4	242.6	—	—
L-Valyl-L-valyl-D-valine	(1) 5.8	232.7	—	—
(1) (2) (3)	(2) 7.5	257.1	—	—
	(3) 5.9	244.2	—	—
L-Valyl-L-valyl-	(1) 5.8	232.5	—	—
(1) (2)	(2) 8.3	249.7	—	—
L-glutamic acid				
L-Leucine	—	222.5	54.4:59.5	—
Glycyl-L-Leucine	—	251.1	—	229.6
L-Leucyl-glycine	—	241.2	54.7:60.1	227.6
L-Leucyl-glycyl-glycine	—	244.1	55.0:60.2	(2) 241.7
(2) (3)				(3) 230.6

where (q) denotes that it appears as AB quartet.

expressed in δ Hz from DSS at 60 MHz.

Results and Discussion

The results obtained for a series of peptides are presented in Table 1. For a given amino-acid residue in the peptides, the spin-spin coupling constants of the α-proton (H) with the β-H, and its chemical shifts, are designated as J_{vic} and δ_{CH_α} respectively. The chemical shifts of methyl groups for the valine and alanine residues, and the methylene group of glycine (δ_{CH_3} and δ_{CH_2}), are also presented when precise measurements were possible.

In this report, the amino-acid residues are numbered in the following way:

NH_2—A—COOH
(1, 3)

NH_2–A—A-COOH
(1) (3)

NH_2–A—A—A-COOH
(1) (2) (3)

where A denotes the amino-acid residue. Therefore, L-val(1,3) indicates L-valine. L-valyl-L-valine consists of two valine residues, L-val(1) and L-val(3).

1) Chemical Shifts. Generally speaking, the chemical shifts of amino-acid protons go to a lower field when peptide bonds are formed. Because an amino acid has both an amino and a carboxyl group, two types of dipeptides can exist; a rather distinct difference in NMR parameters is observable between them.

Let us now summarize the chemical shifts changes of the α-H of L-valine, paying special attention to the δ_{CH_α} of L-val(2) in tripeptides, in Fig. 1. The δ_{CH_α}'s of the amino-acid(1)(L-valine, L-leucine and L-alanine) residues in di- and tripeptides are shown in Fig. 2. Figure 3 shows the δ_{CH_2}'s of glycine peptides. From these figures, the δ_{CH_α} (or δ_{CH_2})'s of a given amino-acid residue in a certain position of the oligopeptides can be seen to have a tendency to cluster within a small range of chemical shift values. The mean values obtained for δ_{CH_α} and δ_{CH_2} are shown in Table 2. The difference, $\Delta\delta$, between the δ_{CH_α} of an amino acid residue in the peptide and that of the free amino acid is also presented in Table 2.

The δ_{CH_2}'s of glycine dipeptides have been reported to be 228.8, and 227.2 Hz from the DSS values for gly(1) and gly(3) respectively.[11] Our results agree fairly well.

A rather large discrepancy is found between

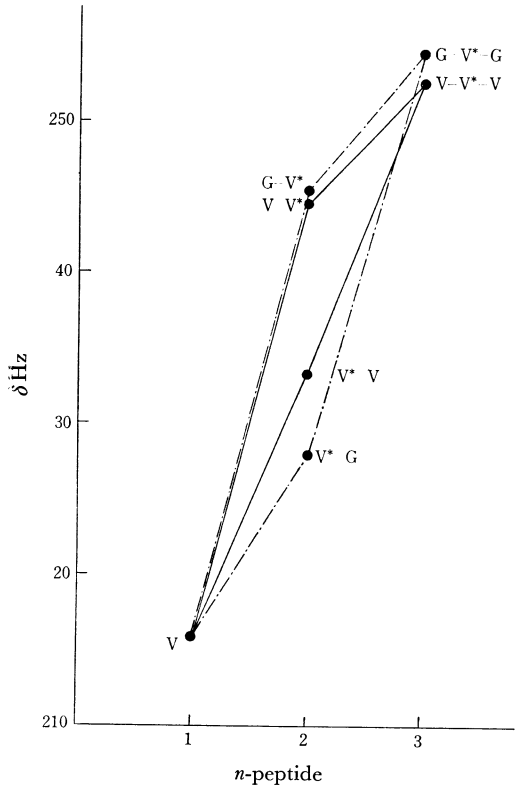

Fig. 1. The change of chemical shifts (δ_{CH_α}) of L-valine residues.
(δ_{CH_α} of the valine residue with an asterisk is shown. G; Glycine, V; Valine)

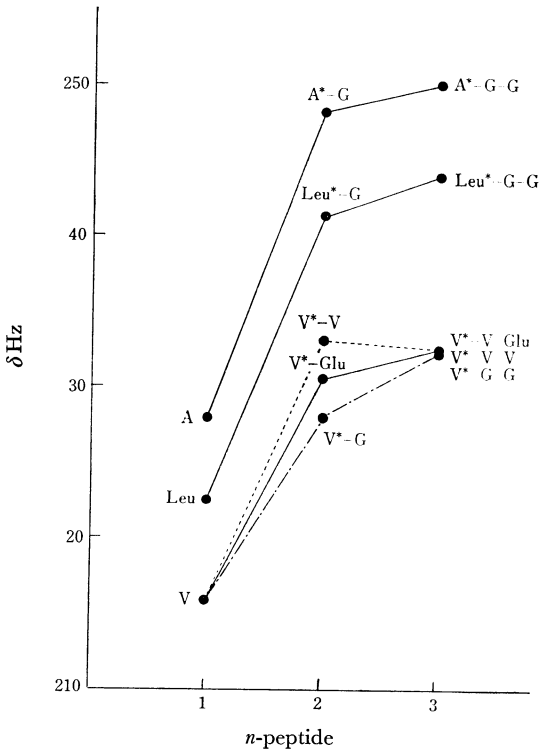

Fig. 2. The change of chemical shifts (δ_{CH_α}) of α-amino acid residues.
(δ_{CH_α} of the amino acid residue with an asterisk is shown. A; Alanine, G; Glycine, Glu; Glutamic acid, Leu; Leucine, V; Valine)

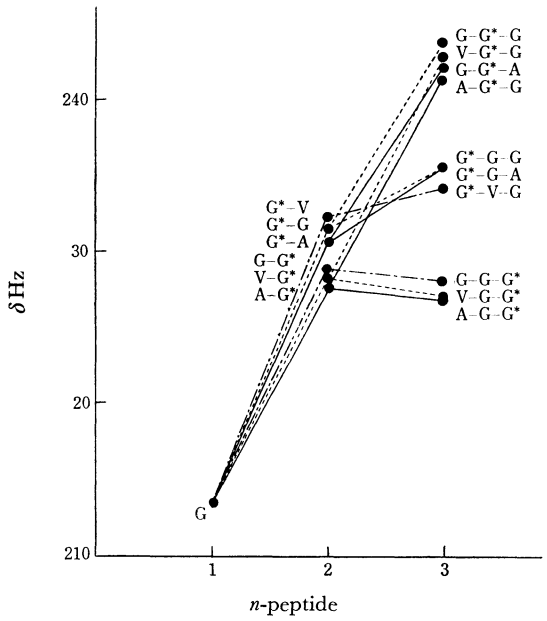

Fig. 3. The change of chemical shifts (δ_{CH_2}) of glycine residues.
(δ_{CH_2} of glycine residue with an asterisk is shown. A; Alanine, G; Glycine, V; Valine)
Values for glycyl-glycyl-alanine were from Ref. 10

TABLE 2. THE AVERAGE CHEMICAL SHIFT VALUES OF THE AMINO ACID RESIDUES IN *n*-PEPTIDES (δ_{CH_α} and δ_{CH_2}) AND THE DIFFERENCES IN THE CHEMICAL SHIFTS WITH RESPECT TO THE FREE AMINO ACID ($\Delta\delta$)

δ_{CH_α} of L-valine peptides

n-Peptides	Amino acid residue	δ_{CH_α} (Hz from DSS)	$\Delta\delta$ (Hz)	Number of cases
1	L-val (1,3)	215.8	0	1
2	L-val (1)	230.6	14.8	3
	L-val (3)	245.0	29.2	2
	L-val (1)	232.4	16.6	4
3	L-val (2)	253.5	37.7	4
	L-val (3)	242.6	27.6	1

δ_{CH_2} of glycine peptides

n-Peptides	Amino acid residue	δ_{CH_2} (Hz from DSS)	$\Delta\delta$ (Hz)	Number of cases
1	Gly (1,3)	213.6	0	1
2	Gly (1)	231.4	17.8	3
	Gly (3)	228.1	14.5	3
3	Gly (1)	234.8	21.2	2
	Gly (2)	242.6	29.0	3
	Gly (3)	227.1	13.5	4

δ_{CH_α} and δ_{CH_2}. That is, the δ_{CH_α} of L-val(3) does not agree with the δ_{CH_2} of gly(3), while the δ_{CH_α} of L-val(1,3) and the δ_{CH_2} of gly(1,3), and those of L-val(1) and gly(1), of dipeptides coincide. This discrepancy can not be explained in terms of the difference in electronegativity; this suggests that the electric field and the magnetic anisotropy effect play an important role in determining the chemical shifts of the peptides.

A rough correlation is found to exist between these chemical shift values; the δ_{CH_α} (or δ_{CH_2}) of the A(1) and A(3) of a given amino acid is almost unchanged through di- and tripeptide, and the δ_{CH_α} (or δ_{CH_2}) of A(2) can easily be estimated by adding the sum of the $\Delta\delta$'s of A(1) and A(3) to the δ_{CH_α} (or δ_{CH_2}) of A(1,3). From this observation, it may be concluded that a tripeptide can be a good model for polypeptides. This conclusion is supported by a comparison of the δ_{CH_2} of the central methylene group of triglycine (243.7 Hz) with the reported[12] δ_{CH_2} value of that of the pentaglycine (240.0 Hz).

2) Spin-spin Coupling Constants. The spin-spin coupling constants of L-valine change when it forms a peptide bond. Figures 4 and 5 show

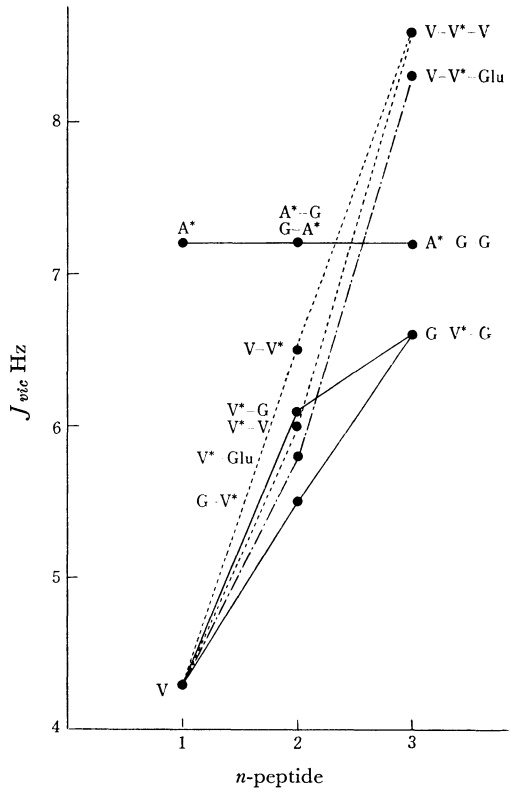

Fig. 4. The change of spin-spin coupling constants (J_{vic}) of L-valine and alanine residues.
(J_{vic} of amino acid residue with an asterisk is shown. A; Alanine, G; Glycine, Glu; Glutamic acid, V; Valine)

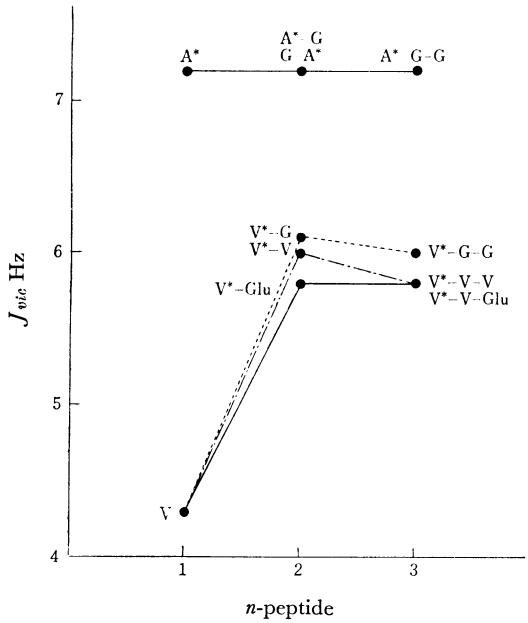

Fig. 5. The change of spin-spin coupling constants (J_{vic}) of L-valine and alanine residues. (J_{vic} of amino acid residue with an asterisk is shown. A; Alanine, G; Glycine, Glu; Glutamic acid, V; Valine)

the J_{vic}'s of L-valine residues. From these figures, rather clear trends are observable; the J_{vic} for L-val(1,3) is 4.3 Hz. The averages of J_{vic} for L-val(1) and L-val(3) in dipeptides are 6.0 and 6.0 Hz respectively, while that for L-val(2) in tripeptides is 7.8 Hz. However, the J_{vic}'s for L-val(1) and L-val(3) hardly change from dipeptide to tripeptide; they are all about 6.0 Hz. That is, the J_{vic} for L-val(1,3) grows larger as its amino and carboxyl groups form peptide bonds. The difference in electronegativity between a free amino (or carboxyl) group and that which formed a peptide bond can not be considered[14] to have such a large effect on the J_{vic} of L-valine. Moreover, the J_{vic} of L-alanine is considered to be influenced by the difference in electronegativity alone and not by the population change in the rotamers,[15-16] because the three rotamers are equivalent. The observed J_{vic} of L-alanine is 7.2 Hz, and it does not change at all when it forms a peptide bond with glycine. From these considerations, it can be concluded that the difference in electronegativity between a free amino (or carboxyl) group and that which formed a peptide bond does not influence the J_{vic} of a given amino-acid residue. Therefore, the J_{vic} of L-valine residues in oligopeptides is mainly affected by the population change of the rotamers. Employing 13.6 and 2.6 Hz as the J_{vic} values of the *trans* and *gauche* rotamer (J_t and J_g)[17], we calculated the populations of the *trans* and *gauche* rotamers (P_t and P_g).

TABLE 3-1. THE POPULATIONS AND THE RELATIVE ENERGIES OF THE ROTATIONAL ISOMERS FOR L-VALINE DERIVATIVES

	J_{vic} (Hz)	P_t	P_g	ΔE (kcal/mol)
L-Val*	4.3	0.15	0.85	−0.63
L-Val*-Gly	6.1	0.31	0.69	−0.06
L-Val*-L-Val	6.0	0.31	0.69	−0.07
L-Val*-L-Glu	5.8	0.29	0.71	−0.11
L-Val*-Gly-Gly	6.0	0.31	0.69	−0.07
L-Val*-L-Val-L-Val	5.8	0.29	0.71	−0.11
L-Val*-L-Val-L-Glu	5.8	0.29	0.71	−0.11

Results for the valine residue with an asterisk are shown.

TABLE 3-2. THE POPULATIONS AND THE RELATIVE ENERGIES OF THE ROTATIONAL ISOMERS FOR L-VALINE DERIVATIVES

	J_{vic} (Hz)	P_t	P_g	ΔE (kcal/mol)
L-Val*	4.3	0.15	0.85	−0.63
Gly-L-Val*	5.5	0.26	0.74	−0.21
L-Val*-L-Val	6.0	0.31	0.69	−0.07
L-Val-L-Val*	6.5	0.36	0.64	0.07
Gly-L-Val*-Gly	6.6	0.36	0.64	0.07
L-Val-L-Val*-L-Val	8.6	0.55	0.45	0.53
L-Val-L-Val*-L-Glu	8.3	0.52	0.48	0.47

Results for the valine residue with an asterisk are shown.

The relative energies of *gauche* rotamers with respect to the *trans* rotamer (ΔE) were obtained, assuming that the two *gauche* rotamers have equal populations. The results are shown in Table 3. It can clearly be seen that the successive formations of peptide bonds to both ends of a valine (amino and carboxyl group) cause the population of the *trans* rotamer to grow larger, thus enlarging the dihedral angle. This phenomenon accords with the one observed hitherto[14] and may be explained in terms of the steric hindrance of the functional groups.

14) R. J. Abraham and K. G. R. Pachler, *Mol. Phys.*, **7**, 165 (1963).

15) H. Ogura, Y. Arata and S. Fujiwara, *J. Mol. Spect.*, **23**, 76 (1967).

16) J. W. Emsley, J. Feeney and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 1, Pergamon Press, New York (1965), p. 561.

17) K. G. R. Pachler, *Spectrochim. Acta*, **20**, 581 (1964).

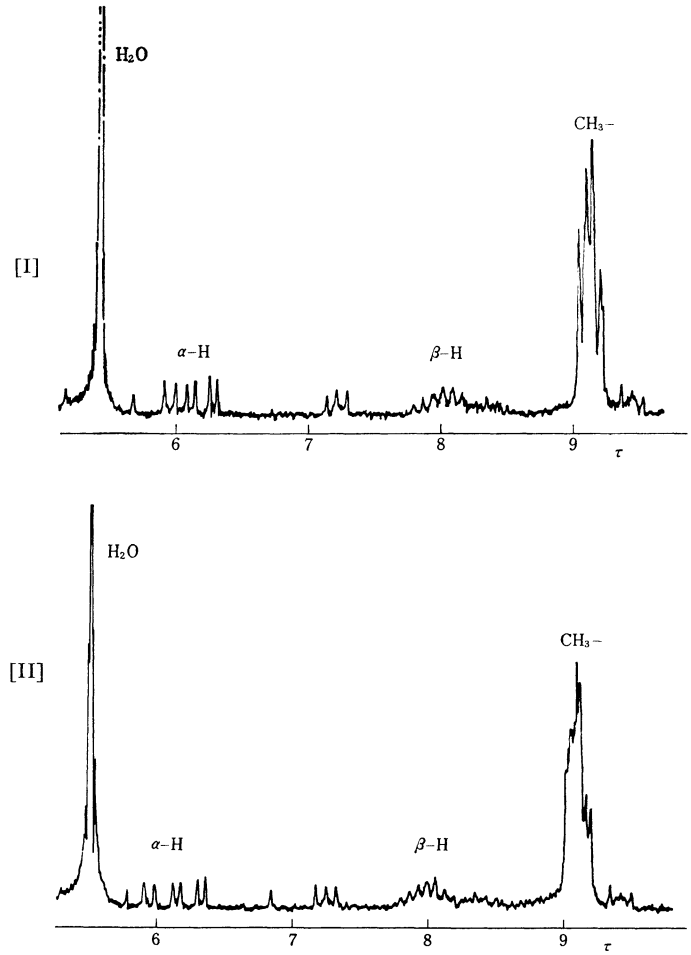

Fig. 6. NMR spectra of L-val-L-val-L-val [I] and L-val-L-val-D-val [II] in D_2O solution (100 MHz)

TABLE 4. THE NMR PARAMETERS, POPULATIONS AND THE RELATIVE ENERGIES OF ROTATIONAL ISOMERS FOR VALINE TRIPEPTIDES

	L-Val (1)	L-Val (2)	D-Val (3)
L-Val-L-Val-L-Val (1) (2) (3) [I]	δ_{CH_α} 232.2 (Hz from DSS)	δ_{CH_α} 252.5 (Hz from DSS)	δ_{CH_α} 242.6 (Hz from DSS)
	J_{vic} 5.8 (Hz)	J_{vic} 8.6 (Hz)	J_{vic} 6.4 (Hz)
	P_t 0.29	P_t 0.55	P_t 0.35
	P_g 0.71	P_g 0.45	P_g 0.65
	ΔE −0.12 (kcal/mol)	ΔE 0.53 (kcal/mol)	ΔE 0.03 (kcal/mol)
	L-Val (1)	L-Val (2)	D-Val (3)
L-Val-L-Val-D-Val (1) (2) (3) [II]	δ_{CH_α} 232.7 (Hz from DSS)	δ_{CH_α} 257.1 (Hz from DSS)	δ_{CH_α} 244.2 (Hz from DSS)
	J_{vic} 5.8 (Hz)	J_{vic} 7.5 (Hz)	J_{vic} 5.9 (Hz)
	P_t 0.29	P_t 0.45	P_t 0.30
	P_g 0.71	P_g 0.55	P_g 0.70
	ΔE −0.12 (kcal/mol)	ΔE 0.29 (kcal/mol)	ΔE −0.09 (kcal/mol)

In conclusion, the J_{vic} of a given amino acid residue is found to be influenced by the one with which it is combined directly; therefore, tripeptide is a good model for polypeptides.

3) The Influence of the Configuration of an Amino Acid Residue on the NMR Parameters of Peptides. Out of the eight diastereoisomers of valyl-valyl-valine, we have chosen two isomers, L-val-L-val-L-val [I] and L-val-L-val-D-val [II], and have studied the influence of optically active residues on the NMR parameters of these diastereoisomers. Figure 6 shows the NMR spectra of I and II at 100 MHz. The results, together with *Pt*, *Pg*, and the ΔE values calculated on the assumptions described above, are shown in Table 4. The influence of the configuration of val(3) is observable in the large differences between the NMR parameters of the val(2)'s of the two isomers.

With I, L-val(2) appears at a higher field and have a larger J_{vic} than L-val(2) of II. However, the NMR parameters of the L-val(1)'s of the two isomers agree very well. These facts show that the configuration of a given amino-acid residue influences its vicinal amino acid residues' NMR parameters and their conformations. However, the influence is very localized and change only the NMR parameters of the amino acid residues combined directly. Therefore, it can be concluded that tripeptide can be a good model compound for polypeptides, even when one considers the influence of the optical isomerism.

The authors are grateful to Professor Shizuo Fujiwara and to Dr's. Yoji Arata and Takeo Yamamoto of the University of Tokyo for stimulating and helpful discussions.