interaction to rationalize this result because in **15**, where the two diasterotopic methyls differ in chemical shift by 2.6 ppm, *a 1,5 CH_3–CH_3 interaction is impossible.* In both **11** and **15**, despite the uncertainties of the average conformation we have deduced, we note that the sterically crowded C-5 methyl in **11** is the upfield one, and in **15** the C-8 methyl (gauche to two carbons, 3 and 7) is upfield from C-6 (gauche only to C-7), both manifestations of the familiar γ effect. In **10** the two diasterotopic methyls are only 0.9 ppm apart and the apparently more crowded (6) appears at low field.

Conclusion

Valuable information on the average solution conformations of acyclic molecules can be obtained by the use of ^1H and ^{13}C shift reagents. The reagents are complementary, but Yb(dpm)$_3$ and ^{13}C spectroscopy are more readily utilized than Eu(dpm)$_3$ and proton spectroscopy. Great caution must be used in interpreting the results because the shift reagent can distort the conformation of the substrate being examined. Diastereotopic atoms can be assigned with assurance.

Acknowledgment. Support of this work through grants from the National Institutes of Health (GM-10224-11), the National Science Foundation, and the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged. We would also like to thank Professors Paul A. Dobosh and John Durso for help with computer programs.

Supplementary Material Available. Three tables of data will appear following these pages in the microfilm edition of this volume of the journal. Table I gives the dihedral angles and torsion angles used to define the conformations of the 18 alcohols as well as the lanthanide–hydrogen and lanthanide–carbon internuclear distances and the lanthanide–oxygen–atom angles for the 21 atoms in each alcohol. Tables II and III give the proton and carbon chemical shifts and assignments for a 1:1 mole ratio of Eu(dpm)$_3$ to alcohol, the calculated geometric factor using the conformations from Table I, and the calculated correlation coefficient for the geometric factor *vs.* induced shift. Photocopies of the supplementary material from this paper only or microfilm (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-1471.

Conformational Aspects of Polypeptide Structure. XLV. Nuclear Magnetic Resonance Study of Trans–Cis Isomerization in an *N*-Methyl-L-alanine Derivative

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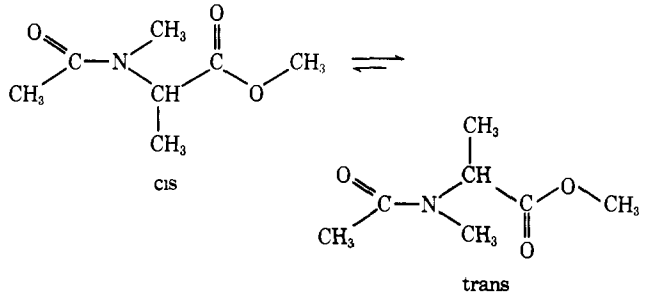
Abstract: Trans–cis isomerism of *N*-acetyl-*N*-methyl-L-alanine methyl ester was studied by using nmr spectroscopy. Kinetic as well as thermodynamic parameters for trans–cis dynamic exchange in various solvents were obtained from computer simulations of the observed resonances at different temperatures. The estimated activation energies as well as enthalpy differences between the two isomers in various solvent systems are similar to those obtained for the other N,N-disubstituted amides. The effects of solvents on the chemical nonequivalence between the trans and cis isomers are also discussed.

Because of the restricted rotation about the C–N amide bond, N-substituted amides exist in two different rotational isomers. During the past decades, nmr spectroscopy has been used to characterize the nature of the restricted rotations of N-alkylated derivatives of formamides as well as acetamides.[2–5] Most of the studies were concerned with cases of equal populations for which kinetic treatments are relatively simple. However, in a few of the amide systems having different amide substituents, nonequivalent populations of the rotational isomers were found.[6–8]

The existence of the distinctive, nonequivalent trans–cis rotational conformers of *N*-acetyl-*N*-methyl-L-alanine methyl ester (I) has been reported by this



N-acetyl-*N*-methyl-L-alanine methyl ester (I)

laboratory.[9] We have also shown that the polymer,

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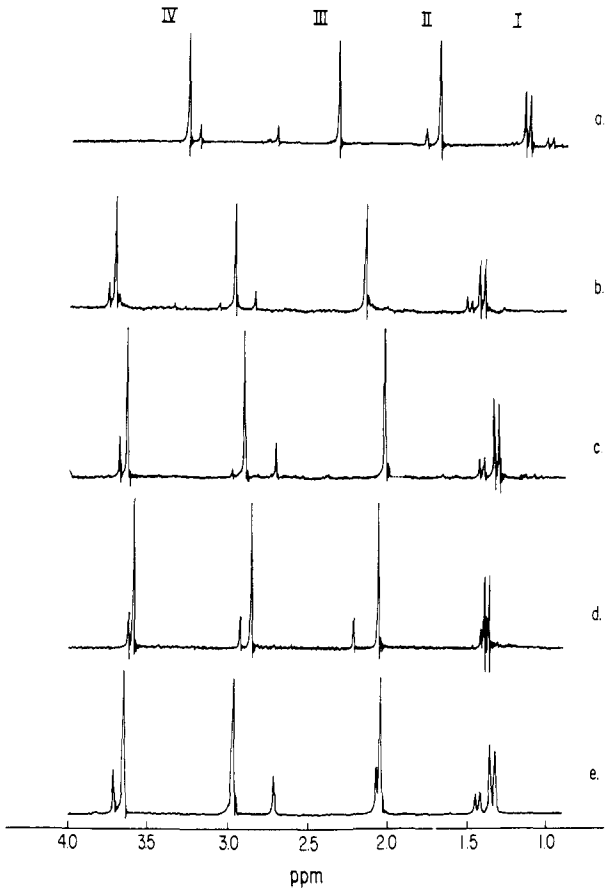

Figure 1. The 220-MHz nmr spectra of *N*-acetyl-*N*-methyl-L-alanine methyl ester in various solvents: (a) benzene-d_6, (b) chloroform-*d*, (c) acetonitrile-d_3, (d) pyridine-d_5, (e) neat liquid, respectively. Peaks I, II, III, and IV arise from C-CH_3, CH_3-CO, N-CH_3, and O-CH_3 proton resonances, respectively. The C_α-H resonance is not included in the figure. Solvent peaks have been eliminated in CD_3CN at (c).

poly(*N*-methyl-L-alanine), exists in an all-trans conformation in some helix-supporting solvents.[10] In this paper, we report a detailed nmr kinetic study of the C–N restricted rotation of this model compound in various solvents and experimental conditions.

Experimental Section

Materials. *N*-Acetyl-*N*-methyl-L-alanine methyl ester (I) was prepared from *N*-acetyl-L-alanine according to the procedure of Goodman, *et al.*[10] Solvents for the nmr study, deuteriochloroform, benzene-d_6, pyridine-d_5, and acetonitrile-d_3 (isotopic purity $\geq$99.5%) were purchased from Wilmad Glass Co., Inc. The concentration of compound I was 10 mg/ml in each solvent system studied. Tetramethylsilane (TMS) was employed as an internal reference.

Method. All the nmr spectra were recorded on a Varian HR 220 spectrometer equipped with a variable-temperature controller. Line widths were measured at the half-height of the resonance peak and error for the measurement of both the chemical shifts and the line width is about $\pm$0.2 Hz. Ethylene glycol or methanol was used to calibrate the probe temperature before and after each measurement and the temperature was found to be constant to within $\pm$0.5°. To ensure the accuracy of the temperature measurements, a proper correction of the calibration chart was applied.[11,12] Sufficiently low power of R. F. field and proper filtering were used to avoid saturation. The relative populations of the two conformations were determined from the measurements of the spectral area in the two corresponding pairs of resonances.

Computer calculations were performed on the CDC 3600 at the University of California, San Diego. The detailed kinetic treatment is described in the later sections.

Results

Solvent Effects. Partial nmr spectra in several solvent systems at room temperature are presented in Figure 1. In all the systems, four sets of resonance peaks were recorded. Peaks I, II, III, and IV appear as pairs of asymmetric doublets and arise from protons of *C*-methyl, acetyl, *N*-methyl, and methyl ester groups, respectively. The *C*-methyl resonance appears as a set of two doublets because of the spin–spin coupling with the C_α proton.

The assignments of the proton resonance of compound I have been previously reported.[9] In each pair of peaks, the major and the minor resonances were assigned to the trans and cis isomers of this compound, respectively. The chemical shifts of each set of resonances vary significantly with the solvent system studied. In benzene, the compound shifts upfield compared to other solvent systems. The chemical shift difference between the nonequivalent resonances arising from the trans and cis conformers ($\Delta_{t,c}$) is also solvent dependent. These differences are summarized in Table I. The

Table I. Chemical Shift Difference ($\Delta_{t,c}$ in Hz)[a,b] between the Two Conformations for *N*-Acetyl-*N*-methyl-L-alanine Methyl Ester in Various Solvents[c,d]

Solvent	C-CH_3	CH_3-CO	N-CH_3	O-CH_3
Benzene-d_6	32	−18	−85	16
Chloroform-*d*	−18	*e*	28	−9
Acetonitrile-d_3	−21	*f*	43	−11
Pyridine-d_5	−5	−33	−15	−7
Neat liquid[g]	−21	−6	56	−13

[a] Measured at 20° in 220-MHz nmr spectroscopy. [b] $\Delta_{t,c}$ refers to the chemical shift difference between the trans and cis resonance of each functional group of this compound. [c] The C_αH resonance is not included in this table. [d] Sample concentration in the solvents, 10 mg/ml. [e] The nonequivalence for this resonance in $CDCl_3$ is indistinguishable. [f] The cis component of CH_3CO in CD_3CN is hidden inside the solvent bands. [g] Neat liquid is compound I, without solvent.

$\Delta_{t,c}$'s of the four sets of resonances in benzene are 32, −18, −85, and 16 Hz, respectively. These values are larger than those observed in the other solvent systems. In Table I, it is shown that $\Delta_{t,c}$ for the *N*-methyl group is the largest among the four sets of resonance peaks in each system, with the exception of pyridine, where $\Delta_{t,c}$ of the acetyl group is larger than that of the *N*-methyl group.

Although the chemical shift for each pair of resonances varies with the solvent system studied, the line width is relatively insensitive to the change of solvent at room temperature. In a given solvent system, the cis–trans ratio for all four pairs of resonances is constant within experimental error. Thus any of the pairs can be used to establish the cis to trans ratio of isomers.

Temperature Effects. In addition to the general broadening of all lines in the spectrum, the chemical shifts of the resonances were observed to be temperature dependent. With increasing temperature, the resonances shift progressively downfield in *benzene* and

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upfield in chloroform. In general, the cis resonance exhibits a broader line width than the corresponding trans peak at a given temperature in a given solvent system. The lines become broader at higher temperatures. At a temperature near 60°, the cis resonance becomes severely broadened. Above the coalescence temperature, only one rather sharp peak from the rapidly interchanging trans and cis forms is observed. However, the coalescence temperature does vary with the particular set of resonances and the solvent considered. The 220-MHz nmr spectra of this compound in pyridine at several temperatures are shown in Figure 2.

The temperature dependence of the relative populations of the trans and cis resonances was also noted. In benzene, the trans/cis ratio, K, decreases from 4.3 at 20° to 3.0 at 60°. In chloroform, K varies from 4.1 to 2.8 over the same temperature range. However, in the other systems, K is relatively insensitive to change over this temperature range (see Table II). At temperatures near the coalescence range (above 60°), the difference of populations in various solvent systems becomes small. From the plot of log K *vs.* $1/T$, the thermodynamic parameters (*i.e.*, enthalpy and entropy) of the cis–trans isomerization can be obtained. These data are shown in Table III. The enthalpy differences between the trans and cis isomers (ΔH) are relatively small in all the solvent systems. ΔH's vary from −2.1 kcal/mol in deuteriochloroform to −0.7 kcal/mol in neat liquid. ΔG's range from −0.6 to ∼−0.9 kcal/mol in these systems. The largest ΔG was obtained when benzene was employed as the solvent.

Table II. The Equilibrium Constant $K = P_t^{a,b}/P_c$ at Various Temperatures for *N*-Acetyl-*N*-methyl-L-alanine Methyl Ester and in Various Solvents

Solvent	Temp, °C	K	Solvent	Temp, °C	K
Benzene-d_6	20	4.3	Chloroform-d	20	4.1
	22	4.2		22	3.9
	36.5	3.9		36.5	3.8
	38	3.6		39	3.6
	51	3.3		43.5	3.2
	59	3.0		48	3.2
Acetonitrile-d_3	20	3.1		54.5	2.8
	36.5	2.9	Pyridine-d_5	20	3.4
	43	3.0		36	3.2
	54.5	2.7		38	3.1
				43	2.9
Neat liquid	20	3.2		48.5	2.8
	38	3.1			
	62	3.0			

[a] The equilibrium constant K was obtained from the averages of the spectral areas of all four sets of resonance peaks: C–CH_3, CH_3–CO, N–CH_3, and O–CH_3. [b] The relative experimental error is approximately ±15%.

Table III. Thermodynamic Parameters from Temperature Dependence of Trans/Cis Ratios (K) of *N*-Acetyl-*N*-methyl-L-alanine Methyl Ester in Various Solvents

Solvent	ΔH, kcal/mol	ΔS, eu	ΔG_{298}, kcal/mol
Benzene-d_6	−1.7 ± 0.4	−2.8 ± 0.6	−0.9 ± 0.6
Chloroform-d	−2.1 ± 0.2	−4.4 ± 0.5	−0.8 ± 0.4
Acetonitrile-d_3	−0.5 ± 0.2	−0.4 ± 0.3	−0.6 ± 0.3
Pyridine-d_5	−0.6 ± 0.3	−0.2 ± 0.2	−0.6 ± 0.3
Neat liquid	−0.7 ± 0.1	−0.1 ± 0.2	−0.7 ± 0.2

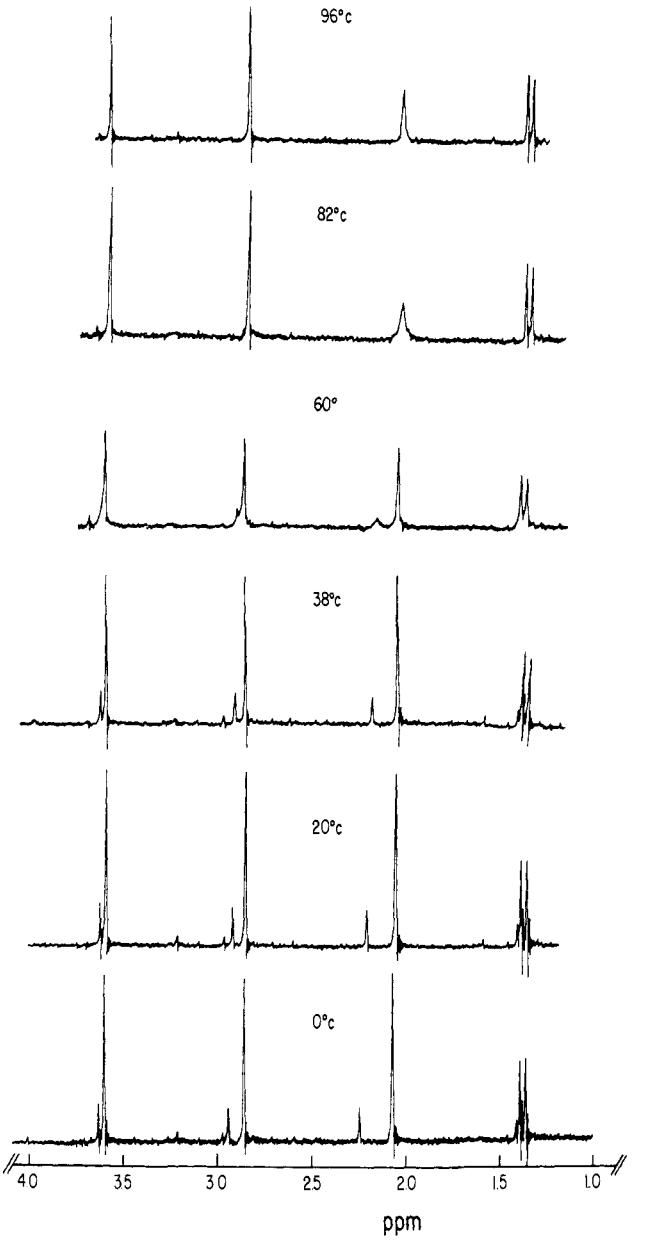

Figure 2. The 220-MHz nmr spectra of *N*-acetyl-*N*-methyl-L-alanine methyl ester in pyridine-d_5 at various temperatures.

Discussion

The asymmetric doublets of each set of resonances of *N*-acetyl-*N*-methyl-L-alanine methyl ester were observed in the various solvents studied, as shown in Figure 1. The chemical shifts of each set of resonances vary significantly with the solvent. This compound exhibits the greatest upfield shift in benzene as compared to other solvents. When the compound is diluted with benzene, the spectra clearly indicate that the resonances shift upfield until a limit of infinite dilution is reached. The peaks associated with the *N*-methyl and acetyl groups of the trans isomer undergo more upfield shifts than the other peaks. Similar distinctive upfield shifts observed for other amide compounds in benzene have been discussed by Hatton and Richards.[13] They suggested that a "collision" complex forms, in which the π-electron cloud of the

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benzene ring interacts with the partial positive charge on the nitrogen and moves as far as possible from the negative charge on the oxygen. LaPlanche and Rogers[14,15] found that the methyl group of an amide trans to the carbonyl–oxygen shows a larger upfield shift on dilution with benzene than the methyl group cis to the carbonyl oxygen. Thus, we are able to confirm our earlier assignment that the major resonance peak arises from the trans amide structure while the minor peak comes from the cis amide structure.

The larger chemical nonequivalence exhibited by the *N*-methyl peak (85 Hz) than by the acetyl peak (18 Hz) also suggests that the benzene molecule is associated with the *N*-methyl group of the amide. Since the stability of "collision" complex is also temperature dependent, it is not surprising that at high temperatures both trans and cis resonances shift downfield in benzene, but not in nonaromatic solvents such as chloroform. The fact that the equilibrium constants in different solvents become similar at higher temperatures suggests that solvations decrease with increasing temperature.

In pyridine, in contrast to benzene, dilution of the compound causes downfield shifts of both the acetyl resonances and the cis *N*-methyl signal. The remaining trans *N*-methyl signal experiences a small upfield shift. It has been suggested[16] that in the pyridine–amide complex, the pyridine is oriented approximately perpendicular with respect to the average plane of the amide group. Such an arrangement would lead to predominantly downfield shifts, as observed, because of the nature of the anisotropy of the magnetic susceptibility of pyridine.

We have shown that the observed asymmetric doublet for each pair of resonances in *N*-acetyl-*N*-methyl-L-alanine methyl ester is attributed to the existence of the two different rotational isomers at room temperature. The gradual increase of line width with increasing temperature (see Figure 2) indicates a slow-to-intermediate rate of interconversion between the two isomers.[17,18] In the limit of slow exchange one should observe broader resonances for minor cis isomers than those observed for the major trans isomer in this temperature range.[17,18] This is indeed in agreement with what we have observed in all solvent systems. For example, in pyridine at temperatures higher than 40°, the line width of the minor peak is 2–3 times broader than that of the major one. Accompanying the changes in line width, the chemical shift separations between the resonances of the two isomers decrease with increasing temperature, especially at temperatures higher than 60°. At 75° the two peaks in each set of resonances are collapsed into one single broad resonance. All of these observations are consistent with the chemical exchange between the trans and cis isomers of this compound.

Since cis–trans isomerism is a chemical exchange process, it is possible to use computer programs to obtain kinetic parameters from the observations. The computer program was written according to the modified Bloch equations for a two-site chemical exchange. Chemical shifts and line widths for the uncoupled, two-site case were simulated according to the total magnetic moment [$M(\omega)$] developed by Gutowsky and Holm,[19,20] where ω_1 is the R.F. field frequency; M_0,

$$M(\omega) = \frac{i\omega_1 M_0[k_t + k_c + \alpha_t P_c + \alpha_c P_t]}{(k_t + \alpha_t)(k_c + \alpha_c) - k_t k_c} \quad (1)$$

the equilibrium magnetic moment; and k_t and k_c are the rate constants for interconversions of the trans and cis conformations, respectively. $\alpha_t = \frac{1}{T_2 t} - i(\omega - \omega_t)$ and $\alpha_c = \frac{1}{T_2 c} - i(\omega - \omega_c)$, where T_2t and T_2c are the transverse relaxation times in the absence of exchange. ω_t and ω_c are the resonance frequencies in radians/sec of the corresponding trans and cis resonances, respectively. P_t and P_c are the relative populations of the trans and cis conformations. The imaginary part (absorption mode) of the total line-shape function was generated by computer under a given set of input parameters. The results were then compared with the observed resonances of this compound.

To ensure the accuracy of the data treatment, several possible contributions to the line width in the absence of exchange should be considered. In the absence of exchange, the line width ($\Delta\nu_0$) of the methyl peaks can be expressed as[21]

$$\Delta\nu_0 = \frac{1}{\pi T_2 \text{inh}} + \frac{1}{\pi T_2 \text{sc}} + \frac{1}{\pi T_2 \text{dd}} \quad (2)$$

These three terms arise from the field inhomogeneity, scalar coupling to ^{14}N in $N-CH_3$ resonances, and dipole–dipole relaxation, respectively. The contribution from field inhomogeneity can be roughly estimated from the line width of TMS, usually 0.4 and 0.6 Hz. The ^{14}N scalar coupling of *N*-methyl protons could contribute to the observed line width if the ^{14}N relaxation time is sufficiently long and scalar coupling is sizable. Shoup, *et al.*,[21] in their study of the restricted rotation of 1-methylcytosine, showed that the contribution can be neglected in the case of two-bond coupling in molecules of this size. The proton dipole–dipole relaxations could contribute 0.1–0.2 Hz at lower temperatures and less at higher temperatures. In our study, after subtracting the inhomogeneity contribution of the observed resonance, the line width varies from 3 to 10 Hz at temperatures higher than 60°. This value is much larger than the possible contribution from dipolar and scalar interactions. Hence, the estimation of these exchange line widths can be justified.

To obtain the accurate kinetic parameters, it is necessary to predetermine the input data such as the chemical shift difference (in the absence of exchange), Δ, of the two corresponding resonance peaks and their relative populations, P_t and P_c, as a function of temperature. When the temperature is well below the

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Table IV. Activation Parameters for the Rotation of *N*-Methyl Group in *N*-Acetyl-*N*-methyl-L-alanine Methyl Ester and in Various Solvents[a,b]

Solvent	E_A, kcal/mol	Log A	$\Delta S^{\ddagger}$, eu	$\Delta H^{\ddagger}_{298}$, kcal/mol	$\Delta G^{\ddagger}_{298}$, kcal/mol
Benzene-d_6	20.5 ± 0.6	15.0 ± 0.4	8.1 ± 1.8	19.9 ± 0.6	17.5 ± 1.1
Chloroform-d	19.9 ± 0.8	14.2 ± 0.5	4.6 ± 2.2	19.3 ± 0.8	17.9 ± 1.4
Acetonitrile-d_3	20.1 ± 1.1	14.5 ± 0.8	5.8 ± 3.6	19.5 ± 1.1	17.8 ± 2.1
Pyridine-d_5	19.6 ± 1.2	14.2 ± 0.8	4.4 ± 3.6	19.0 ± 1.2	17.7 ± 2.2
Neat liquid	18.8 ± 1.2	13.9 ± 0.8	3.1 ± 3.6	18.2 ± 1.2	17.3 ± 2.2

[a] Except in pyridine-d_5, where acetyl resonances were used for computer calculations. [b] Calculated rate constants for the transition from cis → trans exchange.

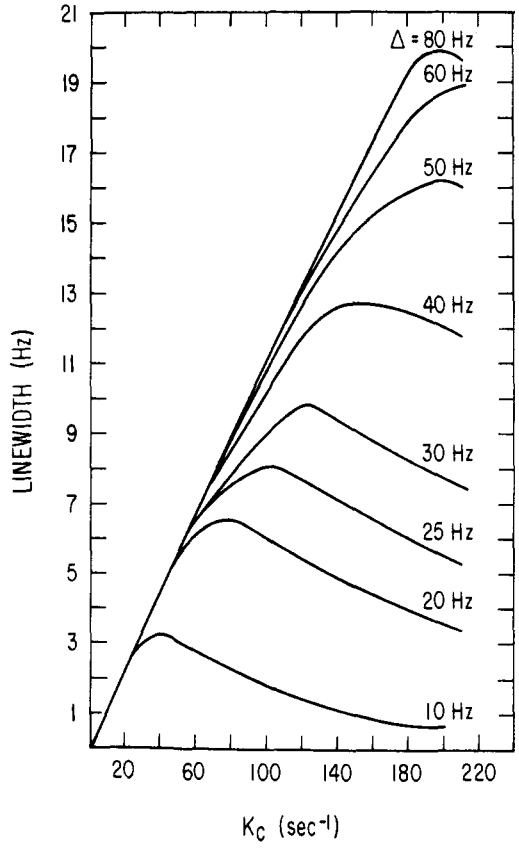

Figure 3. The dependence of the computer-calculated line width for the resonance assigned to the trans isomers as a function of the exchange rate constant, k_c. (Above the coalescence temperature, the calculated line widths represent the combined peaks for both the cis and trans forms.) The results are presented for a series of chemical shift differences, Δ (the values noted with each curve), at the ratio of the two populations. P_{trans}/P_{cis} is equal to 2.85.

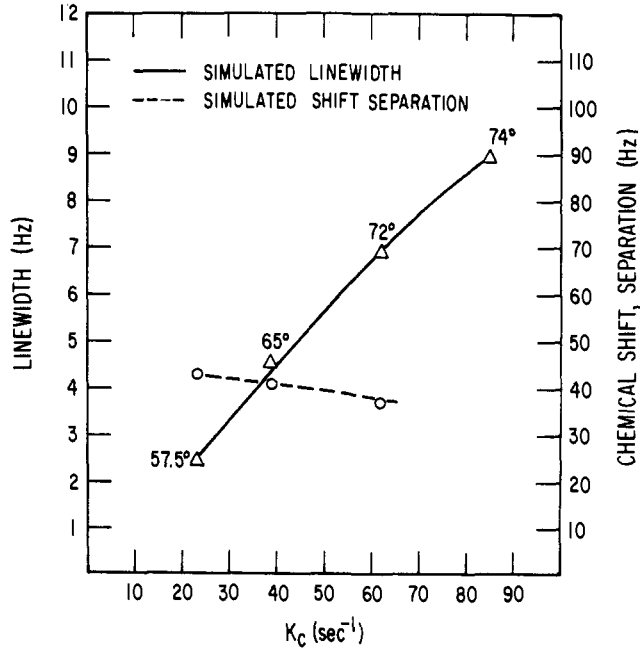

Figure 4. Comparison of the observed (Δ) and the calculated (——) line width as well as the observed (O) and the calculated (---) chemical shift difference for the N-CH_3 resonance in CD_3CN.

coalescence range of the two resonance peaks, the chemical shift difference, Δ, and the relative populations can be accurately measured. These parameters then were extrapolated to the coalescence range. These results were then used as input for the computer program which generated the theoretical line widths and chemical shift difference corresponding to different values of the rate constant for the cis to trans conversion (k_c). Typical results of this calculation are illustrated in Figure 3, where the calculated line width is given as a function of k_c for a range of the chemical shift difference, Δ, at the ratio of the two populations, P_t/P_c, equal to 2.85. Comparison of the theoretical with the experimental spectra permits the correlation of temperatures with the exchange rate constant. The computer best fit of the data was satisfactory only when the rate constants and other parameters were in complete agreement with those from the experimental spectra. A comparison of the observed line widths and the chemical shift difference with the theoretically expected values for the *N*-methyl peak of compound I in CD_3CN is depicted in Figure 4.

Thus, the activation energy for the cis–trans isomerization can be obtained from the rate constants evaluated at various temperatures. A least-squares fit of eq 3 yielded the enthalpy of activation $\Delta H^{\ddagger}$ and

$$k_c = \left|\frac{kT}{h}\right| \exp(-\Delta H^{\ddagger}/RT) \exp(\Delta S^{\ddagger}/R) \qquad (3)$$

entropy of activation $\Delta S^{\ddagger}$, where k and h are Boltzmann and Planck constants, respectively. The results for these calculations in several solvent systems are given in Table IV. The Arrhenius activation energies and frequency factors are also presented in this table.

The activation energy from our results is similar to the values of *N*-acylprolines[22] and other *N*-methylalanine peptides.[23] The elucidated kinetic parameters are close to those obtained in the study of *N*-acetyl-*N*-methyltrinitroaniline[6] and methyl *N*-acetylsarcosinate.[24]

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The values of $\Delta S^{\ddagger}$ are relatively small as compared to those reported for N-alkylated derivatives of formamide and acetamide.[2-5] The variation of $\Delta S^{\ddagger}$ obtained in different solvent systems suggests changes in solvation in proceeding from the ground state to the rotational transition states.[25]

From these studies, we conclude that the splittings observed in nmr for each set of protons are all due to cis–trans isomerism resulting from restricted rotation about the carbonyl–nitrogen amide bond. The results indicate that local minima associated with N–C_α and C_α–C bonds cannot explain the nmr splittings.[26] The coexistence of cis–trans isomers provides a reasonable explanation.

The cis–trans isomerization of the peptide bond has been discussed in detail by Madison and Schellman,[27] Ramachandran,[28] and Andrews[29] from the theoretical point of view. From their calculations, the ground-state energy difference between the cis and the trans isomers is estimated to be less than 2 kcal/mol. Our results agree with the calculated values. In spite of the low energy difference between the two isomers, solvent and intramolecular interactions unique to polypeptides could prevent the formation of the cis isomer in the long chain molecules.[29,30] Thus, it is reasonable to explain the all-trans conformation of poly(*N*-methyl-L-alanine) observed in helix-supporting solvents but with roughly equal cis–trans conformation in a helix-breaking solvent such as trifluoroacetic acid.[10]

Acknowledgment. We gratefully acknowledge financial support for this work by grants from the National Science Foundation (GP 35810) and the National Institutes of Health (GM 18694). One of us (C. Y. Lee) is supported by grants from the American Cancer Society (BC-60-0) to Professor N. O. Kaplan. We thank Dr. Regitze Vold for her many helpful suggestions and discussions in the preparation of this manuscript. Computer assistance from Mr. C. H. Ho is also highly appreciated.

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^{13}C Nuclear Magnetic Resonance Studies of 85% ^{13}C-Enriched Amino Acids. Chemical Shifts, Coupling Constants J_{C-C}, and Conformation

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Abstract: The ^{13}C chemical shifts and the coupling constants J_{C-C} of the amino acids: alanine, valine, leucine, and isoleucine, enriched to 85% in ^{13}C, were studied as a function of pH. The inductive effect on C_β is found to be slight during protonation of the COO^- group and negligible beyond C_γ in the pH range between 1 and 11. The spatial effect of the $\langle NH_3^+, COO^- \rangle$ zone is propagated as far as C_γ and probably further. The four amino acids studied fall into two categories: the first (alanine, leucine) characterized by a C_β carrying two or three protons and the second (valine, isoleucine) by a C_β with only one. The ^{13}C chemical shift variation with pH suggests that the single C_β proton of the second category lies outside the $\langle NH_3^+, COO^- \rangle$ zone, whereas in the first category one of the C_β protons is permanently inside it. Apart from $J_{C_0-C_\alpha}$, which is relatively high (53–60 Hz), the J_{C-C} values range from 31 to 36 Hz. Only $J_{C_0-C_\alpha}$ varies appreciably (by about 6 Hz) and reflects the COO^- group p*K*. On protonation of this group (pH 1–7) the chemical shifts of C_0 and C_α are linear functions of $J_{C_0-C_\alpha}$ and the total electron densities of these carbons vary in the same way for the four amino acids studied.

A large number of articles published in the last few years have stressed the advantages of the ^{13}C nuclear magnetic resonance (^{13}C nmr) technique as a means of studying biological molecules. In the amino acid field, the main parameters defining the chemical shifts of ^{13}C have been established by several authors,[1-4] often using the data of Grant, *et al.*[5] Unfortunately, very few results on the conformation of amino acids exist,[4] which information would lead to a better understanding of the behavior in solution of larger molecules such as peptides and proteins. However, some recent results based on chemical shifts,[6] spin–lattice relaxation times of different species,[7-9] and couplings of the type

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