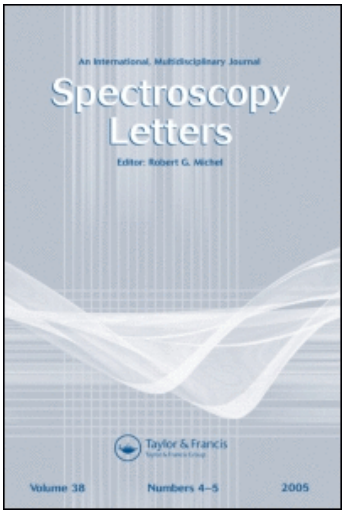

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Solution Conformation of a Pentapeptide by NMR and Molecular Modeling Studies

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SOLUTION CONFORMATION OF A PENTAPEPTIDE BY NMR AND MOLECULAR MODELING STUDIES

Key Words: β-turn, *cis-trans* isomerization, hydrogen bond, 2D NMR, Molecular Modeling

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ABSTRACT

High resolution NMR studies and molecular modeling calculations were performed on a linear pentapeptide BOC-Tyr-Gly-Gly-Pro-Leu-OMe. This molecule exhibits two conformations in the solution state. While the ^{13}C spectrum in nonpolar solvents such as acetonitrile shows only one form, in DMSO-d_6

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and MeOD there are two forms. The appearance of the major (M) and minor (m) resonances is attributed to *cis-trans* isomerization about the X-Pro bond.

The assignments of several proton resonances for the major conformer were made through the combined analysis of two dimensional (2D) homonuclear correlated spectroscopy (COSY) and available data on similar peptides. The solution conformation of the major moiety was probed using the distance constraints obtained from 2D-NOE spectroscopy (NOESY). In addition, other information such as presence of internal hydrogen bonds and dihedral angle values from coupling constants for the amide protons were also used.

INTRODUCTION

We are engaged in extensive studies of several linear peptides that contain proline residue. Proline is a unique amino acid residue in that the side chain folds back and is covalently bound to the preceding peptide nitrogen atom. The five-membered ring thus imposes rigid constraint on the N-C^{α} rotation. The bulky pyrrolidine ring restricts the available conformational space of the preceding amino acid residue. Proline residues have been recognized as

being of special significance due to their strong role on the peptide chain conformation and the process of protein folding. Because of its unique properties, it plays an important role in protein structure and conformation. In many globular proteins, proline residue is exposed to the solvent and causes the helix to kink around the hydrophobic core. In membrane proteins, the inside of proteins may be more polar than the lipid, and in such cases the proline residue may face the interior. In peptides containing proline residues, the oxygen atom of the C=O of the preceding amino acid residue is more electronegative than those preceding other amino acids (1). As a result, this carbonyl group has greater tendency to form stronger hydrogen bonds. This has been shown to be the case in many proline containing β-turns (2) and γ-turns (3).

Proline is the only mammalian residue for which the *cis* peptide bond is energetically accessible. Proline *cis-trans* isomerization is also important in the context of the protein conformational heterogeniety that arises when conformers have either *cis* or *trans* conformation at a particular residue (4). Although *cis* peptide bonds rarely occur in peptides and proteins, peptide moieties containing proline residues exhibit similar stabilities in the *cis* and

trans forms and thus both forms are typically observed (6-11).

NMR studies in the solution state can provide insight into the conformation and dynamics of proline in peptides and proteins. The proline and glycine residues are of interest in protein structure because they are found frequently in β-bend conformations. While Pro-Gly is found to have high propensity for forming a bend, Gly-Pro is found to have a low propensity for such bends (12-14). In addition, Proline is of interest because *cis* conformations have been observed in peptide bonds preceding Pro in small molecules (15-18) and in proteins (19). The pentapeptide studied here provides an opportunity to probe the conformational heterogeneity and possible folded conformation in different solvents. The solvent polarity can change the percentage of *cis* and *trans* conformers and ^{13}C NMR is a powerful tool for investigating the *cis-trans* isomerization about the peptide group.

Proton and carbon-13 NMR were used to assign resonances and to further characterize the major conformation in solution. The results clearly establish the existence of major (M) and minor (m) conformers and their changes with the solvent

polarity. Furthermore the temperature coefficients of the amide proton chemical shifts, the vicinal ^{1}H - ^{1}H coupling constants for the NH-$C^{\alpha}H$ groupings and qualitative Nuclear Overhauser Effects (NOEs) were used to obtain information regarding the preferred conformation of this molecule. Molecular dynamics (MD) and mechanics studies were also performed in conjunction with these experimental observations.

EXPERIMENTAL

The pentapeptide was provided as a gift to the author from Prof. R. Schwyzer's laboratory (E.T.H., Zurich). One dimensional proton and ^{13}C spectra were recorded on a Bruker AM-400 FT NMR spectrometer equipped with an ASPECT 3000 computer and variable temperature equipment. The spectral size varied from 16K to 32K depending upon the particular nucleus studied. The peak positions are given in parts per million (ppm) from TMS. The peptide concentrations range from 5 to 10 mg/ml. The COSY and the NOESY spectra were recorded in the absolute value mode using the Bruker CONOESY pulse sequence on a selective proton probe with a 90° pulse width of 7.5 μs. The experimental setup led to a mixing time of 1 sec for the NOESY experiment. The 2D spectra were obtained

from 512 measurements with t_1 values from 0 to 91 ms. Usually 2048 data points were collected for each value of t_1. Before Fourier transformation, the time domain data matrix was multiplied by a sine or shifted sine bell in both dimensions. In addition, to end up with 1024 X 1024 point data matrix which corresponds to a digital resolution of 5.5 Hz/point, the time domain matrix was expanded to 1024 points in t_1 by zero-filling. The 2D spectra were further improved by symmetrization. All spectra are presented in the absolute value mode.

Molecular Modeling

Molecular mechanics and dynamics calculations were performed using the program QUANTA-CHRAMm (Molecular Simulations, Waltham, MA) and a silicon Graphics work station. The peptide structure was generated using the sequence builder option of QUANTA. The initial linear peptide conformation was minimized using 200 steps of steepest descent and then minimized with the adopted basis Newton-Raphson algorithm. The minimizations were performed using NOE constraints.

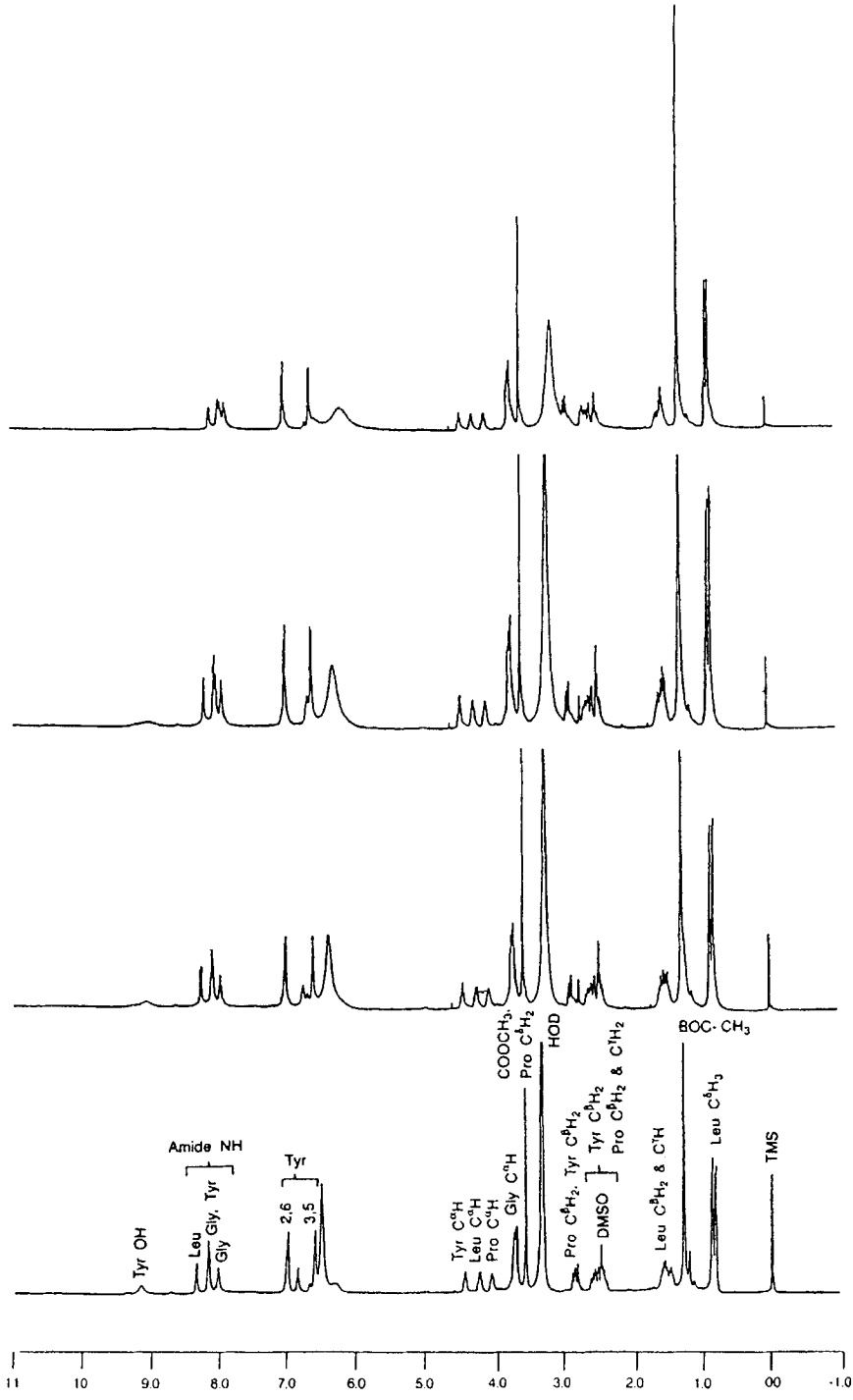
Molecular dynamics (MD) simulations were carried out in vacuo for 10 ps at 300° K with a step size of 0.001 ps. Before the simulation, the initial

structure was heated to 300° K (over 500 steps) and equilibrated for 5 ps.

RESULTS AND DISCUSSION

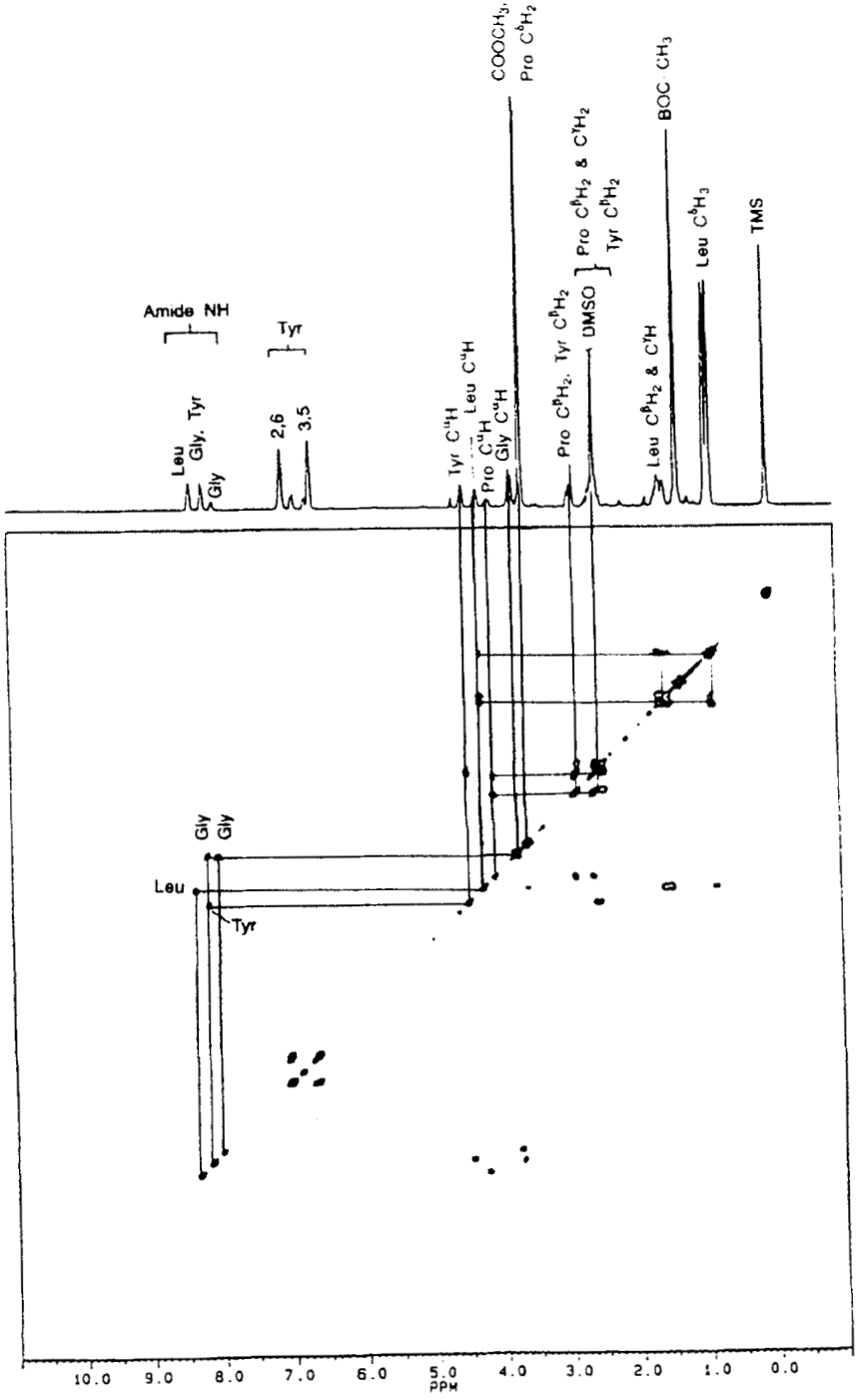
The series of proton spectra in the temperature range 25° to 55° is shown in Fig. 1. The resonance assignments follow from a comparative study of spectra of other proline containing peptides and the analysis of COSY spectra (see Fig. 2). An analysis of the COSY spectrum has allowed the assignment of many amide and α protons in this pentapeptide.

Sequential NOE connectivities between the α proton of a residue i+1 and the amide proton of residue i are used to assign amino acid residues in peptides. Unfortunately in our case very few strong NOEs are seen in the NOESY spectra. This is primarily because of the correlation time dependence of dipolar interactions which can sometimes render an NOE between nuclei with a short internuclear distance barely observable. Thus the NOE effects in the laboratory frame often can be small, with NOESY 2D spectra devoid of any useful connectivities. Although only a few NOEs are observed here, they however provide constraints that are relevant in defining the minimum energy conformation.



The observation of dipolar connectivities between backbone protons from different amino acid residues, particularly those far apart in the primary structure of the molecule, provides tertiary structure information (20). The observation of dipolar connectivities in a 2D NOE spectrum depends on the cross-relaxation rates between the interacting proton spins. The typical 2D NOE spectrum from this pentapeptide moiety with a 1 sec mixing time is shown in Fig. 3. Several NOE cross peaks are seen. The cross peak corresponding to the NOE between the 2,5 and 3,6 protons of the tyrosyl ring can be clearly seen. A strong cross peak between BOC-CH_3 group and Leu-$C^{\delta}H_3$ is observed, which is an indication of a folded peptide moiety. A conservative distance constraint of 3Å is uniformly used between protons that show a modest to strong NOE. An NOE is also observed between the BOC-CH_3 resonance and the one which represents an overlap of the OMe and Pro-$C^{\delta}H_2$ protons. Similarly a weak, but definite, NOE is observed between the Leu-$C^{\delta}H_3$ resonance and that due

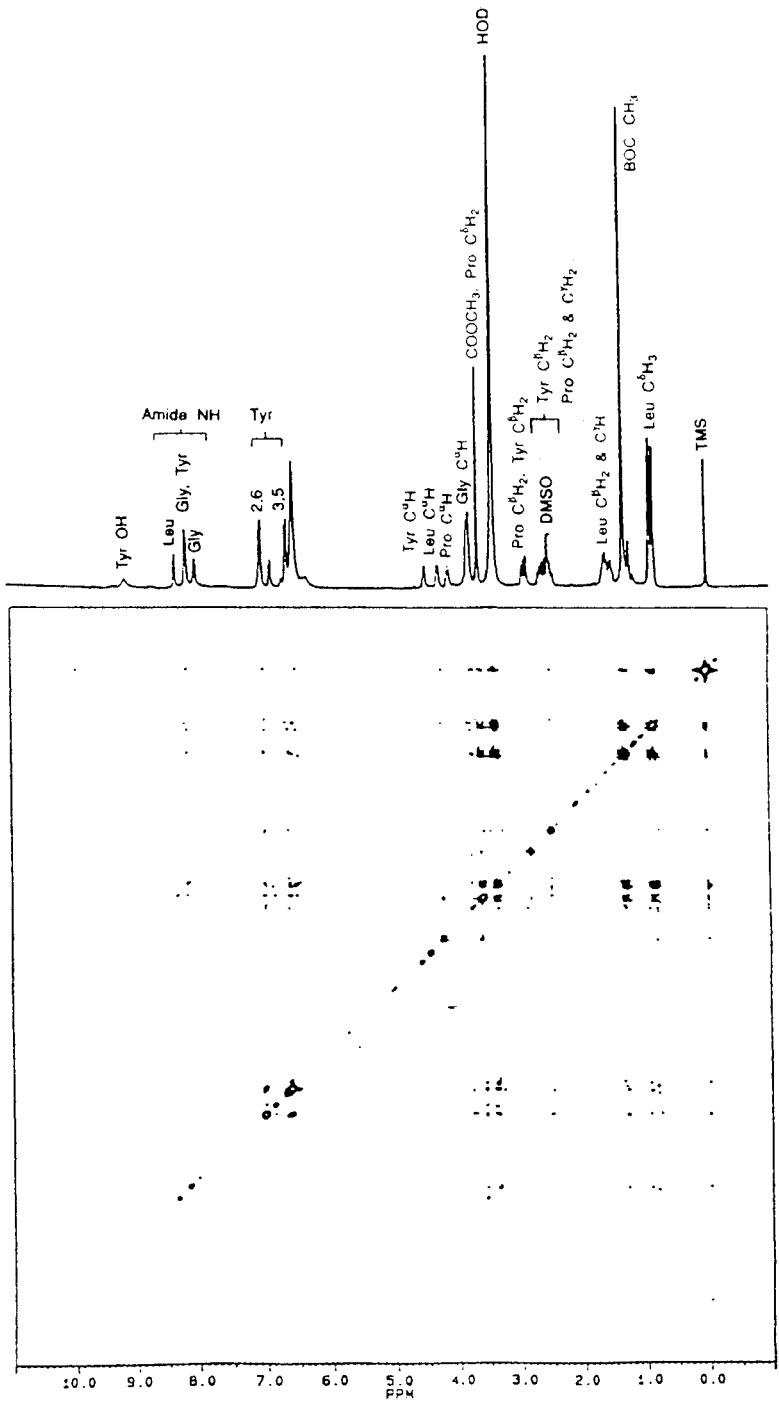
Fig. 1 Proton NMR spectra at 400 MHz of the pentapeptide in the temperature range 25°-55°C.



to the overlap of OMe and Pro-$C^{\delta}H_2$ protons. Also of interest are the internuclear NOE's such as the one between the water protons and those between OMe or Pro-$C^{\delta}H_2$ protons and with BOC-CH_3

The spectrum at 55°C (Fig. 1 top trace) shows minor resonances due both to 2,6 and 3,5 ring protons of tyrosyl residue. No other resonance in the proton spectrum shows such a splitting into major (M) and minor (m) resonances. The chemical shift of γ-carbon resonance is the most reliable way to determine the presence of *cis-trans* isomerization. Since the pentapeptide has an X-Pro bond, the presence of such an isomerization is an interesting possibility. In order to investigate this possibility, the ^{13}C spectrum at ambient temperature was recorded in DMSO-d_6 solution and is shown in Fig. 4. The spectrum shows the doubling of peaks due to tyrosyl ring carbons. All the assignments indicated in this figure are based on the carbon-13 chemical shift data of individual amino acid residues (21) and of proline containing residues (22,23). Probably the most

Fig. 2 COSY spectrum obtained from the CONOESY experiment on the pentapeptide



reliable indicator of peptide bond conformation in X-Pro peptides is the γ-carbon resonance, which appears at 24±1.0 and 22.0± 1.0 ppm from TMS for the *trans* and *cis* conformers respectively. Although minor and major resonances are both observed for the Tyrosyl ring protons, there are no such minor resonances for Pro C^{β}, C^{γ}, and C^{δ} resonances in the carbon spectrum in DMSO-d_6. This observation, although it may not seem consistent with the observation of *cis-trans* isomerization in linear peptides (23-27), is due to insufficient signal-to-noise ratio to clearly observe the minor resonances due to pyrrolidine C^{β} or C^{γ} carbons. The ^{13}C chemical shift of C^{β} and C^{γ} in pyrrolidine rings, in particular, can give clear information on the stereochemistry at this bond (28-29) and the observed chemical shifts for these carbons are consistent with *trans* X-pro peptide bond.

To further characterize the peptide in solution, we make use of both the coupling constants, which can be measured at elevated temperatures (35°-55°C). These are approximately 7.5 Hz for both leucyl and tyrosyl residues.

Fig. 3 NOESY spectrum generated from the CONOESY experiment of the pentapeptide

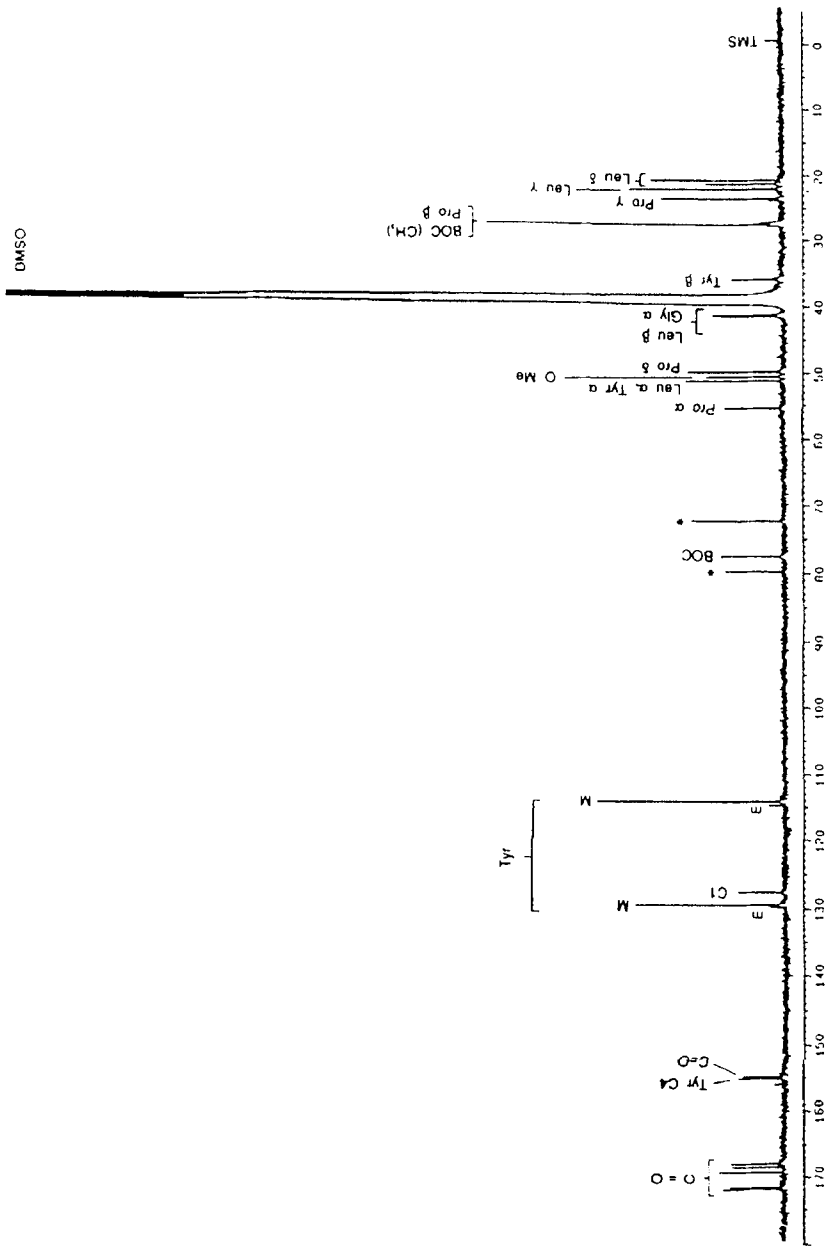


Fig. 4 Proton decoupled C-13 NMR spectrum of the pentapeptide in DMSO-d_6 solution. The peaks denoted by asterisks are from unknown impurities.

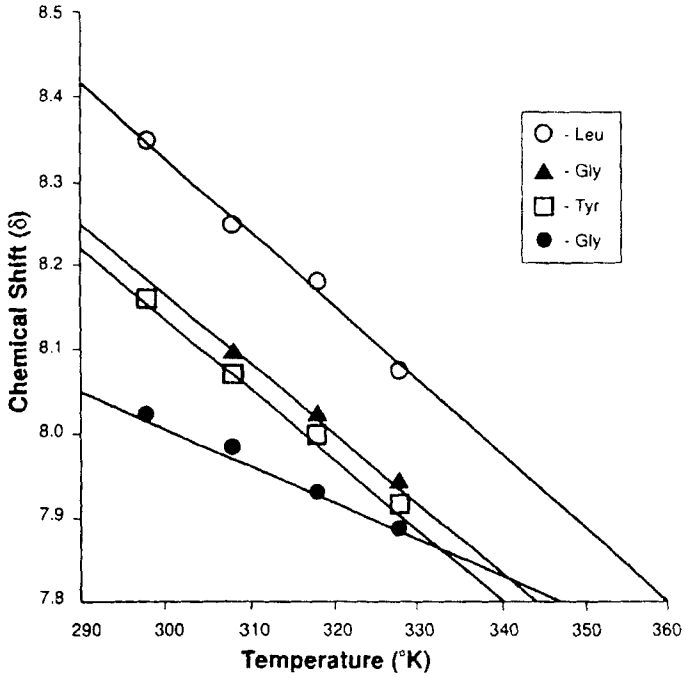


Fig. 5 Diagram showing the variation of chemical shift with respect to temperature.

The temperature variation of the amide resonance is indicative of amide proton accessibility to solvent (30-32). The temperature dependence of chemical shifts of the amide protons determined over the range from 298° to 328° K in DMSO solution is shown in shown in Fig. 5. In all cases, the chemical shifts varied linearly, indicating major conformational changes are not present in the temperature range under study. The $\Delta\delta/\Delta T$ values for the three amide resonances are

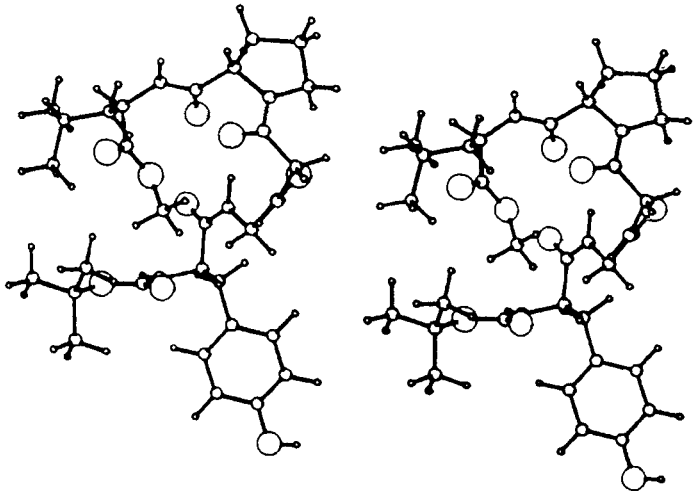


Fig. 6 Stereo view of the predicted conformation of the pentapeptide from the combined molecular mechanics and NMR studies.

roughly -16 x 10^{-3} ppm/deg, while for one of the glycyl residues the value is -4 x 10^{-3} ppm/deg. Temperature coefficients in the range of -3 to -4X10^{-3} ppm K^{-1} have often been interpreted as indicating weakly hydrogen bonded amide groups (33). Thus it appears that the amide NH of one of the glycyl residues is relatively shielded.

The NMR refined structure forms a compact structure with an internal hydrogen bond but does not possess a β turn conformation. A stereo view of the conformation predicted from the experimental constraints and molecular mechanics is shown in Fig.

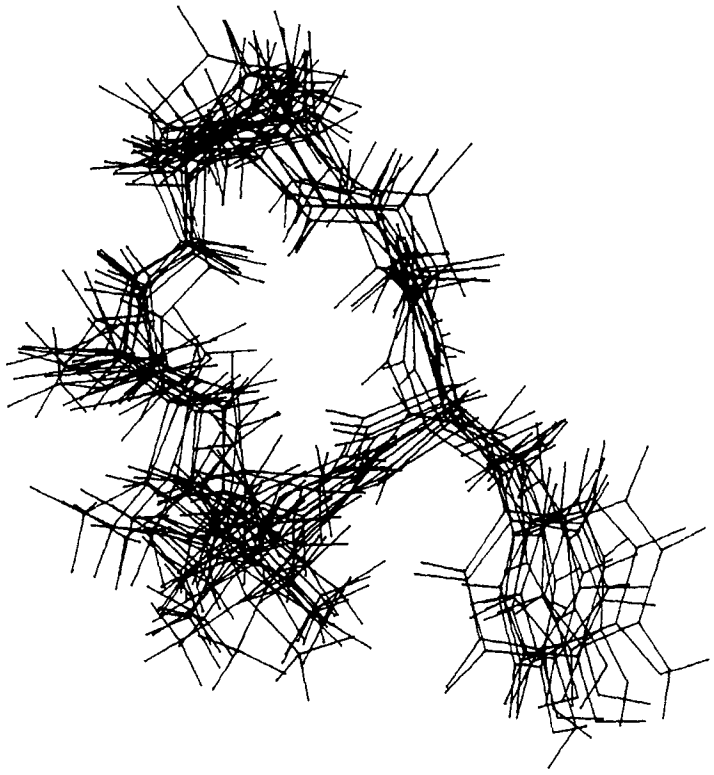


Fig. 7 Superposition of the eleven lowest energy conformations from the molecular dynamics simulation of the pentapeptide.

6. The minimum energy structures taken from the molecular dynamics are shown superimposed in Fig. 7. It is readily seen that there are no significant deviations in the structure, indicating the stability of this conformation. To study whether or not the ratio of the two conformers change with the polarity

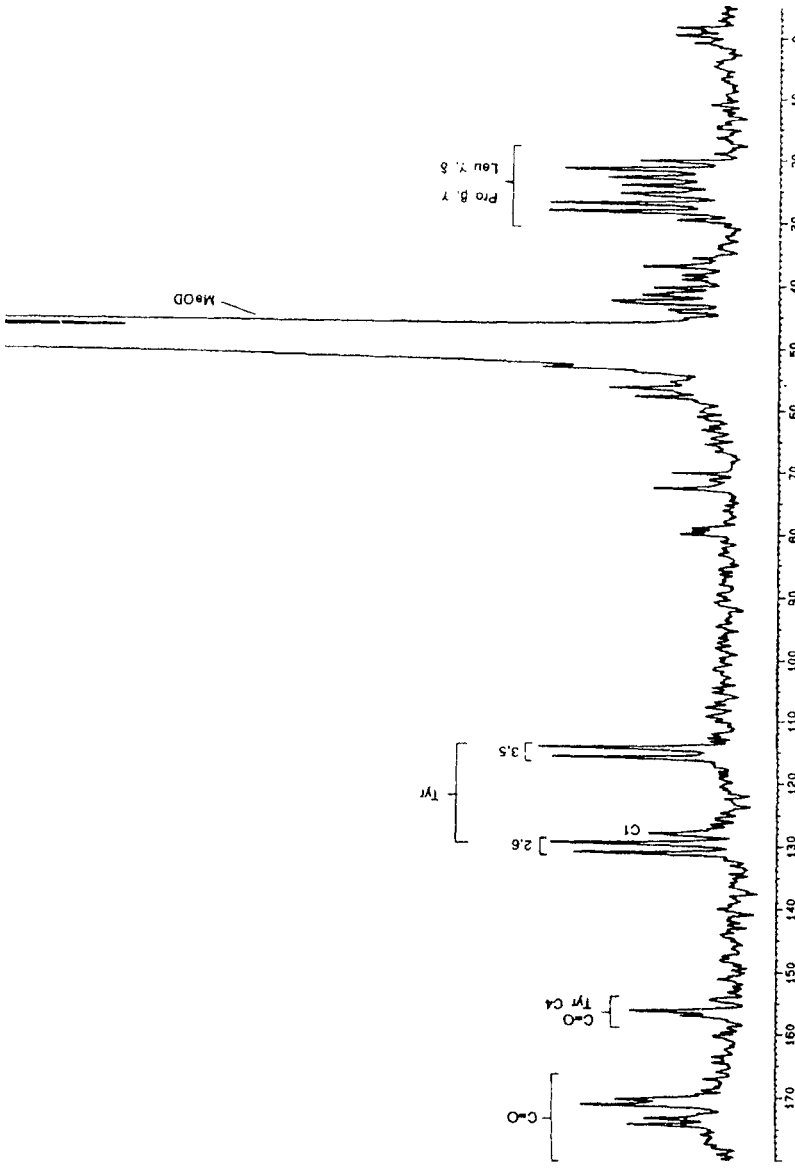


Fig. 8 Proton decoupled C-13 NMR spectrum of the pentapeptide in deuterated methanol.

of the solvent, both the proton and ^{13}C spectra have been recorded in various solvents at ambient temperature. The proton spectra in acetonitrile (CD_3CN) and methanol (MeOD) are indicative of only one type of conformer. An examination of their ^{13}C spectra clearly indicates only one type of species in acetonitrile and two types of species in MeOD. The two conformers in MeOD are roughly in equal ratio (Fig. 8).

CONCLUSIONS

In summary, an energetically stable major conformer is observed in nonpolar solvents. Minor conformers are also observed in DMSO and MeoD solution for the pentapeptide, which appear to arise from *cis-trans* isomerization. The pentapeptide shows the presence of two stable conformers in the form of doubling of peaks for the tyrosyl residue. Furthermore, the ratio of the conformers changes from 16:1 (M:m) in DMSO to roughly 1:1 in MeOD. The molecule exhibits only one type of conformer in nonpolar solvents like CD_3CN.

The NMR refined structure forms a compact structure with an internal hydrogen bond but does not conform to a standard β turn conformation. The

molecular dynamics studies show that this structure is entropically stable.

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