Conformational and Circular Dichroism Studies on N-Acetyl-L-Prolyl-D-Alanyl-Methylamide

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CONFORMATIONAL AND CIRCULAR DICHROISM STUDIES ON N-ACETYL-L-PROLYL-D-ALANYL-METHYLAMIDE

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ABSTRACT

The compact ordered conformations of the molecule N-Acetyl-L-Prolyl-D-Alanyl-Methylamide have been studied by semiempirical energy calculations in vacuum and circular dichroism (CD) in solution.

The presence of ordered structure has been observed in hydrogen bond promoting solvents like trifluoroethanol by CD studies. In hydrogen bond breaking solvents, like trifluoroacetic acid (TFA), significant fraction of the ordered conformers probably assume extended conformation without intramolecular hydrogen bonds and perhaps are in equilibrium with the fraction of compact ordered structures. The trend observed in going from nonpolar to polar solvent is also compatible with the previous NMR studies in solution.

The semiempirical energy calculations have been carried out in the allowed region for β-bends. The flexibility of pyrrolidine ring has been incorporated into the calculations. Representative puckerings, namely, A-type (Cγ-*exo*) and B-type (Cγ-*endo*) have been considered in this study. The results show the B-type to be slightly preferred over the A-type in this tripeptide moiety. The minimum energy conformation predicted from these studies agree only minimally with that found in crystal structure. A better agreement is found after performing the calculations using the geometrical data as observed in the crystal structure of this molecule. Our studies demonstrate that solvent solute interactions are minimal in nonpolar solvents and the predicted minimum energy conformations are preserved at least in nonpolar solvents.

INTRODUCTION

A detailed understanding of the conformation of peptide structures with both L and D amino acid residues are of importance in connection with the studies on cyclic peptides and antibiotics which may consist of either kind of amino acid residues. The key points of reversal in the chain direction in such peptides consisting of both L and D amino acid residues may be expected to be LD or LG sequences (where G denotes a glycyl residue) rather than LL sequences (Chandrasekaran et al., 1973). The LL and LG sequences, however, are found to be important in the folding of polypeptides and proteins. In globular proteins, a chain reversal often enables different parts of the molecule to come close and form a compact structure. Such a chain reversal facilitates ring closures in many cyclic peptides and antibiotics. Chain reversals have been observed in many proteins (Birktoft and Blow, 1972; Blake et al.,., 1967; Lipscomb et al.,., 1976; Kartha et al.,., 1967) and cyclic and linear peptides (Ueki et al.,., 1969; Rudko et al.,., 1971; Karle et al.,., 1970; Zalkin et al.,., 1966; Reed and Johnson, 1973; Brahmachari et al.,., 1981; Ayato et al.,., 1981; Yagi et al.,., 1983; Pichon-Pesme et al.,., 1988; and Ananthanarayanan and Cameron, 1988).

The optimum size required for a compact reversal to occur is just a tripeptide. The earlier studies on three-linked peptide units from purely stereochemical considerations (Venkatchalam, 1970) and later detailed studies from energy considerations have shown that stable folded conformations with a 4—>1 type (IUPAC-IUB Commission on Biochemical Nomenclature, 1970) internal N-H...0 bond are possible. Such conformations are well known as β-bends or β-turns.

The various interesting features of the theoretical predictions were: (i) low energy β-bends can occur near the α-helical conformation and hence a chain reversal can occur in the vicinity of an α-helix (ii) the conformational angle ϕ_2 at C$_2\alpha$ being very close to that for a proline residue, a sequence of the type -pro-x- can be a ready site for a β-bend. Also, it was evident from these studies that while a pyrrolidine ring can be easily accommodated at C$_2\alpha$ in LL bends, it can occur equally well at either C$_2\alpha$ or C$_3\alpha$ in LD bends.

As discussed earlier, β-turns or reverse turns are important structural features in proteins and bioactive peptides and may play an important role in protein folding. There has been a continued interest in such compact secondary structures and has been an active area of research through studies on a variety of model compounds by both theoretical and experimental methods. Several model compounds and macromolecules consisting of such bends have been studied by infrared spectroscopy (IR), (Bandekar and Krimm, 1979; Krimm and Bandekar, 1980; Naik and Krimm, 1984) nuclear magnetic resonance (NMR), (Brahmachari et al., 1981; Narasinga Rao et al., 1983; Ramaprasad et al., 1981; Patel and Tonelli, 1973; Pitner and Urry, 1972; Khaled et al., 1976; Pelton et al., 1988; and Jelicks et al., 1988) and circular dichroism (CD) Kawai and Fasman, 1978; Brahmachri et al., 1979, and Crisma et al., 1984). In addition, several theoretical calculations have been attempted by a variety of minimization techniques to predict the possible conformations. In their studies, Pletnev et al. (1974) have considered a short peptide (Ac-Gly-L-Ala-Gly-NHMe) with intramolecular hydrogen bonds for a detailed theoretical analysis. Zimmerman et al. (1982) have studied in detail the tripeptide H-L-Pro-L-Leu-Gly-NH$_2$. They calculated lowest energy for type II β-bend and was similar to that reported in an X-ray crystal study.

To have an insight into the details of the folded conformation with different side chains at C$_2\alpha$ and C$_3\alpha$ in either L or D configuration a series of spectroscopic and theoretical studies have been undertaken by the author and the results on the molecule N-acetyl-L-prolyl-D-alanyl-methylamide from CD studies and semiempirical energy calculations are presented in this paper. While the circular dichroism studies are compatible with the previous NMR studies on this molecule, semiempirical energy calculations show only marginal agreement with the conformation observed in the solid state.

CD STUDIES

EXPERIMENTAL PROCEDURES

Materials — The tripeptide was given as a gift by Dr. K.S.N. Iyer. It was dried overnight under vacuum in the presence of P$_2$O$_5$ before making the measurements. The purity of solvents were checked by the uv absorption spectra.

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Spectral measurements — Spectra were recorded using the JASCO J-20 spectrometer. The parameters like chart speed, time constant, wavelength expansion and gain contraols were so chosen as to obtain the best signal-to-noise ratio and reproducibility of the spectra. Mean residue ellipticity values are expressed as deg-cm²/decimole and have not been corrected for the effect of refractive index of the solvent. All the measurements were done at ambient temperature (25 °C).

The solution for CD measurements was prepared by dissolving the required amount of the tripeptide in a known volume of the solvent. Cells with a path length of 0.1 to 0.2 cm were employed. The peptide concentrations (expressed as gm per 100 ml of solvent) were around 0.1 to 0.2%. In a given run, the concentration was maintained constant during the searching of the entire accessible wavelength region (210-240 nm). Since there were no signs of aggregation as obtained by our previous NMR studies at even higher concentrations, no concentration dependent CD studies were carried out.

RESULTS AND DISCUSSIONS ON CD STUDIES

The CD spectra of this peptide in both the solvents are recorded in Fig. 1. Only 1 CD extrema, namely $[\theta]_{225} = -7733$, is clear while the peak around $[\theta]_{20}$, can not be obtained from this study. It is interesting to note that the trough at 225 nm is typical of type I and type II β-bend. Further characterization of a particular bend type will require studies similar to those by Brahmachari *et al.* These preliminary studies, however, very well characterize the peptide moiety as an ordered structure.
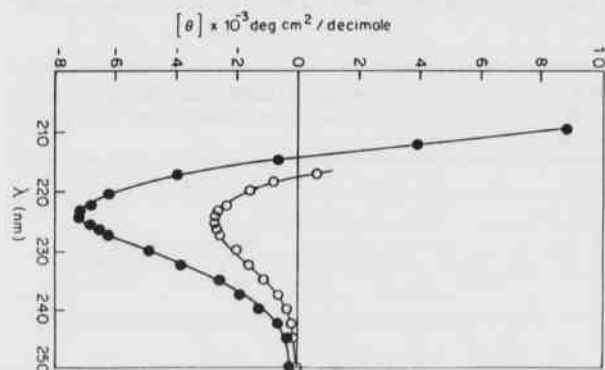
Figure 1. Circular dichroism spectra of the tripeptide in TFE (-•-•-) and TFA (-O-O-).

These studies clearly demonstrate that the $[\theta]_{225}$ decreases in magnitude as the solvent changes from TFE (nonpolar) to TFA the hydrogen bond breaking solvent by about 3-fold. This possibly means that the fraction of ordered β-turns conformers have decreased significantly in going from TFE to TFA.

The results from CD studies may be usefully compared with those of previous NMR studies (Ramaprasad, 1981) in nonpolar solvents like CDCl₃. Both ¹H and ¹³C NMR have shown that the major conformers in CDCl₃ are compact folded β-bends while in more polar solvents, because of *cis-trans* isomerization about the x-pro bond, the fraction of β-bend conformers decreases. The significant decrease in the fraction of ordered structures upon solvent change from TFE to TFA is probably because of random structures generated from the breaking of intra molecular 4—>1 hydrogen bonds and/or cis-trans isomerization about the x-pro bond as observed from NMR.

THEORETICAL

PROCEDURAL DETAILS

A schematic diagram of the molecule is shown in Fig. 2. The IUPAC-IUB conventions (IUPAC-IUB Commission on Biochemical
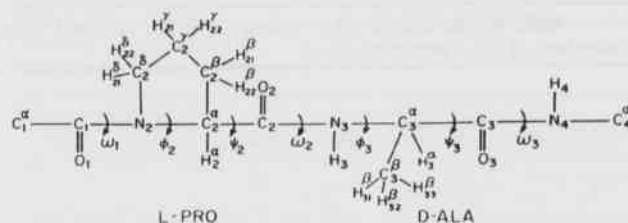
Figure 2. The structural formula of the molecule N-acetyl-L-prolyl-D-alanyl-methylamide. The various backbone dihedral angles are also shown.

Nomenclature, 1970) have been used to denote the conformational angles.

In this study *trans* planar peptides have been used. The hydrogen atoms in the pyrrolidine rings have been fixed by bisecting the appropriate C-C-C or C-C-N angles. The bond angles N_2-$C_2\alpha$-C_2 and N_3-$C_3\alpha$-C_3, in the main chain have been maintained at the expected value of 100°. All the H-C-H bond angles have been taken to the 109.5° and the C-H bond lengths to be 1.1 A°. The hydrogens of the terminal methyl groups at $C_1\alpha$ and $C_4\alpha$ have not been considered separately, but are treated as single 'effective' atoms with increased Van der Waals radii. The methyl hydrogens of the alanine residue have been fixed in the staggered position.

GEOMETRY OF THE PYRROLIDINE RING

The pyrrolidine ring is puckered and this has been taken into account while incorporating the same into the tripeptide system. Ramachandran and colleagues (1968) have classified the puckering into two main categories, namely, type A(C^γ-*exo*) and type B (C^γ-*endo*), and these are illustrated in Fig. 3. They essentially differ in the sign of the dihedral angles about the various bonds of the ring.
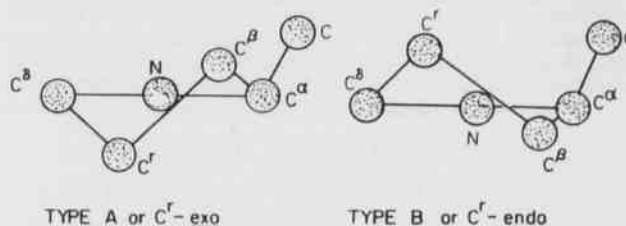
Figure 3. Schematic representation of the two major types of puckering of the pyrrolidine ring.

In the present study, five different representative puckerings have been selected from the list of low energy conformers of the pyrrolidine ring (see Table 2 in Ramachandran, 1970). The selected puckers are designated A_1, A_2, A_3, B_2 and B_3, and they correspond to values in the range −50° to 70°. The endocyclic bond angles and the ring dihedral angles for each puckering is given in Table 1.

THE GENERATION OF THE TRIPEPTIDE

To accommodate the pyrrolidine ring in the main chaing, the angle ϕ_2 at $C_2\alpha$ was assigned an appropriate value using the relation $\phi = \theta$-60°. To generate different tripeptide conformations, the remaining three main chain dihedral angles viz., ψ_2, ϕ_3 and ψ_3, were varied, in the allowed region for an LD bend¹, at intervals of 10°. The criteria now used for selecting 4—>1 hydrogen bonded structures were that the N_4....O_1 distance be between 2.6 and 3.2 Å and the NH$_4$N...O be less than 35°. For each of these conformers the total conformational energy was evaluated.

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Table 1. Bond Angles and the Ring Torsion Angles for the Five Chosen Puckerings of the Pyrrolidine Ring*

Puckering	Bond Angles (in degrees)				Ring Dihedral Angles (in degrees)					Energy kcal/mole
	$N-C^\beta-C^\beta$	$C^\beta-C^\beta-C^\gamma$	$C^\beta-C^\gamma-C^\delta$	$C^\gamma-C^\delta-N$	$C^\delta-N-C^\alpha$	θ	χ^1	χ^2	χ^3	χ^4	
C^γ-exo										
A₁	103	106	106	103	113	10	-25	31	-24	8	12.50
A₂	103	106	105	102	113	0	-20	33	-31	20	12.19
A₃	103	106	106	100	113	-10	-13	31	-35	28	12.98
C^γ-endo										
B₂	103	106	105	102	113	0	20	-33	31	-20	12.16
B₃	103	106	106	103	113	-10	25	-31	24	-8	11.80

* From Ramachandran et al (1930).

EVALUATION OF CONFORMATIONAL ENERGY

The total conformational energy of the molecule was computed using the empirical potential functions (Ramachandran and Sasisekharan, 1968). The total energy is the sum of the contributions from nonbonded and electrostatic interactions and those due to torsional potential as well as the bond angle distortions. The non-bonded energy was evaluated using 6-exp potential functions with the constants given by Chandrasekaran and Balasubramanian (1969). The charges (see Table 2) were computed following the semiempirical methods of Del Re (1963) and Pullman (1963, 1965). The hydrogen bond energy was evaluated according to the method of Ramachandran *et al.* (1971).

Table 2. Patial Charges* (in e.s.u.) for the Molecule of N-Ace-L-Pro-D-Ala-MeA.

Atom	Charges	Atom	Charges
C_1^α	-0.095	N_3	-0.200
C_1	0.339	H_3	0.207
O_1	-0.472	C_3^α	0.050
N_2	-0.049	C_3^β	-0.110
C_2^α	0.040	C_3	0.339
C^β	-0.070	O_3	-0.450
C^γ	-0.070	N_4	-0.185
C^δ	-0.030	H_4	0.196
C_2	0.339	C_4^α	-0.075
O_2	-0.450		

* The charge on hydrogen atoms attached to SP^3-type carbons, on an average, is 0.04 (in e.s.u.).

RESULTS AND DISCUSSION

Examples of the minimum energy conformers for each of the chosen puckerings is given in Table 3. These values range from −1.9 kcal/mole to −3.2 kcal/mole. Among the 5 minimum energy conformers listed in Table 3, the B₃ conformer has the lowest energy of −3.2 kcal/mole. From the same list of values it may be noticed that B₂ and B₃ conformers are energetically more favorable than the corresponding A₂ and A₃ conformers (C$^\gamma$-*exo* type puckers) by 0.4 and 0.7 kcal/mole respectively.

For the type A conformers, the minimum energy for the tripeptide is −2.5 kcal/mole. Similarly, the minimum energy for B-type is −3.2

Table 3. Minimum Energy Conformations of the Tripeptide Molecule for the Five Chosen Puckerings of the Pyrrolidine Ring.

Puckering of Proline	$\phi_2(°)$	$\psi_2(°)$	$\phi_3(°)$	$\psi_3(°)$	Total Energy kcal/mole
C^γ-exo					
A₁	-50	130	110	-40	-1.9
A₂	-60	100	60	40	-2.4
A₃	-70	100	80	30	-2.5
C^γ-endo					
B₂	-60	100	60	40	-2.8
B₃	-70	100	80	30	-3.2

kcal/mole. Thus, between type A and type B the latter is energetically more favorable than the former by about 0.7 kcal/mole. Although, the minimum energy conformation may be expected to occur in the solid state, that is not the only stable conformation which can occur. As such, many low energy conformers extending in energy up to 0.6 kcal/mole from the minimum are recorded in Table 4. It is interesting to com-

Table 4. Characteristics of Low Energy Conformations with the Corresponding Hydrogen Bond Parameters and Their Energies of Stabilization.

Dihedral Angles				Hydrogen Bond Parameters			Total Stabilisation Energy (kcal/mole)
ϕ_2 (°)	ψ_2 (°)	ϕ_3 (°)	ψ_3 (°)	Length $N_4\cdots O_1$ (Å)	Angle $N_4H_4\AA N_4O_1$ (°)	Energy V_{hb} (kcal/mole)	
-70	100	80	30	3.07	16.5	-3.95	-3.2
-70	90	90	30	3.03	15.0	-4.14	-3.2
-70	100	90	30	3.80	23.9	-3.92	-3.1
-70	100	90	20	2.94	13.4	-4.32	-2.9
-70	100	80	20	3.03	7.4	-4.28	-2.9
-70	90	90	20	3.00	4.3	-4.42	-2.9
-70	90	100	30	2.96	22.1	-4.02	-2.9
-70	90	90	40	3.11	27.0	-3.36	-2.8
-70	100	130	-30	2.99	26.8	-3.80	-2.7
-70	100	80	40	3.14	28.0	-3.03	-2.6
-70	110	80	30	3.06	25.5	-3.68	-2.6
-70	90	100	20	2.90	10.8	-4.32	-2.6
-70	110	80	20	3.00	15.9	-4.21	-2.6

pare the minimum energy conformation with that obtained from the crystal structure analysis (Ramaprasad, 1980). On comparing the conformational angles predicted from theory and those obtained from cyrstal structure analysis, there is only an approximate agreement between the two (the one corresponding to crystal structure and one to energy calculations using standard bond lengths and angles). The relative displacement of the two models with respect to the dihedral angles ψ_2 and ψ_3 have been shown diagramatically in Figs. 4 and 5. While drawing these plots, the values of ϕ_2 and ϕ_3 have been kept fixed at values

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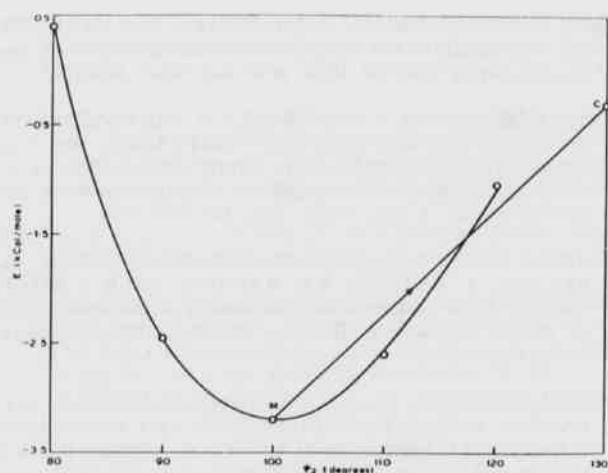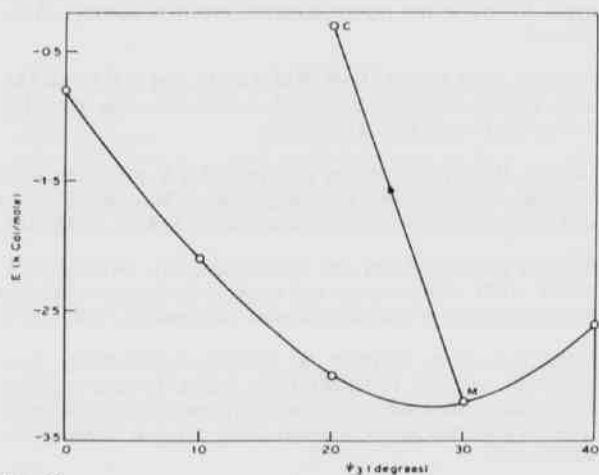Fig. 4

Fig. 5

Figure 4-5. Plots of conformational energy E (computed using the standard bond lengths and bond angles) Vs. ψ_2 and ψ_3 respectively. C and M denote the crystallographically observed and the predicted minimum energy conformations respectively.

Table 5. Characteristic Low Energy Conformers in Regions around the Observed Crystal Structure.

Dihedral Angles				Hydrogen Bond Parameters			Total Stabilisation Energy
ϕ_2 (°)	ψ_2 (°)	ϕ_3 (°)	ψ_3 (°)	Length $N_4\cdots O_1$ (Å)	Angle $N_4H_4\hat{A}N_4O_1$ (°)	Energy V_{hb} (kcal/mole)	(kcal/mole)
-66	107	75	22	2.92	7.2	-4.4	-3.3
-66	107	65	22	3.00	2.6	-4.4	-3.3
-66	97	75	22	3.00	2.1	-4.5	-3.3
-66	107	65	32	3.00	10.8	-4.2	-3.2
-66	97	75	32	3.00	11.5	-4.2	-3.1
-66	107	75	12	2.90	8.4	-4.3	-3.1
-66	107	75	32	3.00	18.9	-4.1	-3.1
-66	97	85	22	2.90	6.1	-4.4	-3.1
-66	117	65	22	2.95	9.1	-4.4	-3.0
-66	97	75	12	3.00	13.8	-4.3	-2.9
-66	97	85	32	3.00	18.6	-4.2	-2.9
-66	997	85	12	2.90	6.7	-4.3	-2.9
-66	107	85	22	2.90	15.2	-4.1	-2.9
-66	107	85	12	2.80	6.1	-4.1	-2.8
-66	117	65	32	3.00	19.2	-4.1	-2.8
-66	117	75	22	2.90	16.5	-4.2	-2.8
-66	117	75	12	2.90	9.6	-4.2	-2.7
-66	107	65	12	3.00	14.8	-4.2	-2.7
-66	127	75	12	2.90	16.2	-4.2	-2.3*

* This energy value corresponds to the conformation observed in the crystal structure.

of about 3 kcal/mole between the minimum energy conformation and the observed one is reduced by 2 kcal/mole on using the geometrical values (excepting ψ_2, ϕ_3, and ψ_3) and the pyrrolidine puckering as observed in the crystal structure. A difference in energy of 1.0 kcal/mole has probably been compensated by intermolecular interactions.
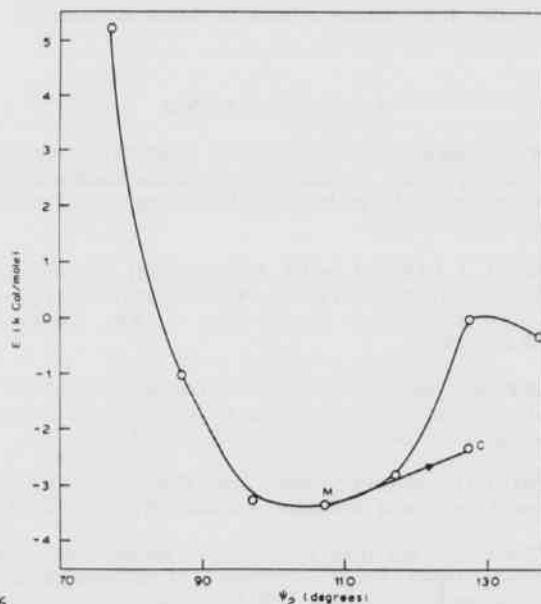

Fig. 6

corresponding to the minimum energy conformation. The differences in the dihedral angles between the X-ray structure and the minimum energy model can be seen in these diagrams wherein both of them have been indicated. This is more clear on comparing the set of conformational angles, namely (ϕ_2, ψ_2) and (ϕ_3, ψ_3) at $C_2\alpha$ and $C_3\alpha$ respectively, obtained from theory and experiment. The values predicted from the theory are ($-70°$, $100°$); ($80°$, $30°$) while those obtained from crystal structure analysis are ($-66°$, $127°$); ($75°$, $12°$).

As a final step in refinement in the calculations the influence of the change in geometry of the molecule on the results was considered. The calculations were performed using the observed pyrrolidine puckering and the data on bond lengths and angles as found in the crystal structure. In these calculations the rotational angles ψ_2, ϕ_3, and ψ_3 alone were varied at intervals of 10° in the allowed region for LD-bends. The low energy values are listed in Table 5. The observed conformation is shifted from the predicted minimum by about 1.0 kcal/mole. The plots of conformational energy (calculated using the crystal structure data) vs. ψ_2 and ψ_3 are shown in Fig. 6 and 7 respectively. The observed conformation is also indicated in this diagram. It is clear that an energy difference

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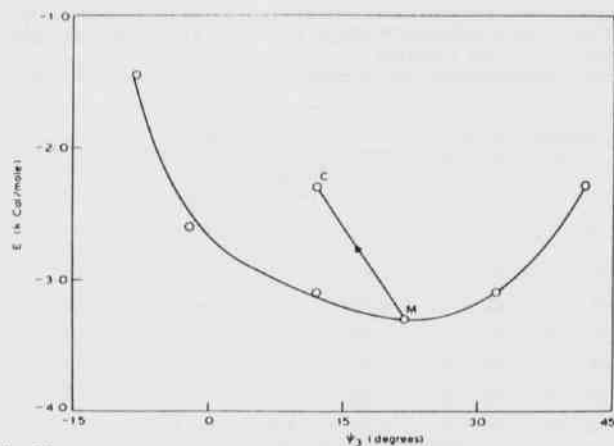## Conformational and Circular Dichroism Studies on N-Acetyl-L-Prolyl-D-Alanyl-Methylamide

Fig. 7

Figure 6-7. Plots of conformational energy, E (computed using the observed bond lengths and bond angles) Vs. ψ_2 and ψ_3 respectively. C and M refer to the crystallographicaly observed and the predicted minimum energy conformations respectively.

CONCLUSIONS

The CD studies demonstrate that the tripeptide is more ordered in TFE than in TFA. In TFA the fraction of ordered structure has been reduced as compared to that in TFE.

The energy calculations with flexible pyrrolidine ring show that the B-type is slightly preferred over the A-type. The results compare only minimally with those from X-ray structure analysis. The theoretical results, however, compare better when geometrical data as observed in the crystal structure are used. These studies point out the need for bond length and angle deviations in similar energy calculations.

ACKNOWLEDGMENTS

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