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# Solvent-Dependent Conformational Distributions of Some Dipeptides

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**Abstract:** CD and NMR measurements, with the aid of conformational energy calculations, have been used to estimate the solvent-dependent conformational distributions of AcProNHMe, AcAlaNHMe, and L-3-acetamidopyrrolidin-2-one. CD, $^{13}C$ chemical shifts, and $^{1}H$ nuclear Overhauser experiments confirm that the intramolecularly hydrogen bonded $C_7$ conformer of AcProNHMe dominates in nonpolar solvents; the same measurements combine to show that $\alpha$-helical and polyproline II-like conformations become increasingly populated in polar solvents. In acetonitrile the three conformers may be about equally populated; in dimethyl sulfoxide the polyproline II form predominates, and in water the $C_7$ conformer is probably absent, but the ratio of the other two forms is not established. Close similarity of the solvent dependence of the AcAlaNHMe CD to that of the more restricted proline derivative suggests that for AcAlaNHMe the dominant $C_7$ conformation in the nonpolar solvents is also replaced largely by $\alpha$-helical and polyproline II-like conformations in polar solvents. In polar solvents, L-3-acetamidopyrrolidin-2-one appears to favor a conformation of high dipole moment in which intramolecular electrostatic interactions are least favorable. Expectation values of the NMR and CD observables were calculated to test assumptions about peptide–solvent interactions. Intramolecular potential functions alone give conformational distributions that correspond reasonably well to deductions from experiments using nonpolar (chloroform, dioxane) solvents. Attenuation of intramolecular electrostatic interactions reduces the predicted population of $C_7$ conformers of the dipeptide models, but better agreement between calculated and observed measurements is obtained if polarization of the solvent by the peptide is also considered. This favors conformations with large dipole moments, such as those for dipeptides that are $\alpha$-helix-like. However, bulk solvent polarization does not differentiate among polar solvents, e.g., between water and acetonitrile. An effective increase in the size of carbonyl oxygens, resulting from hydration, is also considered. Incorporating this asssumption in conformational energy calculations favors $\alpha$-helical and polyproline II-like conformations of dipeptides.

## Introduction

It is well-known that water affects peptide conformation in several ways. Hydration weakens intrapeptide hydrogen bonds and other intramolecular electrostatic interactions, hydrates polar groups, and promotes hydrophobic clustering of nonpolar side chains. To date, however, there is no practical path from these considerations to predictions of peptide conformation in aqueous solutions.

There have already been numerous theoretical and experimental efforts to describe the details of peptide hydration. Lately, quantitative details of interactions between water and peptides in fixed conformations have been explored by Monte Carlo[2] and molecular dynamics[3] techniques. If it becomes computationally feasible to reach equilibrium states for various peptide conformations, calculations of these types should yield valid predictions of conformational distributions in water. There have also been empirical approaches in which it is practical to allow the peptide conformation to vary. These include the hydration-shell models,[4-6] the earliest of which considered peptide interactions with water molecules in a monomolecular layer around the peptide, but not specifically bonded to it. For *N*-acetyl-L-alanine *N'*-methylamide

Table I. Nomenclature and Approximate Dihedral Angles for Peptide Conformers

| conformer | $\phi, \psi$, deg | polymer and protein structure |
|---|---|---|
| $C_5$ | −150, 150 | $\beta$ sheet |
| $P_{II}$ | −80, 150 | polyproline II |
| $C_7$ | −80, 80 | $\gamma$ turn |
| $\alpha_R$ | −80, −50 | right-handed $\alpha$-helix |

(1) (a) University of Illinois Medical Center. (b) Illinois Institute of Technology.

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(AcAlaNHMe), the most commonly discussed model dipeptide unit, this kind of treatment predicts greatest stability in water for $C_5$ conformers. (See Table I for definitions of conformers[7] referred to in this paper.) Recently water molecules specifically hydrogen bonded to the peptide unit have been added to the hydration shell model; $C_5$ conformers are still predicted to be the most stable.[6] In a second method, the conformational energy is made to include interactions with a solvent continuum. A recent study of this type has used terms dependent on the dipole moment, volume, and surface area of the peptide. This has yielded an energy map for AcAlaNHMe that predicts the most stable conformations in water to be near (−110, −70°)[8] rather than in the $C_5$ region.

A third approach to peptide conformation in water has been to calculate the surface area accessible to water molecules for peptide nitrogen and oxygen atoms.[9] No quantitative predictions based on this treatment have been made. Both oxygens of AcAlaNHMe are accessible to water in conformers near the right-handed $\alpha$-helix, the N-terminal oxygen and C-terminal N–H are exposed for a large part of the upper left quadrant of the $\phi$–$\psi$ map, and both N–H units can be hydrated near the left-handed $\alpha$-helix.

Experimental evidence bearing on peptide bond hydration includes the observations made by Wolfenden of the vapor pressures of acetamides in aqueous solution. These yield the conclusion that the hydrophilic character of a peptide bond is chiefly associated with carbonyl solvation.[10] The importance of C=O relative to N—H hydration has also been brought out by the distribution of water in the rubredoxin crystal structure at 1.2-Å resolution. Of the main-chain N–H units in this 54-residue peptide, slightly less than 25% are hydrogen bonded to water, in contrast to slightly more than 50% of the carbonyl oxygens.[11] A recent survey of oligopeptide crystals[12] containing backbone-water hydrogen bonds also showed C=O water association to be more common than N—H water association, and revealed that the distribution of C=O···$O_w$ angles is centered about 138° rather than the expected 120°.[13] The latter observation demonstrated potential interference to peptide bond hydration by other parts of the joined amino acid residues.[12]

There are few observation-based descriptions of the likely changes in conformational distribution that occur on transfer of small acyclic peptides to water from nonpolar organic solvents. Changes in circular dichroism (CD) or optical rotatory dispersion on transfer between solvents are well-known, but interpretation of the optical data alone leaves many ambiguities.

We have been working to define conformational distributions using the intersection of inferences from CD measurements, nuclear magnetic resonance (NMR) measurements, the solvent dependences of these, and their calculated expectation values. The precision of such most ranges from an estimated ±15% for the population of the dominant $C_7$ conformer in chloroform to qualitative estimates of most likely conformers for acyclic peptides in water.

In this paper we report NMR and CD data for *N*-acetyl-L-proline *N'*-methylamide (AcProNHMe), *N*-acetyl-L-alanine *N'*-methylamide (AcAlaNHMe), and L-3-acetamidopyrrolidin-2-one in a series of solvents that includes polar protic, polar aprotic, and nonpolar liquids.[14] For both AcProNHMe and AcAlaNHMe we conclude from experiments that the $C_7$ conformer (see Table I) dominates in nonpolar solvents. For AcProNHMe, $\alpha_R$ and $P_{II}$ forms are increasingly populated in more polar solvents. Both forms are present in acetonitrile, $P_{II}$ is dominant in dimethyl sulfoxide, and the ratio is uncertain in water. A very similar solvent dependence of the CD spectra for AcAlaNHMe argues for closely similar changes for it as solvent polarity increases. In polar solvents L-3-acetamidopyrrolidin-2-one appears to favor a conformation of high dipole moment with unfavorable intramolecular electrostatic interactions. We have compared these conclusions with predictions in the literature as well as predictions made from simple models which include peptide–solvent interactions. These models include specific hydration of N—H or C=O groups and interactions of an effective peptide dipole with the solvent continuum.

## Experimental Section

**Materials.** **$^{15}N$-Acetyl-DL-alanine *N'*-Methylamide.** DL-Alanine 96% enriched in $^{15}N$ (Prochem) was treated in acetic acid with excess acetic anhydride (50 °C, 15 min). The solution was evaporated at reduced pressure. The residue was dissolved in hot water to destroy mixed anhydrides, again concentrated, and dried by azeotropic distillation using toluene. The $^1H$ NMR spectrum, and its integral, of the crystalline residue confirmed quantitative conversion to the *N*-acetyl derivative. The product was dissolved in dry pyridine and treated with a slight excess of methyl isocyanate. After 1 h at room temperature, $CO_2$ evolution having ceased, the pyridine was removed under reduced pressure and the residue was crystallized from ethyl acetate. The NMR spectrum and its integral confirmed the structure of the *N'*-methylamide.

We have used this procedure, treatment with methyl isocyanate in pyridine, for the conversion of a number of other peptide acids to *N*-methylamides.

L-3-Acetamidopyrrolidin-2-one was prepared from L-2,4-diaminobutyric acid (Vega-Fox) according to Wilkinson.[15]

*N*-Acetyl-L-amino acid *N'*-methylamide derivatives of alanine and proline were obtained from Bachem and recrystallized from ethyl acetate before use.

**Nuclear Magnetic Resonance.** $^1H$ NMR spectra of solutions in water and most other solvents were obtained using the rapid-scan correlation mode of the 250-MHz spectrometer of the Carnegie-Mellon NMR Facility for Biomedical Studies. In a few cases, a Varian CFT-20 instrument, operating at 80 MHz, was used.

Vicinal H–N–$C^\alpha$–H coupling constants of peptides are generally obtained from the peptide proton (N–H) resonances. However, for low molecular weight amides in water, the N–H resonances are usually broadened sufficiently to obscure the splitting of interest. The broadening is not, under most conditions, the result of chemical exchange; it results from the quadrupole relaxation modulation of the coupling between the proton and the directly bound $^{14}N$ nucleus. In this work $^{15}N$-acetylalanine *N'*-methylamide was used to obtain precise coupling constant data. Figure 1 compares the N–H resonances of the normal and isotopically enriched species in water and in methanol. Comparison of the line widths of the two substances in a variety of solvents shows that the broadening in the $^{14}N$ compound is inversely related to solvent viscosity, a result consistent with the expected dependence on rotational correlation time for the nitrogen-containing group.[16]

Proton nuclear Overhauser enhancements (NOEs) in solutions of multiply recrystallized AcProNHMe were made on degassed, sealed samples using the NTC 360 spectrometer of the Purdue University Biochemical Magnetic Resonance Laboratory. The enhancements in $CDCl_3$, $CD_3CN$, and $CD_3SOCD_3$ were obtained from the transformed difference of free induction decays collected during irradiation of the N–H line(s) and during irradiation 200–500 Hz from the nearest N–H line. An experiment in $H_2O$ was carried out using the rapid-scan correlation mode, scanning upfield from above the water resonance, and subtracting the two raw data sets before correlation.

Carbon-13 data were obtained on a Varian CFT-20 instrument at 20 MHz, except for very dilute solutions in chloroform, where measurements were made with the NTC 150 instrument of the Purdue facility.

Measurements of NMR spectra were made at concentrations (given in the tables) sufficiently low that association could be considered unimportant. In most cases it was demonstrated that the property under observation did not change when the concentration of peptide was increased or decreased two- to fivefold. In one favorable case for associ-

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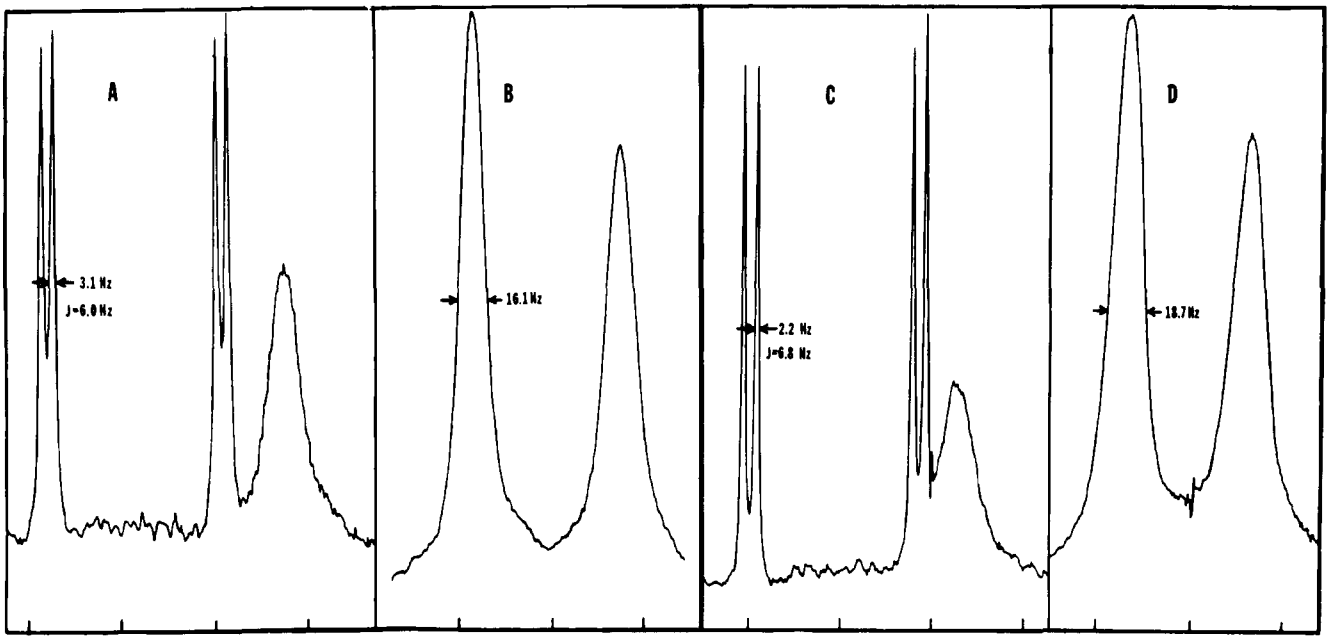


**Figure 1.** N–H resonances of *N*-acetyl-($^{15}$N)alanine *N'*-methylamide and the normal $^{14}$N compound in water and in methanol at about 10 mg/mL and 30 °C. The spectra were recorded without artificial line broadening using the rapid scan correlation mode at 250 MHz. Full widths at half-maximum are indicated on the traces. Scale divisions are 50 Hz. Nitrogen isotope and solvent: A, $^{15}$N, $H_2O$; B, $^{14}$N, $H_2O$; C, $^{15}$N, $CH_3OH$; D, $^{14}$N, $CH_3OH$.

ation between amide groups, *cyclo*-(Pro-Gly), we have shown by vapor pressure osmometry that the dimerization constant is 15 $M^{-1}$ in chloroform at 25 °C.[17] This peptide would be more than 80% monomeric at concentrations less than 0.01 M; we assumed similar association for the compounds under study in the present work, and measurements were restricted to concentrations less than 0.01 M for chloroform solutions.

**Circular Dichroism Measurements.** CD spectra were measured using a Jasco J-40A and/or a Cary 60 with Model 6001 CD attachment. Both instruments were calibrated with solutions made from dry *d*-10-camphorsulfonic acid (Aldrich, Lot KA-81867) which had been three times sublimed under vacuum at 125 °C. Peptide solutions were 0.03–8.0 × $10^{-3}$ M, made up in spectral grade solvents that had been dried over activated molecular sieves. The chloroform used was the Matheson Coleman and Bell product, stabilized with pentene rather than ethanol. Quartz cells with path lengths of 0.01–1.0 cm were used, and spectra were run at ambient temperature, about 22 °C.

**Calculated Circular Dichroism.** The rotatory strength and wavelength of each molecular transition were computed by the configuration interaction method of Schellman and co-workers[18] for each peptide conformation.

Two sets of optical parameters were used for the chromophores: *water parameters*, with original transition wavelengths of 215 nm for the n–$\pi^*$ band and 187 (monosubstituted amide) or 198 nm (disubstituted amide) for the $\pi$–$\pi^*$ band, and *cyclohexane parameters*, with original transition wavelengths of 230 nm for the n–$\pi^*$ band and 185 or 200 nm for the $\pi$–$\pi^*$ bands of mono- or disubstituted amides, respectively. The other parameters in these sets are given in ref 18b.

In calculating expected CD spectra of AcProNHMe and acetamidopyrrolidinone, the cyclohexane parameters were used for the standard potential with $\epsilon = 1$ (see below). The water parameters were used with all other potentials. AcAlaNHMe CD spectra were calculated using only the water parameters.

The molecular coordinates used for calculating CD spectra were those used in computing potential energies (see below).

The expectation values of rotatory strength were obtained by calculating

$$\langle R \rangle = \frac{\sum_{\phi,\psi} R_{\phi,\psi} \exp(-E_{\phi,\psi}/RT)}{\sum_{\phi,\psi} \exp(-E_{\phi,\psi}/RT)}$$

where the conformational energies and the calculated value of the observable were obtained at 10° angular intervals and the temperature was taken as 25 °C.

**Calculation of Expected NMR Observables.** We have used the correlation derived by Ramachandran et al.[19] to relate the H–N–C$^\alpha$–H coupling constant in peptides to the corresponding dihedral angles, viz.

$$^3J_{HNC^\alpha H}\ (Hz) = 6.55 \cos^2\theta - 1.55 \cos\theta + 1.35$$

where $\theta = |60° - \phi|$. Reservations have been expressed about this correlation on the grounds that it relies on observed couplings involving cis peptide bonds for small values of $\theta$. However, a closely similar relationship, $^3J = 5.41 \cos^2\theta - 1.27 \cos\theta + 2.17$, has recently been derived by DeMarco et al.[20] using only observations on trans peptides, including examples with small $\theta$. The two correlations agree within their estimated errors. DeMarco et al.[20] have presented the objections of other proposed correlations.

The difference in chemical shift between C$^\beta$ and C$^\gamma$ in carbon-13 spectra ($\Delta\delta_{\beta\gamma}$) was related to the C$^\beta$–C$^\alpha$–C'–O dihedral angle $\theta$ ($\theta = \psi - 60°$) through the expression $\Delta\delta_{\beta\gamma}$ (ppm) = $0.036|\theta| + 0.73$ proposed for trans peptides by Siemion et al.[21]

Expectation values of $^3J_{HNC^\alpha H}$ and $\Delta\delta_{\beta\gamma}$ were calculated as described for rotatory strengths using the Boltzmann weighting determined by potential-energy calculations.

Predicted nuclear Overhauser enhancements for the *H*–C$^\alpha$–C'(O)–N–*H* system of AcProNHMe were calculated using the expression given by Glickson et al.[22]

$$F_i(S) = \frac{F_H(H) \sum_s r_{is}^{-6}}{\sum_s r_{is}^{-6} + \sum_n r_{in}^{-6}}$$

where $r_{is}$ is the distance between observed and irradiated protons and $r_{in}$ is the distance between observed and unirradiated protons. Extreme narrowing ($F_H(H) = 0.5$ in the above expression) could be assumed. The effective $\tau_c$ for $AcProNH_2$ in $D_2O$ is 2–4 × $10^{-11}$ s,[23] so that at 360 MHz $\omega\tau_c$ is still small, about 0.07. Two sets of interproton distances were used in the calculation, based on the crystal structure coordinates of either *cyclo*-(Ala-Pro-D-Phe)$_2$[24] or AcProNHMe.[25] Proton coordinates were adjusted to make the C–H bond distances 1.10 Å, the N–H bond distance 1.0 Å, and the C–C–H angles of the proline ring 111°. The coordinates

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from the first case gave a maximum NOE at $\psi = 120°$, for example, of 21%, from the second, 27%. The means of the two calculations are cited in the Results section.

**Conformational Energies.** For AcAlaNHMe our computer programs were adapted to use the method of the empirical conformational energy program for peptides (ECEPP) of Scheraga and co-workers.[26] This method includes a Lennard-Jones 6-12 potential in which the repulsive term is halved for 1–4 interactions, a Coulombic potential using charge monopoles on the atoms, and a hydrogen-bonding potential. We did not use any torsional potentials. The atomic coordinates, monopole charges, and Lennard-Jones parameters were those of ECEPP.

For AcProNHMe, conformational energies were taken from a previous study[27] which included flexibility of the bonded skeleton through the consistent force field (CFF) method of Lifson and co-workers.[28] Additional computations were carried out to approximate the effects of polar solvents (see below) using the ECEPP method and atomic coordinates based on a crystal structure of Leung and Marsh.[29]

For acetamidopyrrolidinone, the CFF method was utilized for all computations with the parameters previously reported[27] and monopole charges based on Smyth's bond moments.[30] The CFF calculations on the pyrrolidinone give two minimum-energy ring conformations. Only one of these is indicated by NMR data and that conformation is used in subsequent calculations of conformational distributions and expectation values.

The variations we applied to the above calculations to approximate the influences of polar solvents or water on peptide conformations are described below.

**A. Standard Potential.** This refers to the ECEPP or CFF potentials as described. The intramolecular electrostatic energy was maximized or neglected by setting the effective dielectric constant for intrapeptide Coulombic interactions at 1 or ∞.

**B. Solvated Dipole.** The potential energy of interaction between a solvent continuum and the net molecular dipole of the peptide was added to the energy computed from the standard potential with $\epsilon = \infty$. The solvent continuum–peptide dipole energy was computed assuming a point dipole in the center of a spherical cavity, using the expression

$$U\ (\text{kcal/mol}) = -14.4\ \frac{\mu^2}{R^3}\ \frac{\epsilon - 1}{2\epsilon + 1}$$

where $\mu$ is the peptide dipole moment in debyes, $R$ is the cavity radius in angstroms, and $\epsilon$ is the dielectric constant of the continuum.[31] A dielectric constant of 80, appropriate to water, was used for this term. $R$ was taken as half the $C^{\alpha(0)}$–$C^{\alpha(2)}$ separation plus the van der Waals radius of a carbon atom, where the two $C^{\alpha}$'s are the terminal methyl carbons of AcProNHMe and AcAlaNHMe, and the methyl and $C^5$ carbons of acetamidopyrrolidinone. The dipole moment was calculated as the sum over all atoms of $q_i r_i$, where $q_i$ is the atomic change (esu × $10^{10}$) and $r_i$ is the position vector in angstroms.

**C. Big Oxygen.** The standard potential with $\epsilon = \infty$ was used except that the van der Waals radius of oxygen was increased by 1 Å to emphasize conformers in which there is space about the carbonyl oxygens for the approach of solvent molecules.

**D. Waters on Oxygen.** The standard potential was used for a supermolecule containing one water molecule attached by a linear hydrogen bond to each carbonyl oxygen in the plane of the peptide. In accord with the observed preferences for peptide carbonyl hydration in crystals[12] the C′=O···$O_w$ angle was set at 140° and the O···$O_w$ distance at 2.8 Å. The water molecules were placed on the N side of the acetyl carbonyl and the $C^{\alpha}$ side of the amino acid carbonyl so that they would have the maximum effect on the conformational distribution.

**E. Oxygen on N–H.** The standard potential was used for a supermolecule in which an oxygen atom is attached collinearly to each peptide N–H at an N···O distance of 2.8 Å.

## Results and Discussion

***N*-Acetyl-L-proline *N*′-Methylamide.** In proline peptides the N–$C^{\alpha}$ dihedral angle, $\phi$, is restricted by the pyrrolidine ring to near −60°, and the principal degree of torsional freedom is in the dihedral angle $\psi$ about the $C^{\alpha}$–C′ bond. Infrared studies of AcProNHMe have shown that in dilute solution in carbon tetrachloride 90% of the amide protons are hydrogen bonded.[32] Circular dichroism spectra in nonpolar solvents show a large negative n–$\pi^*$ band which has maximum ellipticity in cyclohexane solution. From these data the existence of a dominant, hydrogen-bonded $C_7$ conformer (Table I) in nonpolar solvents has been inferred. The contribution of NMR spectroscopy for this compound has been to define the ratio of cis and trans isomers of the acetyl–proline bond.[33,34] Trans is favored.

In the present study, CD spectra of AcProNHMe were measured in a variety of solvents. The spectra are compared in Figure 2. For solutions in water, dioxane, and cyclohexane (not shown) our ellipticities were about 25% greater than those reported previously.[33] The n–$\pi^*$ CD band near 225 nm is largest in cyclohexane and chloroform, less in dioxane, and still less in acetonitrile. In the protic solvents, water, methanol, and *tert*-butyl

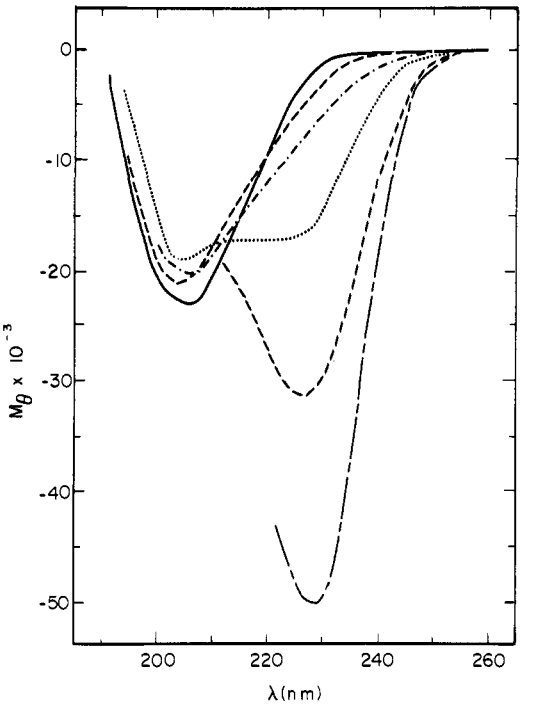


**Figure 2.** Circular dichroism spectra (molar ellipticity) for AcProNHMe in the following solvents: water, —; methanol, - - -; *tert*-butyl alcohol, - · - · -; acetonitrile, ···; 1,4-dioxane, ~~~; chloroform, — · — · —.

Table II. $^{13}C^{\beta}$–$^{13}C^{\gamma}$ Chemical Shift Difference and $H^{\alpha}$ NOE for Acetylproline Amide and *N*-Methylamide in Various Solvents

| solvent | | concn, M | % trans[a] | S($C^{\beta}$–$C^{\gamma}$) trans | S($C^{\beta}$–$C^{\gamma}$) cis | $F_{H^{\alpha}}$–(NH)[b] |
|---|---|---|---|---|---|---|
| $D_2O$ | ($-NH_2$) | 0.25 | 75 | 5.93 | 9.21 | |
| | ($-NHCH_3$) | 0.25 | 70 | 5.81 | 9.05 | |
| $CD_3SOCD_3$ | ($-NH_2$) | 0.25 | 65 | 5.43 | 9.19 | |
| | ($-NHCH_3$) | 0.06 | 65 | 5.40 | 9.15 | 0.09 |
| $CD_3OD$ | ($-NH_2$) | 0.17 | 75 | 5.64 | 9.42 | |
| | ($-NHCH_3$) | 0.25 | 75 | 5.52 | 9.28 | |
| $CD_3CN$ | ($-NHCH_3$) | 0.25 | 80 | 4.72 | 9.08 | 0.07 |
| $CDCl_3$ | ($-NHCH_3$) | 0.008 | 80 | 2.01 | | 0.05 |
| $C_4H_8O_2$[c] | ($-NHCH_3$) | 0.20 | 75 | 3.13 | 9.03 | |

[a] Rounded to nearest 5%. [b] Nuclear Overhauser enhancement of α proton of trans form upon irradiation of corresponding N–H resonance. Solutions 0.03 M. [c] 1,4-Dioxane.

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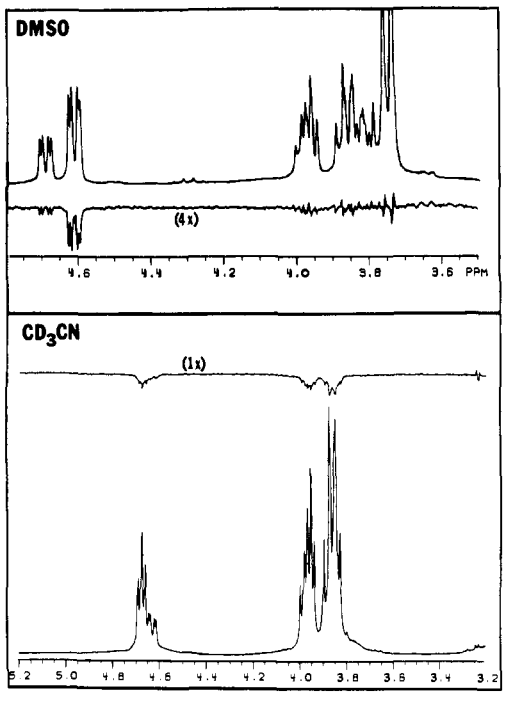


**Figure 3.** Nuclear Overhauser enhancements on irradiating the amide proton of AcProNHMe in dimethyl-$d_6$ sulfoxide and acetonitrile-$d_3$. The $\alpha$- and $\delta$-proton resonances at 360 MHz are shown. In obtaining the positive spectra, decoupling power was applied off the amide proton (N–H) resonances. The negative traces are the transformed differences between the accumulated free induction decays with off N–H resonance irradiation and irradiation on the N–H resonances. (The enhancement is positive.) In the dimethyl sulfoxide case only the amide proton of the major (trans) component is irradiated; the small effect observed for the $\alpha$ proton of the cis form is the result of exchange. The NOE of the major $\alpha$-proton resonance is 9%; an estimate of the maximum NOE for the $\delta$-proton resonance region or the $\beta$-, $\gamma$-proton region (not shown) is 1%. For the $CD_3CN$ solution amide protons of both cis and trans forms are simultaneously irradiated, since the resonances are broad and overlap. The NOE for the $\alpha$ protons is 7% trans, 5% cis and for the total $\delta$ protons 5%. For the $\beta$, $\gamma$ regions (not shown) it is 5–6% (total).

alcohol, there is no minimum in the n–$\pi^*$ region, and the spectra are dominated by a $\pi$–$\pi^*$ band near 200 nm. Curve fitting revealed $R_{n-\pi^*} = -0.02$ DM in water.[33] If a large negative n–$\pi^*$ band reflects the $C_7$ conformation of AcProNHMe, that conformation is depopulated as the polarity or hydrogen-bonding ability of the solvent is increased.

We have been able to parallel these CD data with $^{13}C$ chemical shift observations and proton nuclear Overhauser effects which also reflect solvent-dependent changes in the conformational distribution of AcProNHMe.

Siemion, Wieland, and Pook proposed a correlation between $\psi$ and the $C^\beta$–$C^\gamma$ chemical shift difference $\Delta\delta_{\beta\gamma}$ for both cis and trans proline peptides.[21] We measured $\Delta\delta_{\beta\gamma}$ for AcProNHMe (and $AcProNH_2$) in a range of solvents (Table II). Notably, $\Delta\delta_{\beta\gamma}$ for the trans isomer varies from 5.8 ppm in water to 2.0 ppm in chloroform. The decrease in this parameter in the series of solvents water, methanol, acetonitrile, dioxane, chloroform parallels the increase in the negative n–$\pi^*$ CD band for that series. For the five solvents, $\Delta\delta_{\beta\gamma}$ vs. the molar ellipticity at 225 nm fits a line with a correlation coefficient of 0.99. Dimethyl sulfoxide, for which CD data cannot be obtained, shows a value of $\Delta\delta_{\beta\gamma}$ close to that of methanol. Further, because the fraction of cis isomers is always between 20 and 30%, and the value of $\Delta\delta_{\beta\gamma}$ for the cis isomer remains effectively constant, the measurements suggest that the contribution of the cis isomer to the CD spectra is nearly constant.

It is extremely likely, therefore, that both the CD and $^{13}C$ chemical shift observations indicate chiefly the distribution of the $\psi$ dihedral angle of the trans form of AcProNHMe.

To obtain further information about the $\psi$ distribution, we measured the nuclear Overhauser enhancement (NOE) of the

Table III. Computed Conformational Properties for *trans*-AcProNHMe

| potential function[a] | $\epsilon$ | $\langle\Delta\delta_{\beta\gamma}\rangle$, ppm | $\langle R_{n-\pi^*}\rangle$, DM | % of population for $\psi$[b] centered at −50° ($\alpha_R$) | 80° ($C_7$) | 150° ($P_{II}$) |
|---|---|---|---|---|---|---|
| standard | 1 | 1.7 | −0.094 | 7 | 88 | 5 |
| standard | ∞ | 3.7 | −0.003 | 12 | 14 | 74 |
| solvated dipole | ∞ | 4.2 | −0.025 | 62 | 6 | 32 |
| big oxygen | ∞ | 4.2 | −0.030 | 93 | 0 | 7 |
| oxygen on N-H | ∞ | 3.5 | +0.016 | 0 | 0 | 100 |
| observed: | | | | | | |
| in chloroform | | 2.0 | −0.40 | | | |
| in water | | 5.8 | −0.02 | | | |

[a] Standard potential from ref 27 computed by the CFF method for the two ring conformers. Energy for solvation of dipole computed using coordinates of Leung and Marsh (ref 29, $\phi = -68°$) and added to standard CFF energy with $\epsilon = \infty$. Energies for big oxygen on N-H potentials computed by the ECEPP method using Leung and Marsh coordinates. [b] Includes population within ±30° of given $\psi$ values.

$\alpha$-proton resonance when the amide-proton (N–H) resonance is irradiated. The measurement was made for the solvents with which amide protons cannot exchange: dimethyl sulfoxide, acetonitrile, and chloroform. The results are shown in Table II. For comparison, the calculated NOEs for various conformers are approximately 20% for $P_{II}$, 25% for $\psi$ = 120° (closest approach of $\alpha$ and amide protons), 10% for $C_7$, and 2% for $\alpha_R$.

The observed 5–9% enhancements at the $\alpha$ proton (Figure 3, Table II), while less in any case than those calculated for the $C_7$ or $P_{II}$ conformations, are in the direction of an increased population of the latter form in the more polar solvents. More significant is the fact that comparable enhancements are also observed for the multiplets of the $\beta + \gamma$ (5–6%) and $\delta$ (5%) protons in acetonitrile, but not in dimethyl sulfoxide or chloroform (<1%). Attempts at quantitative interpretation are probably not worthwhile, but only the $\alpha_R$ range of conformations should give rise to enhancements of the $\beta$, $\gamma$, and $\delta$ resonances. In the $\alpha_R$ conformation the N–H proton is close to the $\beta$, $\gamma$, and $\delta$ protons on one side of the pyrrolidine ring. In the other conformations it is not. The observations of such enhancement is consistent, then, with an increased contribution of the $\alpha_R$ conformation in acetonitrile. The absence of observed ($\beta + \gamma$) and $\delta$ proton enhancements in dimethyl sulfoxide suggests that the $\alpha_R$ conformation is absent in that solvent. In dimethyl sulfoxide, a basic solvent, N–H–solvent hydrogen bonding is more important than in acetonitrile. Solvation of the N–H is hindered by the pyrrolidine ring in the $\alpha_R$ form but not in the $P_{II}$ form.

The intense resonance of water precluded observation of an NOE for the $\alpha$ proton in that solvent, but using the rapid-scan correlation method it was possible to examine the ($\beta + \gamma$) and $\delta$ resonances. No (<1%) NOE was detected for these in water. This result may indicate unimportance of the $\alpha_R$ form in water, as it does for dimethyl sulfoxide solutions. However, interpretation of the NOEs in water is highly tentative since the 110 M solvent protons may provide the principal relaxation path and intramolecular NOEs might be negligible for any conformation.

Conformational energy calculations from previous work and those using the additional parameter sets described in the Experimental Section yield predicted $\psi$ distributions for AcProNHMe and expectation values for the rotatory strength and $C^\beta$–$C^\gamma$ chemical shift difference. These results appear in Table III, where they may be compared with observations. The potential energy vs. $\psi$ plots for most of the parameter sets are given in Figures 4A and 4B. Among the models tested, the standard potential with $\epsilon$ = 1 places nearly 90% of the molecules in the $C_7$ region, and predicts the most intense negative n–$\pi^*$ band and the smallest chemical shift difference. That this model most nearly matches the observations in chloroform solution is not an unexpected result.

As Figures 4C and 4D show, any departure from a distribution centered around the $C_7$ conformation will tend to decrease the

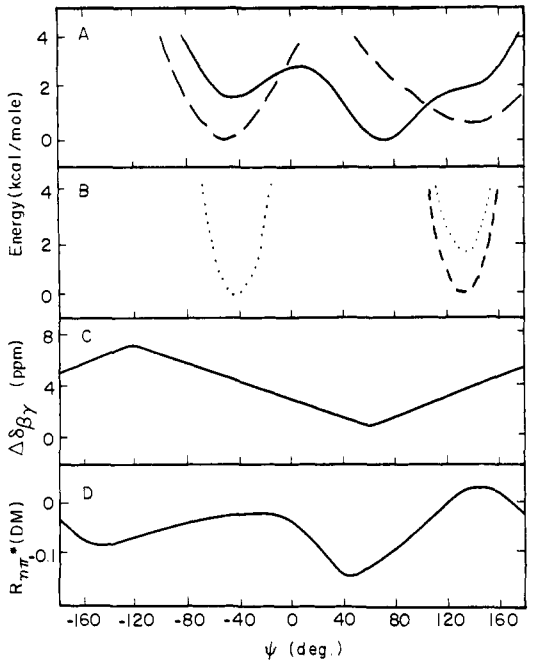


**Figure 4.** Computer conformational properties for AcProNHMe. (A) Intramolecular potential energy computed by the CFF method using the standard potential ($\epsilon = 1$),[27] lower energy of two ring conformers plotted —; solvated dipole model, dipole solvation energy calculated using Leung and Marsh coordinates added to standard CFF energy ($\epsilon = \infty$) ---. (B) Intramolecular energies computed by the ECEPP method using Leung and Marsh coordinates: big oxygen potential, ···; oxygen on N–H potential, - - -. (C) Predicted carbon-13 chemical shift difference $\Delta\delta_{\beta\gamma}$ (ref 21). (D) Predicted n–$\pi^*$ rotatory strength using cyclohexane optical parameters and CFF-calculated coordinates with $\phi = -53°$.

negative n–$\pi^*$ rotatory strength and increase $\Delta\delta_{\beta\gamma}$. This will move the predicted values toward those observed for solutions in polar solvents. The standard potential ($\epsilon = \infty$) and modifications that we examined tend to populate $P_{II}$ and/or $\alpha_R$ regions, but unfortunately, these regions are not readily distinguished on the basis of $\Delta\delta_{\beta\gamma}$.

The nuclear Overhauser enhancements indicate that in acetonitrile both $P_{II}$ ($\alpha$-H NOE) and $\alpha_R$ ($\beta$-, $\gamma$-, $\delta$-H NOE) regions are populated, and the magnitude of the negative n–$\pi^*$ band for solutions in acetonitrile suggests that perhaps 30% remains in the $C_7$ conformation. Assuming that the experimental data for acetonitrile can be read that the three regions are populated about equally, reference to Table III suggests that a combination of the standard and solvated dipole potentials with properly adjusted values of the dielectric-constant parameter could describe the situation.

The importance of $P_{II}$ forms in dimethyl sulfoxide, indicated by the NOE observations, is predicted by the oxygen on the N–H model, as might be anticipated.

For aqueous solutions the n–$\pi^*$ CD and the $\beta$–$\gamma$ carbon chemical shift data combine to suggest that the $C_7$ conformation is absent. The lack of a positve n–$\pi^*$ band indicates that the population is not entirely in the $P_{II}$ form, but it is not possible to estimate the balance between $P_{II}$ and $\alpha_R$ forms.

These results for AcProNHMe are important to the interpretation of the measurements made on AcAlaNHMe.

***N*-Acetyl-L-alanine *N'*-Methylamide.** The prototype dipeptide model, with torsional freedom about both N–$C^\alpha$ ($\phi$) and $C^\alpha$–C' ($\psi$), has been the subject of numerous studies. Investigations of its optical activity[35] include a spectrum extended into the vacuum ultraviolet region.[36] Nielsen and Schellman[18b] suggested that a small positive n–$\pi^*$ rotatory strength for AcAlaNHMe in water

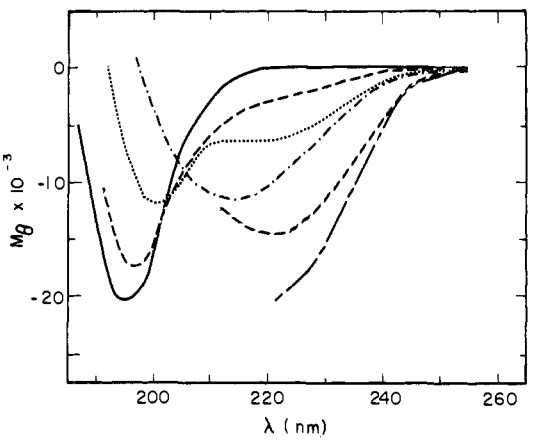


**Figure 5.** Circular dichroism spectra for AcAlaNHMe in the following solvents: water, —; methanol, - - -; acetonitrile, ···; *tert*-butyl alcohol, - · - ·; 1,4-dioxane, – – –; chloroform, — · — ·.

indicates a population of $P_{II}$ conformers. This is the principal allowed conformational region for which $R_{n-\pi^*}$ is predicted to be positive. (The left-handed $\alpha$-helical region is the only other.) In dioxane $R_{n-\pi^*}$ is negative, and conformers such as $C_7$ and/or $C_5$ were inferred from this. Analyses of the N–H stretching absorptions of AcAlaNHMe and other dipeptide models in dilute carbon tetrachloride solution have been made,[32b,c] and it is concluded that under these conditions the molecule exists about 70% in the $C_7$ form[32b,c] and about 30% in the $C_5$ form.[32c,d] The $C_7$ form is reduced to about 25% in chloroform according to one study[32b] and to 50% in dichloroethane according to another.[37] The H–N–$C^\alpha$–H proton–proton coupling constants have been interpreted as being consistent with the dominance of the $C_7$ conformation in chloroform, but it should be noted that the coupling constants are not very different in other organic solvents, e.g., dimethyl sulfoxide.

Our CD spectra of AcAlaNHMe in the range of solvents are shown in Figure 5. Their strong similarity to the spectra for AcProNHMe (Figure 2) should be noted. In the light of the corroborative $C^\beta$–$C^\gamma$ chemical shift evidence it is certain that the negative n–$\pi^*$ band shown in AcProNHMe in nonpolar solvents is associated with the $C_7$ conformation. It is the simplest assumption that this band is also associated with the $C_7$ conformation in AcAlaNHMe. In fact, the maximum negative $R_{n-\pi^*}$ for AcAlaNHMe is calculated to occur at the $C_7$ conformation. The molar ellipticity of AcAlaNHMe in chloroform is -20 000° near 220 nm. This is about half the magnitude of the n–$\pi^*$ band observed for the proline analogue, in agreement with infrared data that suggests a smaller fraction of $C_7$ conformation for the acyclic compound.[32] The n–$\pi^*$ band of AcAlaNHMe decreases in the order chloroform, dioxane, *tert*-butyl alcohol, acetonitrile, methanol, water. Except for inversion of the *tert*-butyl alcohol–acetonitrile pair, this order is the same as was observed for AcProNHMe, and is consistent with the expected depopulation of the $C_7$ conformation as intramolecular hydrogen bonding becomes less important relative to interactions with solvent.

In the most polar solvents (water, methanol, and acetonitrile), there is a substantial negative $\pi$–$\pi^*$ band for AcAlaNHMe, just as for AcProNHMe. In water and methanol this band is about the same intensity, -20 000°, for the two compounds. Taken with the parallel reduction of the negative n–$\pi^*$ band in polar solutions of the two molecules, this suggests that there may be similar distributions about $\phi$ and $\psi$ for the alanine and proline peptides in polar solvents; i.e., that the most important change in going from chloroform to water is probably in the distribution of $\psi$. A shift from $C_7$ to $C_5$ would only coincidentally produce the same changes in $\pi$–$\pi^*$ rotatory strength as those observed for AcProNHMe, where such a shift is impossible.

Table IV gives the H–N–$C^\alpha$–H coupling constants for AcAlaNHMe in our solvent series, measured from the N–H proton

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Table IV. $HNC^{\alpha}H$ Coupling Constant of AcAlaNHMe in Various Solvents

| solvent | $^3J_{HNC^{\alpha}H}$, Hz (concn, M)[a] | solvent | $^3J_{HNC^{\alpha}H}$, Hz (concn, M)[a] |
|---|---|---|---|
| $H_2O$ | 6.0 (0.09) | $(CH_3)_3COH$ | 7.6 (0.09) |
| $CD_3SOCD_3$ | 7.6 (0.09) | $CDCl_3$ | 7.5 (0.0007) |
| $CD_3OH$ | 6.8 (0.09) | $C_4H_8O_2$[b] | 8.0 (0.02) |
| $CD_3CN$ | 7.3 (0.006) | | |

[a] Temperature about 30 °C, 250-MHz correlation spectroscopy; direct observation of $^{15}N$-H resonance, $J \pm 0.05$ Hz. [b] 1,4-Dioxane.

resonances of material prepared from $^{15}N$-enriched alanine. As Figure 1 shows, there are no uncertainties because of overlap of the doublet components. The coupling constant, $J_{obsd}$, is 7.5 Hz in dilute chloroform solution, which would correspond to $\phi = -90$ or $-150°$ if a single conformation is important. The $-90°$ value is consistent with the preponderance of the $C_7$ conformer that is to be inferred from the CD data, and the $-150°$ value corresponds to the $C_5$ form that has been postulated from infrared data. $J_{obsd}$ remains between 7.3 and 8.0 Hz in all of the solvents save methanol (6.8 Hz) and water (6.0 Hz). It should be noted that the dimethyl sulfoxide values, therefore, cannot be taken as characteristic of aqueous solutions for computational purposes. The decrease on going to water must indicate either a slight shift in the weighted average $\langle\phi\rangle$ to near $-75°$ or a major shift in $\langle\phi\rangle$ with considerable contribution from molecules near $-165°$. As just argued, similarity of AcAlaNHMe and AcProNHMe CD spectra in water strongly suggests the smaller shift in $\langle\phi\rangle$, to retain most of the AcAlaNHMe molecules in conformations allowable also for AcProNHMe. It appears that the effect of water is to reduce the population in the vicinity of $\phi = -120°$, where the coupling constant is at a maximum. This may occur because close $H^{\alpha}\cdots O{=}C$ approach in the vicinity of $\phi = -120°$ may hinder hydration of the acetyl carbonyl oxygen.

Qualitatively, the experimental data suggest to us that the $C_7$ population of AcAlaNHMe in chloroform goes to $\alpha_R$ and $P_{II}$ forms in water, and that any $C_5$ population present remains approximately constant. $R_{n-\pi^*}$ has a small negative value for conformations in the $\alpha_R$ region, so that contributions from the $P_{II}$ region are necessary if $R_{n-\pi^*}$ is to become positive, as observed in water at 25 °C[18b] and in ethanol at −90 °C.[38] Reference to a $\phi$–$\psi$ energy map with electrostatic energies removed, e.g., Figure 6B (see also the $J$ vs. $\phi$ plot of Figure 6A), shows that a shift to $\psi$ near $-50$ or $150°$ will provide more allowable conformations with less negative values of $\phi$ and thus could contribute to smaller values of $J_{obsd}$.

To identify factors influencing the conformational distribution in polar solvents, especially water, we made conformational energy calculations to obtain expectation values of $J_{HNC^{\alpha}H}$ and $R_{n-\pi^*}$ for several models of solvent interaction. The results appear in Table V and Figure 6. The $\phi$–$\psi$ plots in Figure 6 contain only the $-180° < \phi < 0°$ range, since, as Table V shows, the predicted population in the left-handed $\alpha$-helical region is negligible in all cases.

As in the proline case, the expectation values when the distribution was calculated using the standard potential and $\epsilon = 1$ are the best match to the observations of the chloroform solutions: $J_{obsd} = 7.5$, $\langle J\rangle = 7.4$ Hz and $R_{obsd} = -0.16$, $\langle R_{n-\pi^*}\rangle = -0.13$ DM. ($\langle R\rangle$ depends on the optical parameter set. Since only water parameters (see Experimental Section) were used for AcAlaNHMe, the apparent better agreement here than for AcProNHMe is not significant.) The predicted 57% $C_7$ conformer is also in rough agreement with the estimates from infrared spectra.[32,37] The observations in aqueous solutions, $R_{n-\pi^*} = +0.02$ DM, $J = 6.0$ Hz, were not so closely matched by any of the models.

Three of the models reduce $\langle J\rangle$ and $\langle R_{n-\pi^*}\rangle$ to approach the observed values in aqueous solution and retain 70% or more of the molecules in the range of $\phi$ accessible also to proline, and are thus most consistent with the experimental data. These are the solvated dipole (case 3 of Table V), big oxygen (case 4), and waters on oxygen models (case 6), and they probably contain the important elements of a useful peptide hydration model. Neglect of intramolecular electrostatic interactions and solvent (case 2) produces a rather even distribution of 80% of the molecules over the upper left quadrant of the $\phi$–$\psi$ map, which is very similar to the result of the solvation shell plus specific hydration model of Nemethy, Hodes, and Scheraga,[6] and does not reduce $\langle J\rangle$ or $\langle R_{n-\pi^*}\rangle$ so effectively as the first mentioned three models. An estimate made from the published maps of Hopfinger's hydration shell model[39] emphasizes the $C_5$ conformation (case 7 of Table V).

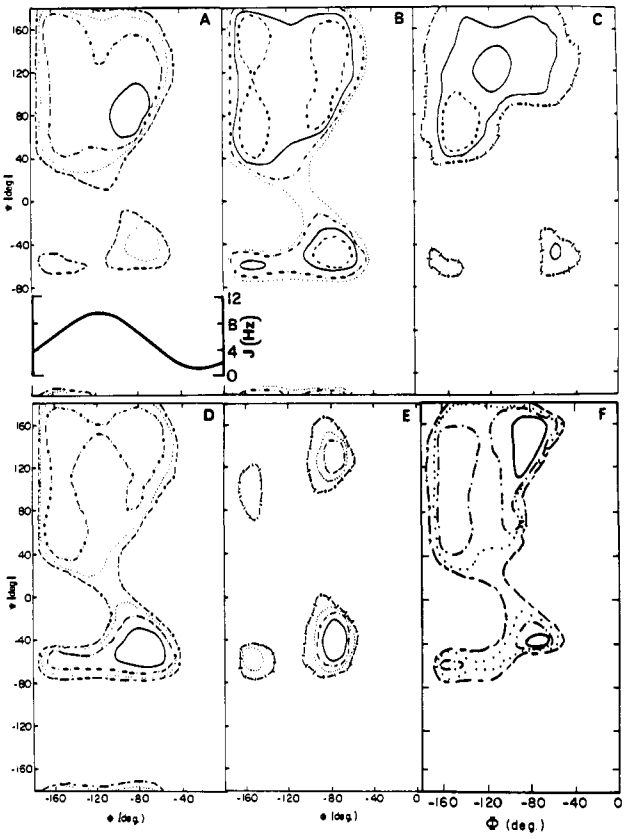


Figure 6. Potential-energy contours computed for AcAlaNHMe by the ECEPP method. The height in kcal/mol above the minimum is indicated by lines of different style: ---, 0.5; —, 1; - - -, 2; ···, 3; — · —, 4; - - -, 5. (A) Standard potential ($\epsilon = 1$). Predicted $^3J_{HNC^{\alpha}H}$ inset, ref 19. (B) Standard potential ($\epsilon = \infty$). (C) Oxygen on N–H. (D) Solvated dipole. (E) Big oxygen. (F) Waters on oxygen.

**L-3-Acetamidopyrrolidin-2-one** has a molecular geometry that constrains $\psi$ to the region near $-140°$ and precludes intramolecular hydrogen bonds. The principal degree of freedom is in the $\phi$ torsion. The optical rotatory dispersion (ORD) in water and in dioxane was studied by Nielsen and Schellman[18b] and $R_{n-\pi^*}$ was reported to be −0.19 DM in water and −0.03 DM in dioxane. $R_{\pi-\pi^*}$ was given as less than 0.01 DM in either solvent. From these optical properties and computed conformational energies, Nielsen and Schellman concluded that conformers with $\phi \sim -100°$ are important in water, and that a mixture of conformers with $\phi \approx 180$ and $60°$ occurs in dioxane. The weak rotatory strength in dioxane was described as resulting from cancellation of oppositely signed contributions from the $\sim 180$ and $\sim 60°$ conformations.

Circular dichroism spectra of acetamidopyrrolidinone are presented in Figure 7. They are in agreement with the ORD results for water and dioxane. In water the molar ellipticity of the $n$–$\pi^*$ band is $-24\,000°$ at 210 nm. In other solvents there is a progressive decrease in its magnitude, in the order water,

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Table V. Computed Conformational Properties for AcAlaNHMe

| potential function | ⟨J⟩, Hz[a] | ⟨$R_{n-\pi^*}$⟩, DM | % population in regions[b] $C_5$ | $P_{II}$ | $2_1$ | $C_7$ | brdg | $\alpha'$ | $\alpha_R$ | $\alpha_L$ |
|---|---|---|---|---|---|---|---|---|---|---|
| standard ($\epsilon = 1$) | 7.4 | −0.13 | 16 | 10 | 14 | 57 | 1 | 0 | 1 | 0 |
| standard ($\epsilon = \infty$) | 7.2 | −0.07 | 21 | 24 | 22 | 15 | 2 | 2 | 12 | 1 |
| solvated dipole | 6.8 | −0.05 | 11 | 14 | 11 | 4 | 0 | 5 | 50 | 2 |
| big oxygen | 6.2 | −0.04 | 0 | 10 | 0 | 1 | 0 | 2 | 88 | 0 |
| oxygen on N–H | 7.4 | −0.07 | 16 | 27 | 44 | 9 | 0 | 0 | 2 | 0 |
| waters on oxygen | 7.1 | −0.02 | 15 | 54 | 14 | 5 | 0 | 1 | 10 | 0 |
| solvation shell[39] | 7.3 | −0.07 | 40 | 15 | 12 | 11 | 5 | 12 | 3 | 0 |
| observed: | | | | | | | | | | |
| chloroform | 7.5 | −0.16 | | | | | | | | |
| water | 6.0 | +0.02 | | | | | | | | |

[a] $^3J_{HNC^\alpha H}$. [b] Regions defined as ($\phi - 30°$ to $\phi + 30°$, $\psi - 30°$ to $\psi + 30°$. ($\phi$, $\psi$) at the center of each region: $C_5$ (−150, 150°), $P_{II}$ (−80, 150°), $2_1$ (−150, 80°), $C_7$ (−80, 80°), brdg (−110, 10°), $\alpha'$ (−150, −50°), $\alpha_R$ (−80, −50°), and $\alpha_L$ (60, 60°).

Table VI. Vicinal Proton-Proton Coupling Constants for Acetamidopyrrolidinone in Various Solvents[a]

| solvent | concn, M | $^3J_{HNC^\alpha H}$ | $^3J_{HC^3C^4H}$ |
|---|---|---|---|
| $H_2O$ | 0.1 | 7.9 | Σ = 19.3 |
| $CD_3SOCD_3$ | 0.035 | 8.0 | 8.5, 10.5 |
| $CD_3OH$ | 0.07 | 8.3 | 8.6, 10.2 |
| $CD_3CN$ | 0.007 | 8.0 | 8.0, 10.5 |
| $CD_3COCD_3$ | 0.035 | 7.8 | 7.8, 10.6 |
| $(CH_3)_3COH$ | 0.07 | 7.6 | |
| $CDCl_3$ | 0.008 | 5.3[c] | 8.8, 10.9 |
| $C_4H_8O_2$[b] | 0.0035 | 6.1 | 8.2, 10.7 |

[a] Coupling constants in Hz, ±0.2 Hz, obtained from α(3)-proton resonance. [b] 1,4-Dioxane. [c] 5.5 ± 0.5 Hz at $8 \times 10^{-4}$ M.

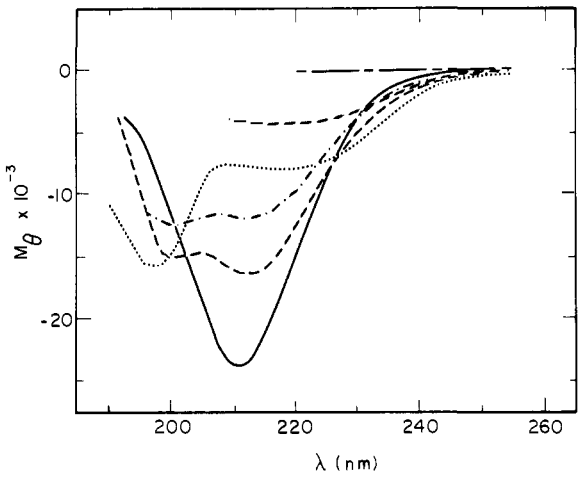


Figure 7. Circular dichroism spectra for acetamidopyrrolidinone in the following solvents: water, —; methanol, - - -; *tert*-butyl alcohol, - · - · -; acetonitrile, ···; 1,4-dioxane, – – –; chloroform, — · — · —.

methanol, *tert*-butyl alcohol, acetonitrile, dioxane, chloroform. This sequence of solvent dependence is the reverse of that shown by AcProNHMe. In chloroform the n–π* ellipticity is near zero. In acetonitrile a distinct negative π–π* band is visible at 200 nm.

$^1$H NMR studies of acetamidopyrrolidinone (Table VI) show that the vicinal H–$C^3$–$C^4$–H coupling constants are, within error, independent of solvent, and that therefore the ring conformation is constant. The observed couplings, ca. 8 and 10.5 Hz, indicate a positive $C^2$–$C^3$–$C^4$–$C^5$ dihedral angle of 20–30° for the pyrrolidinone ring,[40] which corresponds to the ring conformer observed in crystals of 2-oxopyrrolidine-5-carboxamide (pyroglutamide).[41]

(40) Kopple, K. D.; Wiley, G. R.; Tauke, R. *Biopolymers* **1973**, *12*, 627–636.
(41) Molin-Case, J. A.; Fleischer, E.; Urry, D. W. *J. Am. Chem. Soc.* **1970**, *92*, 4728–4730.

Table VII. Computed Conformational Properties for Acetamidopyrrolidinone

| potential function | ε | ⟨J⟩, Hz[a] | ⟨$R_{n-\pi^*}$⟩, DM | % population for φ centered at[b] −160° | −70° | +60° |
|---|---|---|---|---|---|---|
| standard | 1 | 5.7 | 0.02 | 87 | 9 | 4 |
| standard | ∞ | 6.6 | −0.02 | 53 | 41 | 6 |
| solvated dipole | ∞ | 6.7 | −0.05 | 30 | 65 | 5 |
| big oxygen | ∞ | 6.4 | 0.01 | 68 | 32 | 0 |
| big oxygen + solvated dipole | ∞ | 6.8 | −0.01 | 54 | 46 | 0 |

[a] $^3J_{HNC^\alpha H}$. [b] Includes population within region ±40° from given value.

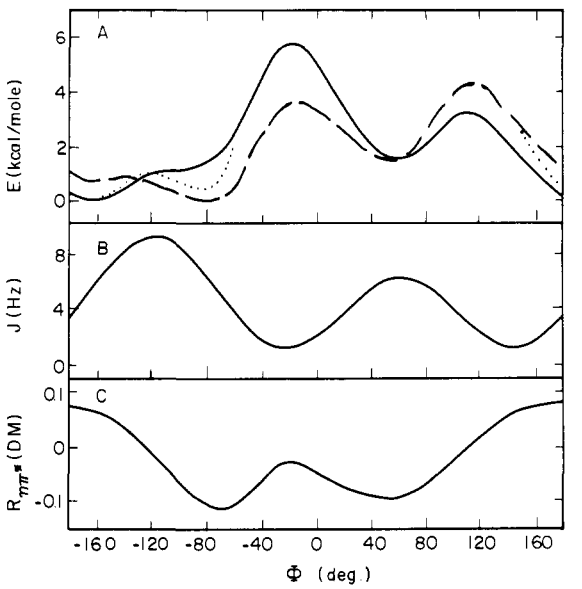


Figure 8. Computed conformational properties for acetamidopyrrolidinone. (A) Intramolecular potential energies computed by the CFF method: standard potential ($\epsilon$ = 1), —; solvated dipole, - - -; big oxygen, ···. (B) Predicted $^3J_{HNC^\alpha H}$ (ref 19). (C) Predicted n–π* rotatory strength using water optical parameters.

The same ring conformer is predicted to be the more stable for acetamidopyrrolidinone in the $160° < \phi < -40°$ range.

The H–N–$C^\alpha$–H coupling constant is observed to be near 8 Hz in all of the polar solvents, even though the CD spectra signal considerable variation in the conformational distribution. Only in chloroform and dioxane does the observed coupling constant reflect changes in the conformational distribution.

Our observations are consistent with earlier conformational conclusions of Nielsen and Schellman.[18b] We have attempted to reproduce the experimental conformational distribution through

computations. Steric interactions are minimal for regions near −160 and −70°. In the region near −70° there is electrostatic repulsion between carbonyl oxygens; in none of the regions is there significant intramolecular electrostatic attraction. Table VII lists the computed distributions and expectation values of $R_{n-\pi^*}$ and $J$, and Figure 8 shows the computed potential energies, coupling constants, and rotatory strengths as a function of $\phi$.

Values of $\langle R_{n-\pi^*} \rangle$ and $\langle J \rangle$ computed using the standard CFF potential with $\epsilon = 1$ are similar to experimental observations in nonpolar solvents. $\langle J \rangle$ is 5.7 Hz and $J_{obsd}$ values are 5.3 Hz in chloroform and 6.1 Hz in dioxane. $\langle R_{n-\pi^*} \rangle$ is 0.02 DM, and the observed values are about zero in chloroform and −0.03 DM in dioxane.

Neglect of intramolecular electrostatic interactions ($\epsilon = \infty$) in the remaining models shifts molecules to the minimum near −70° and $\langle R_{n-\pi^*} \rangle$ becomes more negative in agreement with observations. Although $\langle J \rangle$ increases, the predicted values fall considerably short of those observed in polar solvents. The experimental evidence, large negative $R_{n-\pi^*}$ and $J \sim 8$ Hz, suggest the dominance of the −120 and −80° conformational region (see Figure 8). The solvated dipole model has the largest population, 65%, in this region, but none of the distributions is sharply enough centered there to predict the correct NMR and CD. Our computations indicate that attenuation of intramolecular electrostatic repulsion, solvent polarization, and solvation volume requirements of the carbonyl groups could all contribute to the stability of this region.

## Conclusions

The changes in conformational distribution that we infer when the dipeptide models under study are transferred from chloroform to water are summarized below.

1. CD spectra, $^{13}C$ NMR spectra, and $^1H$ NOE experiments confirm earlier conclusions from CD and infrared work that the trans form of AcProNHMe is mostly in the intramolecularly hydrogen bonded $C_7$ conformation in the nonpolar solvents. The same measurements indicate that the distribution shifts in polar solvents to populate both the $\alpha_R$ and $P_{II}$ regions. In acetonitrile the three conformations are probably present about equally. In dimethyl sulfoxide the $P_{II}$ form is favored. In water the $C_7$ conformer is absent, but the ratio of the other two forms is not established.

2. The CD spectra of AcAlaNHMe almost exactly parallel the shape and solvent dependence of those of AcProNHMe, although the intensities are reduced. From this it seems likely that the principal change in conformations of AcAlaNHMe on going between chloroform and water is that the population of the $C_7$ region, which is the most important single conformation in chloroform, is redistributed in water between the $\alpha_R$ and $P_{II}$ regions. The observed values of H–N–C$^\alpha$–H coupling for AcAlaNHMe are consistent with $\phi$ values allowable for proline.

3. A null n–π* CD band in chloroform, which becomes increasingly negative as solvent polarity is increased, in combination with conformational energy calculations, suggests that L-3-acetamidopyrrolidin-2-one is largely in forms with $\phi$ near −160 and +60° in chloroform and dioxane, but that an appreciable fraction shifts toward $-120° < \phi < -80°$ in water. H–N–C$^\alpha$–H coupling data show no specific effects of water as solvent and are ambiguous about the conformational shift. However, the NMR and CD data are consistent with $\phi \sim -100°$ for the aqueous conformer.

4. In each case the changes in population distribution indicated by the CD spectra vary in the solvent sequence chloroform (most vacuum-like), dioxane, acetonitrile, or *tert*-butyl alcohol, methanol, water (most strongly interacting).

The correspondence between these inferences from experiment and the predictions of the solvation models we tested is summarized below.

1. The standard intramolecular potential ($\epsilon = 1$) yields predictions in accord with experiment in inert media such as chloroform solution. Conformers which have low steric and electrostatic energies dominate.

2. Attenuation of intramolecular electrostatic interactions depopulates hydrogen-bonded conformers and permits others which are favored sterically but disfavored electrostatically. This prediction is in qualitative agreement with observations upon transfer of peptides from nonpolar to polar solvents, but fails to focus the population in specific regions which are indicated experimentally.

3. Inclusion of the polarization energy of the dielectric surrounding the peptide (solvated dipole model) concentrates the peptide population in conformers with large dipole moments (such as $\alpha_R$ and $P_{II}$) which are observed in all polar solvents, but does not differentiate among polar solvents.

4. Addition of specific solvent–peptide interactions differentiates solvents of similar polarity. In aqueous solution, hydration of the C=O groups is more effective than hydration of the N—H groups in giving a population distribution in accord with inferences from experiment. The carbonyl groups are most accessible to solvent in the $\alpha_R$ and $P_{II}$ conformers.

The two factors which we have identified, solvent polarization and solvation volume, should be included with intramolecular interactions in a simple model for predicting peptide conformation in water. In emphasizing the $P_{II}$ and $\alpha_R$ regions in water, this model differs from the hydration shell models in an eventually testable prediction. It will be of particular interest to test the effect of these factors in other conformationally constrained molecules, and in larger peptides where energy differences among conformations may be more pronounced.

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