Conformational Properties of 2,4-Methanoproline (2-Carboxy-2,4-methanopyrrolidine) in Peptides: Evidence for 2,4-Methanopyrrolidine Asymmetry Based on Solid-State X-ray Crystallography, ^{1}H NMR in Aqueous Solution, and CNDO/2 Conformational Energy Calculations

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Abstract: The crystal structure of the terminally blocked amino acid *N*-acetyl-2,4-methanoproline-*N'*-methylamide (Ac-2,4-MePro-NHMe) has been determined by X-ray crystallography ($R = 0.05$). In the solid state, the Ac-2,4-MePro peptide group is trans, and slightly nonplanar ($\omega_0 = -178.4°$). The unit cell was found to contain two conformations of Ac-2,4-MePro-NHMe which are characterized by the values of the backbone dihedral angles ϕ ($\pm 29°$) and ψ ($\mp 114.6°$), and by distortions of the side-chain 2,4-methanopyrrolidine ring conformation. These two conformations are related to each other as mirror images. ^{1}H NMR spectroscopy was used to identify the 2,4-methanopyrrolidine asymmetry in Ac-L-Tyr-2,4-Me-Pro-NHMe in water. CNDO/2 conformational energy calculations on Ac-2,4-MePro-NHMe and related analogues indicate that the preference for asymmetric side-chain conformations and nonzero values of ϕ arises primarily from inter-residue interactions, and particularly from unfavorable interactions between the carbonyl group of the preceding peptide with the carbonyl group of the symmetric 2,4-MePro residue (e.g., unfavorable nonbonded 1–4 C'···C' interactions). These results indicate that proper modeling of the conformational properties of 2,4-MePro in peptides requires that its backbone (ϕ) and side-chain conformational chirality be taken into account.

1. Introduction

Proline and hydroxyproline are unique among the commonly occurring amino acids found in proteins in so far as they are the only ones that form tertiary peptide bonds (rather than secondary peptide bonds) and have cyclic side-chain structures. These structural properties of proline and its derivatives result in unique constraints on the conformational space of sequences containing proline or hydroxyproline.[1-6] For example, L-proline constrains the conformational space of the preceding L-amino acid residue in an amino acid sequence so as to disfavor α-helical backbone conformations.[1,3] On the other hand, X-Pro-Y sequences[7] with trans X-Pro peptide bonds and X-Pro sequences with cis X-Pro peptide bonds have a strong tendency to form type-I[8,9] and type-VI[8] β-bends at Pro-Y[3,10] and at X-Pro,[6] respectively. These well-understood conformational properties of proline in peptides suggest that chemical analogues of proline[11-15] may be useful in molecular engineering approaches to designing polypeptides with desired structures and functions.

In a recent paper,[15] we described solution NMR studies which demonstrate that replacement of L-proline in small peptides with the bicyclic proline analogue 2,4-methanoproline[16-18] results in selective stabilization of the trans tertiary peptide bond conformation. These results suggest that 2,4-MePro[18] may be a useful L-proline analogue for polypeptide molecular design. For this reason, further studies of the conformational properties of 2,4-MePro in peptides are now in progress in our laboratory.

In order to carry out energy calculations to explore the conformational properties of 2,4-MePro in peptides,[19] reliable values of the fixed bond lengths and bond angles for 2,4-MePro in a peptide must be obtained from X-ray crystallography. Although a crystal structure for the free amino acid (i.e., without peptide bonds) has been reported elsewhere,[16] these data are inadequate as input for conformational energy calculations since several essential bond lengths and bond angles can be obtained only from the crystal structures of molecules containing peptide bonds (e.g., the C'–N–C$^\alpha$ bond angle for an X-2,4-MePro peptide bond cannot be parameterized properly from the structure of the free amino acid). For this reason, the terminally blocked amino acid Ac-2,4-MePro-NHMe[18] was crystallized and its structure was determined by X-ray crystallography. In the course of this analysis, it was observed that the peptide Ac-2,4-MePro-NHMe exists in two conformations. In these conformations, the side chain (2,4-methanopyrrolidine) lacks a plane of symmetry. The two conformations are mirror images of each other. This has important consequences for the conformational properties of 2,4-MePro in peptides. A method for detecting this conformational distortion in peptides by ^{1}H NMR is proposed and used to provide evidence for structural asymmetry in Ac-L-Tyr-2,4-MePro-NHMe in aqueous solution. In addition, CNDO/2 calculations are presented and compared with experimental data in order to identify the

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(1) Schimmel, P. R.; Flory, P. J. *J. Mol. Biol.* **1968**, *34*, 105.
(2) Zimmerman, S. S.; Pottle, M. S.; Némethy, G.; Scheraga, H. A. *Macromolecules* **1977**, *10*, 1.
(3) Zimmerman, S. S. Scheraga, H. A. *Biopolymers* **1977**, *16*, 811.
(4) Némethy, G.; Scheraga, H. A. *Biopolymers* **1982**, *21*, 1535.
(5) Vásquez, M.; Nemethy, G.; Scheraga, H. A. *Macromolecules* **1983**, *16*, 1043.
(6) Oka, M.; Montelione, G. T.; Scheraga, H. A. *J. Am. Chem. Soc.* **1984**, *106*, 7959.
(7) We use the notation X-Pro-Y to designate a tripeptide sequence of L-amino acids, where X always refers to the L-amino acid preceding L-proline, and Y to the L-amino acid following L-proline.
(8) The classification of β-bend types used here is described in ref 9.
(9) Lewis, P. N.; Momany, F. A.; Scheraga, H. A. *Biochim. Biophys. Acta* **1973**, *303*, 211.
(10) Chain reversals involving four residues may be characterized by the values of the dihedral angles ϕ_{i+1}, ψ_{i+1}, ϕ_{i+2}, ψ_{i+2}, where i is the index of the first residue on the amino terminal side of the bend sequence.
(11) Galardy, R. E.; Alger, J. R.; Liakopoulou-Kyriakides, M. *Int. J. Peptide Protein Res.* **1982**, *19*, 123.
(12) Delaney, N. G.; Madison, V. *Int. J. Peptide Protein Res.* **1982**, *19*, 543.
(13) Delaney, N. G.; Madison, V. *J. Am. Chem. Soc.* **1982**, *104*, 6635.
(14) Flippen-Anderson, J. L.; Gilardi, R.; Karle, I. L.; Frey, M. H.; Opella, S. J.; Gierasch, L. M.; Goodman, M.; Madison, V.; Delaney, N. G. *J. Am. Chem. Soc.* **1983**, *105*, 6609.
(15) Montelione, G. T.; Hughes, P.; Clardy, J.; Scheraga, H. A. *J. Am. Chem. Soc.* **1986**, *108*, 6765.
(16) Bell, E. A.; Qureshi, M. Y.; Pryce, R. J.; Janzen, D. H.; Lemke, P.; Clardy, J. *J. Am. Chem. Soc.* **1980**, *102*, 1409.
(17) Hughes, P.; Martin, M.; Clardy, J. *Tetrahedron Lett.* **1980**, *21*, 4579.
(18) Throughout the text, we have adopted the abbreviation 2,4-MePro for the α-amino acid 2,4-methanoproline (2-carboxy-methanopyrrolidine), and Ac- and -NHMe for terminal acetyl and *N*-methyl groups, respectively.
(19) Piela, L.; Némethy, G.; Scheraga, H. A. *J. Am. Chem. Soc.* following paper in this issue.

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physical basis of the asymmetry of 2,4-MePro in peptides.

2. Methods

2.1. Materials. The syntheses and characterization of the peptides Ac-2,4-MePro-NHMe and Ac-L-Tyr-2,4-MePro-NHMe are described elsewhere.[15]

2.2. Nomenclature. We have followed the standard IUPAC–IUB polypeptide nomenclature[20] for the 2,4-MePro residue,[21,22] except that (in describing the results of NMR experiments) the C^{β} protons $H^{\beta 11}$, $H^{\beta 12}$, $H^{\beta 21}$, and $H^{\beta 22}$ are designated $H^{\beta 1,endo}$, $H^{\beta 1,exo}$, $H^{\beta 2,exo}$, and $H^{\beta 2,endo}$, respectively, where exo and endo refer to the position of the β-methylene proton with respect to the larger ring of the bicyclic methanopyrrolidine side chain. The residue with which each atom is associated is designated by a subscript.[20] For Ac-2,4-MePro-NHMe, the subscripts 0, 1, and 2 refer to the acetyl N-terminal group, the 2,4-MePro residue, and the methylamide C-terminal group, respectively.

2.3. X-ray Crystallography. Ac-2,4-MePro-NHMe was crystallized by cooling a warm saturated solution of the peptide in ethyl acetate to 4 °C. A single crystal of approximate dimensions 0.5 mm × 0.5 mm × 0.5 mm was used for data collection. X-ray diffraction data were collected by using a computer-controlled four-circle diffractometer (Syntex $P2_1$), with variable-speed 1° ω-scan and graphite monochromated Cu Kα radiation (1.541 78 Å).

Data were collected to a nominal resolution of 0.79 Å ($2\theta \leq 114°$, and $136° \leq 2\theta \leq 156°$). During data collection, three reflections were monitored regularly at intervals of every 50 measurements. These check reflections indicated that no significant decay of the crystal occurred during the diffraction measurements. Out of a total of 1916 noncheck reflections, 1671 (87%) were judged to be reliably observed.[23]

A phasing model was constructed[24] by using the multisolution weighted tangent formula procedure. A full-matrix least-squares refinement with anisotropic thermal parameters for nonhydrogen atoms and isotropic (fixed) hydrogen atoms converged to a model for which the standard unweighted crystallographic residual (R) was 0.05. All hydrogen atoms were identified from difference Fourier synthesis at various stages of refinement.

2.4. Nuclear Magnetic Resonance Spectroscopy. ^1H NMR spectra were recorded from samples in 5-mm diameter NMR tubes at 300 MHz with a Brüker WM-300 spectrometer equipped with an Aspect 2000A computer. The probe temperature was thermostated at 25 ± 1 °C. Samples were prepared at a concentration of 5 mg/mL in deuterium oxide (99.96% D, Stohler). Chemical shifts in D_2O are reported relative to $\delta_{HOD} = 4.78$ ppm. Resolution enhancement of high-resolution one-dimensional spectra was carried out by using a Gaussian line-shape transformation.[25]

Two-dimensional, two-quantum filtered proton correlated spectra (2QF-COSY) were obtained using methods described by Piantini et al.[26] Quadrature detection in the t_1 domain was obtained by time proportional phase incrementation.[27,28] Details of data acquisition and processing are identical with those described previously.[15]

2.5. CNDO/2 Calculations. Quantum-mechanical calculations of the (valence) electronic energy were carried out by using the standard CNDO/2 (complete neglect of differential overlap) algorithm of the molecular orbital procedure.[29-31] The program has been modified slightly by adding the Mulliken overlap population analysis.[32] The molecular orbitals and total energy were calculated for the Ac-2,4-MePro-NHMe molecule using the positions of heavy (i.e., C, N, and O) atoms determined by X-ray crystallography. Since X-ray crystallographic data are not very sensitive to hydrogen atom positions, idealized[33] rather than the experimentally obtained positions of hydrogen atoms were used in these calculations; i.e., CH and NH bond lengths were assumed to be equal to their standard values[33-35] of 1.09 and 1.00 Å, respectively.

To show which interactions determine the observed asymmetry in the Ac-2,4-MePro-NHMe molecule, CNDO/2 calculations were also carried out for the two molecules N-acetyl-2,4-methanopyrrolidine and N-protonated 2,4-methanopyrrolidine. N-Acetyl-2,4-methanopyrrolidine has a single hydrogen atom in place of the rotatable peptide unit $(C'O)_1$-NHMe of Ac-2,4-MePro-NHMe. The position of this hydrogen atom (with 1.09-Å bond length) was oriented so that the C_1^{α}–H bond vector was directed along the C_1^{α}–C'_1 bond vector of the original structure. For the N-protonated 2,4-methanopyrrolidine molecule, the CH_3CO– group of the peptide N-acetyl-2,4-methanopyrrolidine was replaced with two hydrogen atoms (with 1.00-Å bond length) bonded to the N_1 nitrogen atom in such a way as to ensure symmetry of their positions with respect to the C_1^{α}–C'_1–C_1^{δ} plane, the HNH bond angle being 126.5°. This is a standard value which is also used for the HCH bond angles.[34,35] The geometry of the N-protonated methanopyrrolidine ring was adopted from the X-ray crystal structure of 2-carboxy-2,4-methanopyrrolidine zwitterion.[16]

CNDO/2 calculations were also carried out for Ac-2,4-MePro-NHMe, Ac-2,4-methanopyrrolidine, and N-protonated 2,4-methanopyrrolidine with symmetrized geometry. Details of the symmetrization procedure and bond lengths, bond angles, and dihedral angles for the symmetrized methanopyrrolidine geometry are presented in the accompanying paper.[19,36] This symmetrized conformation does not necessarily correspond to the symmetric structure of lowest energy.

Table I. Dihedral Angles for Ac-2,4-MePro-NHMe

Backbone Dihedral Angles (deg)		
C_0^{α}–C'_0–N_1–C_1^{α}	ω_0	−178.4
O_0–C'_0–N_1–C_1^{α}		4.1
C'_0–N_1–C_1^{α}–C'_1	ϕ_1	−29.0
N_1–C_1^{α}–C'_1–N_2	ψ_1	114.6
C_1^{α}–C'_1–N_2–C_2^{α}	ω_1	179.2
O'_1–C'_1–N_2–C_2^{α}		4.9
Side-Chain Dihedral Angles (deg)		
N_1–C_1^{α}–$C_1^{\beta 1}$–C_1^{γ}	$\chi_1^{1,1}$	64.5
N_1–C_1^{α}–$C_1^{\beta 2}$–C_1^{γ}	$\chi_1^{1,2}$	−65.1
C_1^{α}–$C_1^{\beta 1}$–C_1^{γ}–C_1^{δ}	$\chi_1^{2,1}$	−65.0
C_1^{α}–$C_1^{\beta 2}$–C_1^{γ}–C_1^{δ}	$\chi_1^{2,2}$	67.7
$C_1^{\beta 1}$–C_1^{γ}–C_1^{δ}–N_1	$\chi_1^{3,1}$	42.5
$C_1^{\beta 2}$–C_1^{γ}–C_1^{δ}–N_1	$\chi_1^{3,2}$	−46.5
C_1^{α}–N_1–C_1^{δ}–C_1^{γ}	χ_1^{4}	+2.0

(20) IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry* **1970**, *9*, 3471.

(21) It should be noted that the following parallel notation is used elsewhere:[15] $C^{\beta 1} \equiv C^{\beta,pro\text{-}R}$, $C^{\beta 2} \equiv C^{\beta,pro\text{-}S}$, $H^{\delta 1} \equiv C^{\delta}H^{pro\text{-}S}$, $H^{\delta 2} \equiv C^{\delta}H^{pro\text{-}R}$, where the *pro-R* and *pro-S* descriptions refer to the prochirality[22] relationships of the C^{β} atoms with respect to the C^{α} atom and of the H^{δ} protons with respect to the C^{δ} atom. For the NMR measurements, the prochirality assignments are not available. Instead the upfield (u) resonance of the prochiral protons are designated $H^{\beta,endo,u}$, $H^{\beta,exo,u}$, and $H^{\delta,u}$ while the downfield (d) prochiral protons are $H^{\beta,endo,d}$, $H^{\beta,exo,d}$, and $H^{\delta,d}$.

(22) Hanson, K. R. *J. Am. Chem. Soc.* **1966**, *88*, 2731.

(23) The principal programs used were the REDUCE and UNIQUE data-reduction programs written by M. E. Leoniwicz, Cornell University, 1978.

(24) The principal programs used were: MULTAN80, a system of computer programs for the automatic solution of crystal structures from X-ray diffraction data (locally modified to perform all Fourier calculations) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, University of york, England, 1978; BLS78A, an anisotropic block diagonal least-squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; ORTEP, a crystallographic illustration program written by C. K. Johnson, Oak Ridge; and BOND, a program to calculate molecular parameters written by K. Hirotsu, Cornell University.

(25) Ferrige, A. G.; Lindon, J. C. *J. Magn. Reson.* **1978**, *31*, 337.

(26) Piantini, U.; Sørensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800.

(27) Redfield, A. G.; Kunz, S. D. *J. Magn. Reson.* **1975**, *19*, 250.

(28) Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967.

(29) Pople, J. A.; Santry, D. P.; Segal, G. A. *J. Chem. Phys.* **1965**, *43*, S129.

(30) Pople, J. A.; Segal, G. A. *J. Chem. Phys.* **1965**, *43*, S136.

(31) Pople, J. A.; Segal, G. A. *J. Chem. Phys.* **1966**, *44*, 3289.

(32) Mulliken, R. S. *J. Chem. Phys.* **1955**, *23*, 1833.

(33) Momany, F. A.; Carruthers, L. M.; McGuire, R. F; Scheraga, H. A. *J. Phys. Chem.* **1974**, *78*, 1595.

(34) Momany, F. A.; McGuire, R. F.; Burgess, A. W.; Scheraga, H. A. *J. Phys. Chem.* **1975**, *79*, 2361.

(35) Némethy, G.; Pottle, M. S.; Scheraga, H. A. *J. Phys. Chem.* **1983**, *87*, 1883.

(36) For each geometry (experimental and symmetrized), ψ_1 was allowed to vary and two minima were found, one for a positive and the other for a negative value of ψ_1. The energy difference cited in the text is the value computed by CNDO/2 for the lowest energy conformations (obtained by optimization of ψ_1) for the experimental and the symmetrized geometries of the molecule. For the symmetrized molecule, both minima (at $\phi_1 = 0°$, $\psi_1 = \pm 66.0°$) are of equal depth. For the experimental geometry, the minimum occurs at $(\phi_1, \psi_1) = (+29°, +32.5°)$ or at $(\phi_1, \psi_1) = (-29°, -32.5°)$ for the two mirror-image conformations. For the conformation with $\phi_1 = -29°$, the geometry determined by X-ray crystallography corresponds to a positive value of ψ_1 (+114.6°), while the present CNDO computation and the independent ECEPP results[19] indicate that the conformation with a negative ψ_1 (−32.5°) is energetically preferred. A possible reason for this difference is discussed in the accompanying paper.[19]

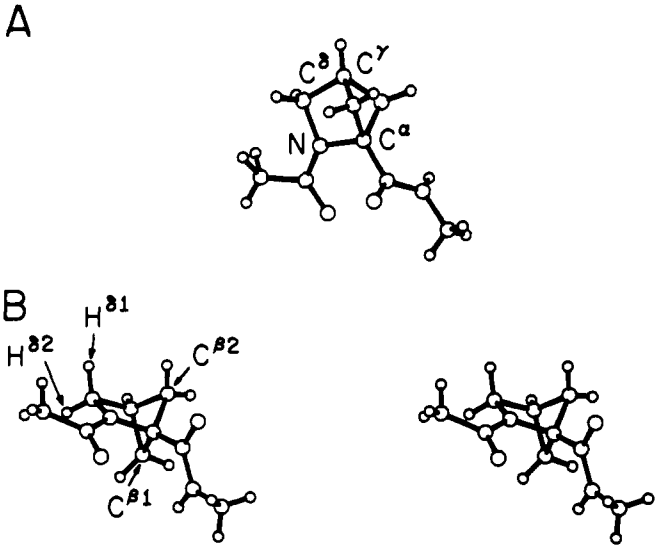

Figure 1. (A) ORTEP picture of one conformation of Ac-2,4-MePro-NHMe based on atomic coordinates obtained from X-ray crystallographic structure determination. (B) ORTEP stereo picture of the same conformation as in (A), shown so as to emphasize the asymmetry in the bicyclic ring structure.

In the present paper, we did not attempt to optimize the geometry of the isolated molecule under study. Instead, we have assumed that its geometry is probably similar to that observed in the crystal (with the positions of hydrogen atoms being determined independently, as described above). The geometry observed experimentally may be considered to result from "distortion" of the corresponding symmetrized structure. The experimental and symmetrized structures are related by a set of changes in bond lengths, bond angles, and dihedral angles. This set of changes is described by the vector Δ. To study distortions smaller or larger than those observed experimentally, a series of geometries was generated using different sets of changes given by $\Delta(\eta) = \eta\Delta$. The value of the parameter $\eta = 0$ corresponds to the assumed symmetrized geometry ($\Delta = 0$), while $\eta = 1.0$ corresponds to the experimental one (except for the positions of hydrogen atoms). Variation of the parameter η for the molecule under study produces a series of geometries for which the CNDO/2 energy can be calculated. It enables one to find the optimal value of η (generally different from $\eta = 1.0$) corresponding to the lowest calculated energy. However, we recognize that this is not a complete search of the conformational space.

3. Results

3.1. Crystal Structure of Ac-2,4-MePro-NHMe. Preliminary diffraction data showed that the crystal belongs to the monoclinic crystal system with point group symmetry $2/m$. The unit cell dimensions were determined by a least-squares fitting of 15 diffractometer measured reflections. The observed cell dimensions are: $a = 7.5232$ (25) Å, $b = 14.2803$ (35) Å, $c = 9.0659$ (09) Å; $\alpha = 90.021$ (15)°, $\beta = 100.755$ (20)°, $\gamma = 89.965$ (23)°; cell volume = 956.89 (41) Å^3. A calculated density of 1.26 g·cm^{-3} for the molecular formula $C_9N_2O_2H_{14}$ and a molecular weight of 182, indicated the presence of four molecules per unit cell. The presence of the systematic extinctions $0k0$ ($k = 2n + 1$) and $h0l$ ($l = 2n + 1$) uniquely determined the space group to be $P2_1/c$.

It was observed that the individual molecules in the unit cell do not possess a plane of symmetry, although there are no chiral atoms in the molecules. On the other hand, the space group symmetry $P2_1/c$ implies the existence of a glide plane in the unit cell. This is possible only if two of the four molecules in the unit cell have conformations that are mirror images of the conformations of the other two molecules.

Stereoscopic projections of the X-ray structure of one conformation of Ac-2,4-MePro-NHMe are shown in Figure 1. Standard[20] backbone and side-chain dihedral angles for this conformation are presented in Table I. These molecular parameters are reported for one of the two mirror-image conformations present in the unit cell. The other conformation can be generated by using symmetry operations on the fractional coordinates (reported in Table 1S of the Supplementary Material[37]).

Table II. Values of Some Geometric Parameters from the Crystal Structure of Ac-2,4-MePro-NHMe Which Indicate the Asymmetry

Bond Lengths (Å)		
$C_1^{\beta 1}$–C_1^{γ}		1.551 (3)[a]
$C_1^{\beta 2}$–C_1^{γ}		1.536 (3)
Bond Angles (deg)		
C_1^{α}–$C_1^{\beta 1}$–C_1^{γ}		81.9 (1)[a]
C_1^{α}–$C_1^{\beta 2}$–C_1^{γ}		82.4 (1)
$C_1^{\beta 1}$–C_1^{γ}–C_1^{δ}		103.1 (2)
$C_1^{\beta 2}$–C_1^{γ}–C_1^{δ}		100.5 (2)
Dihedral Angles (deg)		
C'_0–N_1–C_1^{α}–C'_1	ϕ_1	−29.0[b]
C_1^{α}–N_1–C_1^{δ}–C_1^{γ}	χ_1^4	+2.0[b]
C_1^{α}–$C_1^{\beta 1}$–C_1^{γ}–C_1^{δ}	$\chi_1^{2,1}$	−65.0
C_1^{α}–$C_1^{\beta 2}$–C_1^{γ}–C_1^{δ}	$\chi_1^{2,2}$	67.7
$C_1^{\beta 1}$–C_1^{γ}–C_1^{δ}–N_1	$\chi_1^{3,1}$	42.5
$C_1^{\beta 2}$–C_1^{γ}–C_1^{δ}–N_1	$\chi_1^{3,2}$	−46.5
$C_1^{\beta 1}$–C_1^{α}–N_1–C_1^{δ}		−45.6
$C_1^{\beta 2}$–C_1^{α}–N_1–C_1^{δ}		42.4

[a] Values in parentheses represent standard deviations in the last reported significant figure. [b] The dihedral angle for a symmetric conformation would be 0°.

The C–H bond lengths are observed to be in the range of 0.96 ± 0.05 Å, except for the $C_1^{\beta 2}$–$H_1^{\beta 21}$ bond (bond length 0.82 Å).

In the solid state, both the Ac-(2,4-MePro) and (2,4-MePro)-NHMe peptide bonds have a trans conformation as they do in the predominant solution conformation.[15] The C_0^{α}–C'_0–N_1–C_1^{α} dihedral angle of −178.41° and the O_0–C'_0–N_1–C_1^{α} dihedral angle of 4.1° indicate that the Ac-(2,4-MePro) peptide group is not planar. The nonplanarity of the peptide group is due in part to pyramidalization at the N_1 atom. No pyramidalization about C'_0 atom was detected.

The bicyclic pyrrolidine ring of the 2,4-MePro residue lacks a plane of symmetry. This asymmetry manifests itself in several ways which are summarized in Table II. Some bond lengths and bond angles involving the $C^{\beta 1}$ and $C^{\beta 2}$ atoms, which would be expected to be equal in a symmetric conformation, are found to be unequal. In addition, the dihedral angles ϕ (i.e., C'_0–N_1–C_1^{α}–C'_1) and χ^4 (i.e., C_1^{α}–N_1–C_1^{δ}–C_1^{γ}) are nonzero. These two dihedral angles would be zero for a symmetric side-chain conformation.

The observed values of ϕ and ψ for this conformation are −29.0 and +114.6°, respectively. For the mirror-image conformation, ϕ and ψ have values opposite in sign, i.e., +29.0 and −114.6°, respectively. Similarly, the values for other dihedral angles in Table I change in sign but remain the same in magnitude. The bond lengths and bond angles which do not involve prochiral atoms are the same for both conformations. To describe the conformation which is a mirror image of the one described in the tables, the superscripts (1 and 2) on prochiral atoms have to be interchanged.

The atoms in the bicyclic pyrrolidine ring of 2,4-methanoproline also show considerable deviation from tetrahedral geometry. This is clearly illustrated by the range of bond angles in the side chain, viz., 81.7 to 103.9°. These deviations from tetrahedral geometry indicate strain in the ring.

A single intermolecular hydrogen bond was observed, beteween O_0 and N_2 with an O···N distance of 2.83 Å. A list of all intermolecular contacts less than 3.5 Å is presented as supplementary material[37] (Table 4S).

3.2. Evidence for 2,4-Methanopyrrolidine Asymmetry in Ac-L-Tyr-2,4-MePro-NHMe in Aqueous Solution from ^{1}H NMR Spectroscopy. The X-ray diffraction data and analysis described in the preceding section provide evidence for two conformations of the 2,4-methanopyrrolidine side chain of Ac-2,4-MePro-NHMe in the solid state. These two mirro-image conformations are

(37) See paragraph at end of paper regarding supplementary material.

Table III. Results of CNDO/2 Conformational Energy Calculations

molecule	E(asymm) − E(symm)[a] (kcal/mol)
Ac-2,4-MePro-NHMe	−1.0
Ac-2,4-MePro	+0.8
$^{+}H_2N$-2,4-MePro	+3.4

[a] Energy difference between asymmetric (η = 1.0) and symmetric (η = 0.0) molecular conformations. Negative values indicate that the asymmetric conformation is preferred.

expected to be isoenergetic in solution. Hence, it is difficult to obtain experimental evidence in solution for the two conformers of trans Ac-2,4-MePro-NHMe directly. Conformational energy calculations, described in the accompanying paper,[19] indicate that incorporation of 2,4-methanoproline into a peptide containing one or more chiral atoms (e.g., a peptide containing L or D amino acids) results in a preferential stabilization of one chiral side-chain conformation (and correlated ϕ_1 backbone conformation). Analysis of coupling constants within the 2,4-methanopyrrolidine side chain, representing a statistical average for the two nondegenerate 2,4-methanopyrrolidine mirror-image conformations, should therefore provide evidence for conformations with asymmetric side chains.

The set of interproton couplings within the methanopyrrolidine side chain of Ac-L-Tyr-2,4-MePro-NHMe was assigned[15] using 2QF-COSY and measured in one-dimensional ^{1}H NMR spectra. These measurements were obtained from spectra recorded with 64 000 data points and a spectral width of 2500 Hz, resulting in a nominal resolution of ≤0.08 Hz. The spectra were resolution-enhanced by Lorentzian to Gaussian line-shape transformation,[25] resulting in line widths at half-height of ca. 1.0 Hz. Comparisons between pairs of splittings manifesting the same coupling constant indicate a precision of ±0.05 Hz (for coupling >1 Hz). However, in several independently recorded spectra the splittings arising from $H^{\delta,u}$...$H^{\beta,exo,d}$ and $H^{\delta,d}$...$H^{\beta,exo,u}$ long-range couplings[21] range from 1.15 to 1.45 and 1.40 to 1.65 Hz, respectively. For a symmetric methanopyrrolidine ring conformation, these couplings should be identical (within the digital resolution of ±0.08 Hz). On the other hand, the long-range couplings are expected to be sensitive to ensemble-averaged asymmetry if they arise in part from direct electronic interactions[38] between the prochiral faces of the bicyclic ring, e.g., interactions between the antibonding "sp^3" orbitals of the $C^{\beta 1}H^{exo}$ and $C^{\delta}H^{1}$ bonds compared to antibonding interactions of the $C^{\beta 2}H^{exo}$ and $C^{\delta}H^{2}$ bonds whose relative orientations (Figure 1B) depend on the conformation of 2,4-methanopyrrolidine. The experimental differences in the H^{δ}···$H^{\beta,exo}$ couplings suggest asymmetry in the bicyclic ring and are *consistent* with theoretical calculations[19] which indicate a preferential stabilization of one chiral methanoproline side-chain conformation in Ac-L-Tyr-2,4-MePro-NHMe. The differences, however, are small and the ranges are overlapping so that a definite statement about the side-chain asymmetry cannot be drawn from these data. For this reason, CNDO/2 conformational energy calculations (described in the next section) were also used to provide corrobating evidence that methanopyrrolidine asymmetry arises from interactions inherent to 2,4-MePro in peptides.

3.3. CNDO/2 Conformational Energy Calculations. The ^{1}H NMR data presented above for Ac-L-Tyr-2,4-MePro-NHMe are consistent with the view that the asymmetry of Ac-2,4-MePro-NHMe in the solid state arises from intramolecular interactions rather than from intermolecular crystal packing effects. This conclusion was corroborated by CNDO/2 conformational energy calculations on Ac-2,4-MePro-NHMe, *N*-acetyl-2,4-methanopyrrolidine, and N-protonated 2,4-methanopyrrolidine. The results of these calculations are summarized in Table III.

For Ac-2,4-MePro-NHMe, the calculations show that the asymmetric side-chain conformation of the crystal structure has a lower energy than the corresponding structure with a symmetrized side-chain conformation (Table III). On the other hand, for both Ac-2,4-methanopyrrolidine and N-protonated methanopyrrolidine, a symmetric conformation is energetically favored (Table III). CNDO/2 conformational energies were also calculated for different values of the distortion parameter η (which is described in the Methods section). For Ac-2,4-methanopyrrolidine and N-protonated 2,4-methanopyrrolidine, the optimized values of η are ~0.0 and ~0.1, respectively, indicating little or no tendency for methanopyrrolidine asymmetry. These theoretical results indicate that the methanopyrrolidine asymmetry of Ac-2,4-MePro-NHMe arises from intramolecular interactions which are not present in either Ac-2,4-methanopyrrolidine or in N-protonated 2,4-methanopyrrolidine. Examination of the crystal structure (see Figure 1B) suggests that nonbonded interactions between the carbonyl groups of 2,4-MePro and the preceding peptide bond which are unfavorable for the backbone dihedral angle ϕ = 0° can account for the observed asymmetry when 2,4-MePro is incorporated into a polypeptide. This conclusion is supported by the CNDO/2 calculations on Ac-2,4-MePro-NHMe which indicate unfavorable interactions between the acetyl and carboxyamide groups and (to a smaller degree) between the carboxyamide and β-methylene groups of the symmetrized conformation which are partially relieved when the asymmetric conformation is adopted.

4. Discussion

A comparison of the geometries of Ac-2,4-MePro-NHMe with that of Ac-L-Pro-NHMe[39] was made to evaluate the effect of introducing the methylene bridge into the pyrrolidine side chain of Ac-L-Pro-NHMe. These comparisons are summarized in Tables 2S and 3S of the supplementary material.[37] The N_2···O_0 intermolecular hydrogen bonding pattern in the two crystals is similar.[39]

All the bond angles interior to the pyrrolidine ring are smaller in Ac-2,4-MePro-NHMe compared with the corresponding bond angles in Ac-L-Pro-NHMe (the magnitude of the difference in corresponding angles ranges from 1.1 to 22.8°). The bond angles C'_0–N_1–C_1^{α} (MePro, 126.9°; Pro, 121.4°), C'_1–C_1^{α}–N_1 (MePro, 116.6°; Pro, 114.3°), and C'_1–C_1^{α}–C_1^{β} (MePro, 128.3, 118.5°; Pro, 111.5°), all three of which are external to the pyrrolidine ring, are larger in Ac-2,4-MePro-NHMe than in Ac-L-Pro-NHMe. The atoms in the bicyclic pyrrolidine ring of Ac-2,4-MePro-NHMe also show greater deviation from tetrahedral geometry (ranging from 81.9 to 103.1°) than the correspoding atoms of Ac-L-Pro-NHMe (102.8 to 104.7°). These bond angle comparisons indicate the strain which is inherent in the bicyclic pyrrolidine ring.

The magnitude of ϕ in Ac-2,4-MePro-NHMe (±29°) is smaller than that in the crystal structure[39] of Ac–Pro–NHMe (−76.3°). This is as expected. In Ac–MePro–NHMe, the two symmetric β-methylene groups tend to force ϕ toward 0°. However, this is opposed by unfavorable steric interactions which are relieved when the peptide adopts an asymmetric conformation with a larger value of ϕ. The balance of these forces results in the observed ϕ = ±29°. In Ac-L-Pro-NHMe, there is no interaction which favors a zero value of ϕ since there is only one β-methylene. This leaves the peptide free to adopt a conformation which is determined by optimal ring closure and interactions between the end groups, both of which favor a nonzero value of ϕ.

Replacement of L-proline in small peptides by the bicyclic proline analogue 2,4-methanoproline results in selective (>98%) stabilization of trans X-Pro peptide bond conformers in solution.[15] Other proline analogues for which the trans peptide bond connecting the analogue to the preceding residue is stabilized selectively include 5-oxo-L-proline[11] and 2-methylproline.[13,14] Unfortunately, incorporation of 5-oxo-L-proline into a polypeptide results in the formation of an imide functional group, which is highly susceptible to base-catalyzed hydrolysis in aqueous solution.[11] 5-Oxo-L-proline is, therefore, an unacceptable analogue for polypeptide molecular design. Both 2,4-methanoproline and 2-methylproline are stable in aqueous solution. In the solid state,

(38) Barfield, M.; Della, E. W.; Pigou, P. E.; Water, S. R. *J. Am. Chem. Soc.* **1982**, *104*, 3549.

(39) Matsuzaki, T.; Iitaka, Y. *Acta Crystallogr., Sect. B* **1971**, *27*, 507.

Ac-L-2-MePro-NHMe[14] has backbone dihedral angles $\phi = -60.5°$ and $\psi = -24.9°$, similar to those of Ac-L-pro-NHMe[39] ($\phi = -76.3°$, $\psi = -15.9°$), while in Ac-2,4-MePro-NHMe, $\phi = \pm 29°$. For this reason, 2-methylproline[13,14] may be a better proline analogue than 2,4-methanoproline for selectively stabilizing X-pro peptide bonds while simultaneously maintaining a ϕ,ψ conformational space similar to that of trans L-Pro. On the other hand, replacement of L-Pro with 2,4-MePro selectively stabilizes the trans peptide bond and also contrains $\phi = \pm 29°$. Hence, both 2-MePro and 2,4-MePro should be useful proline analogues for polypeptide molecular design.

An attempt was made to compare the 2,4-methanopyrrolidine structure with structures which have been determined for similar bicyclic rings. To our knowledge, there is no atomic resolution molecular structure available for the corresponding nitrogen-containing 2-azabicyclo[2.1.1]hexane. For bicyclo[2.1.1]hexane, in which the nitrogen atom is replaced by carbon, the molecular structure based on gas-phase electron diffraction[40] has been criticized by subsequent ab initio calculations[41] and by comparisons of the calculated photoelectron spectrum with that observed experimentally.[42] For both the electron diffraction[40] and ab initio calculations, a symmetric bicyclic structure was assumed a priori, while the X-ray diffraction data presented in this paper clearly indicate an asymmetric methanopyrrolidine structure for Ac-2,4-MePro-NHMe.

(40) Chiang, J. F. *J. Am. Chem. Soc.* **1971**, *93*, 5044.
(41) Wiberg, K. B.; Wendoloski, J. J. *J. Am. Chem. Soc.* **1982**, *104*, 5679.
(42) Della, E. W.; Pigou, P. E. *J. Electron Spectrosc. Relat. Phenom.* **1984**, *33*, 163.

The experimental and theoretical results presented in this paper indicate that proper modeling of the conformational properties of 2,4-MePro in peptides requires that its backbone (i.e., ϕ) and side-chain conformational chirality be taken into account. Although the introduction of the 2,4-methylene bridge into L-Pro results in selective stabilization of the trans peptide bond conformation,[15] the constraints of $\phi = \pm 29°$ restrict 2,4-MePro to a conformational space different from that of L-Pro. In particular, $\phi = \pm 29°$ prevents 2,4-MePro from adopting some extended conformations which are accessible to L-Pro. The effects of these contraints on the conformational properties of 2,4-MePro compared with L-Pro in polypeptides are addressed with conformational energy calculations in the accompanying paper.[19]

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Supplementary Material Available: Table 1S, fractional coordinates and thermal parameters for Ac-2,4-MePro-NHMe; Table 2S, comparison of bond lengths in Ac-2,4-MePro-NHMe, Ac-Pro-NHMe, and 2,4-MePro; Table 3S, comparison of bond angles in Ac-2,4-MePro-NHMe, Ac-Pro-NHMe, and 2,4-MePro; Table 4S, hydrogen bonds and other short intermolecular distances in the Ac-2,4-MePro-NHMe crystal (5 pages). Ordering information is given on any current masthead page.

Conformational Properties of 2,4-Methanoproline (2-Carboxy-2,4-methanopyrrolidine) in Peptides: Theoretical Conformational Energy Analysis of Restrictions of the Polypeptide Chain Conformation

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Abstract: The α-amino acid 2,4-methanoproline (2-carboxy-2,4-methanopyrrolidine, 2,4-MePro) can serve as an analogue of proline in studies of the folding of globular proteins and of collagen structure and function. Conformational energy computations have been carried out on the terminally blocked residue Ac-2,4-MePro-NHMe and on the dipeptides Ac-2,4-MePro-X-NHMe and Ac-X-2,4-MePro-NHMe, where X = L-Ala or L-Tyr. The trans form of the peptide bond preceding the 2,4-MePro residue is strongly stabilized over the cis form, by at least 5.9 kcal/mol, in agreement with experimental NMR studies of this residue, and in contrast to the cis/trans equilibrium in prolyl peptides (where the trans form is preferred by only about 2 kcal/mol). The value of the dihedral angle ψ also is more strongly constrained than in prolyl peptides. The conformational constraints exerted by the 2,4-MePro and Pro residues on the residue *following* them in a dipeptide are similar, except that preferences for partially folded (such as A and D) conformations, compared with more extended (such as E and F) conformations, are stronger in the case of 2,4-MePro. On the other hand, Pro strongly constrains the conformational freedom of the residue *preceding* it in a dipeptide, while such constraints are much less severe in the case of a residue preceding 2,4-MePro. The probability of bend formation in both the Ala-Pro and Pro-Ala dipeptides is strongly enhanced by substitution of 2,4-MePro for Pro. Therefore, the 2,4-MePro residue can serve as a model for Pro where bulkiness and rigidity of the polypeptide chain is required, with selective stabilization of the trans peptide bond, if some change of the conformational preferences of the neighboring residues can be allowed.

1. Introduction

The rare achiral amino acid[2] 2,4-methanoproline (2-carboxy-2,4-methanopyrrolidine) occurs naturally as a free amino acid in some plants.[3,4] It is not one of the naturally occurring amino acid components of proteins. It is of potential importance, however, in conformational studies of proteins and peptides as an analogue of proline, especially in the study of the cis–trans isomerization about the peptide bond preceding proline. It has

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(2) Abbreviations used: Ac, *N*-acetyl terminal group; 2,4-MePro, 2,4-methanoprolyl residue; NMR, nuclear magnetic resonance.
(3) Bell, E. A.; Qureshi, M. Y.; Pryce, R. J.; Janzen, D. H.; Lemke, P.; Clardy, J. *J. Am. Chem. Soc.* **1980**, *102*, 1409–1412.
(4) Hughes, P.; Martin, M.; Clardy, J. *Tetrahedron Lett.* **1980**, *21*, 4579–4580.

0002-7863/87/1509-4477$01.50/0