Conformations of Cyclic Heptapeptides: Crystal Structure and Computational Studies of Evolidine

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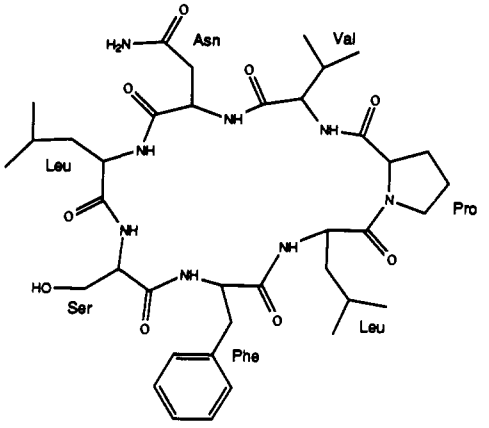
Abstract: Evolidine, *cyclo*(Ser-Phe-Leu-Pro-Val-Asn-Leu), crystallized as a tetrahydrate from aqueous ethanol in a triclinic cell of dimensions a = 11.370 (3) Å, b = 11.705 (4) Å, c = 9.058 (6) Å, α = 94.56 (7)°, β = 111.50 (5)°, γ = 73.23 (2)° with one formula unit in space group $P1$. Least-squares refinement of 3484 observed data ($I > 3\sigma(I)$) led to residuals R = 0.054 and R_w = 0.061. The cyclic heptapeptide backbone includes two β-turns, one of type I at Leu-Ser and one of type VI(a), which incorporates a cis peptide bond, at Leu-Pro. Transannular backbone hydrogen bonds occur between Phe N—H and Asn C′=O and between Phe C′=O and both Val N—H and Asn N—H. This structure corresponds to the minimal version of a classical β-bulge as identified in proteins. The backbone conformation is close to that proposed from solution NMR data of evolidine (Kopple, K. D. *Biopolymers* **1971**, *10*, 1139; Peishoff, C. E.; Bean, J. W.; Kopple, K. D. J. Am. Chem. Soc., following paper in this issue) and parallels that from both a crystal structure of the heptapeptide ilamycin, which has a different sequence, and the proposed solution conformation of a third cyclic heptapeptide containing no N-alkylated residues. These parallels, together with computations, suggest that the two β-turn backbone incorporating a bulge can be a favorable motif for cyclic heptapeptides.

Introduction

Cyclic peptides are of interest in and of themselves[1] and have consistently been used as models for studying recurring structural features of proteins.[2] Synthetic and naturally occurring cyclic peptides have been the subject of many conformational studies with the cyclic penta- and hexapeptides receiving the most attention. Octapeptides and larger rings have also been studied; however there have been no systematic examinations of cyclic heptapeptides. Evolidine, (**1**), a cyclic heptapeptide of sequence



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cyclo(Ser-Phe-Leu-Pro-Val-Asn-Leu), was first isolated from the leaves of *Evodia xanthoxyloides* and characterized by Eastwood, Hughes, and Ritchie.[3] Suggested conformations of the peptide backbone were deduced from solution ^{1}H NMR studies in 1971[4] (and in the following paper[4c]), but a solid-state conformation was never reported. Evolidine is one of but a few cyclic heptapeptides that have been characterized structurally either through nuclear magnetic resonance or X-ray diffraction work; others include ilamycin B_1,[5] a dolastatin 3 analogue,[6] cycloheptasarcosine,[7] four other synthetic peptides including *cyclo*(Ala-Ile-Val-Ser(Bzl)-Aib-Phe-Gly),[8] and the mycotoxin rhizonin A.[9]

The structure of ilamycin B_1, *cyclo*(1-(1,1-dimethyl-2-propen-2-yl)-L-Trp-*N*-methyl-L-Leu-3-nitro-L-Tyr-L-Ala-*N*-methyl-L-Leu-L-Leu-L-2-amino-4-(*E*)-hexenoic acid), and a possible parallelism with that of evolidine, attracted our attention because of our interest in adding to the library of well-defined, conformationally restricted, cyclic peptide backbones. An exceptional correspondence between the molecular conformation of ilamycin B_1 observed in the crystal[5a] and the structure derived by solution ^{1}H NMR methods[5b] is observed. In that structure, the peptide bonds involving the N-methylated residues are cis and reside in type VI turns. Additionally, there are three transannular hydrogen bonding interactions. These combined features suggest a fairly stable and rigid peptide backbone conformation that could represent a generalized cyclic heptapeptide framework of considerable interest.

We were thus interested in determining the three-dimensional structure of evolidine, which was predicted[3] to contain only one cis peptide bond (at the Pro residue) yet also showed solvent shielded peptide protons at Asn and Phe, suggesting that transannular hydrogen bonding analogous to that observed for ilamycin B_1 would be observed. Confirmation of this structure would illustrate the second of three generalized geometries containing two β-turns (cis-cis, cis-trans, and trans-trans) which might be envisioned as possible templates for the cyclic heptapeptide backbone. The same motif may also be accessible to a structure incorporating only trans peptide bonds. For example, *cyclo*[-Ala-Ile-Val-Ser(Bzl)-Aib-Phe-Gly-], which contains no N-alkylated residues, has been suggested to maintain a preferred

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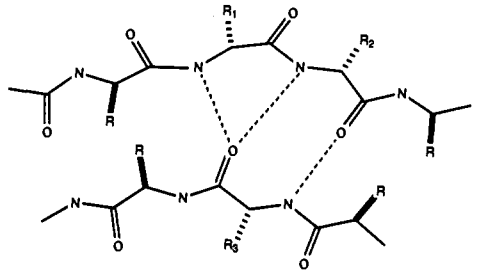
backbone conformation with type I turns at the Aib-Phe and Ala-Ile junctures.[8] In contrast, the rhizonin A structure,[9] which contains all-trans peptide bonds, but three N-methylated and three D residues, displays only one β-turn (type II).

Cyclic heptapeptides containing two β-turns may mimic the β-bulges that occur as nonrepetitive secondary structure features in proteins, most frequently within antiparallel sheets.[10] In contrast to the normal β-strand arrangement in which a one-to-one overlay of residues between the two strands exists, in a β-bulge two consecutive residues of one strand lie opposite one on the other, as illustrated in the classical β-bulge schematic, **2**. Many examples of bulges are documented in protein structures[10] underscoring their importance as a secondary structure feature.



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As defined by Richardson,[10b] a classical β-bulge displays a pair of convergent hydrogen bonds from N—H's of adjacent residues on one strand to C=O of the residue opposite, as in **2**. Side chains of all three residues are on the same side of the β-sheet. These features result in a bending of the β-sheet and, as with turns, may affect its directionality. Bulges also enhance the right-handed twist of β-strands since residue 1 adopts an approximate α-helical conformation on average ($\phi_1/\psi_1 = -100°/-45°$) while residues 2 and 3 maintain extended conformations with $\phi_2/\psi_2 = -140°/160°$ and $\phi_3/\psi_3 = -100°/130°$.

An uneven number of residues in a cyclic oligopeptide may predispose it toward similar overlap of two residues opposite one, given a tendency for β-turn formation. Indeed the resemblance of the ilamycin B_1 structure to the β-bulge motif is striking and our anticipation was that the evolidine structure could mimic this same motif while incorporating only one cis peptide bond. Of particular further relevance to mimicry of naturally occurring β-bulges would be the identification of (or demonstration that) cyclic peptides without cis peptide bonds also can accommodate these features. To this end we have studied computationally a *cyclo*(Ala_7) model based on the evolidine structure, constrained to have all-trans bonds, to explore its potential correspondence to the β-bulge model.

Experimental Section

X-ray Diffraction. Crystals of the tetrahydrate were grown by slow evaporation from aqueous ethanol. An irregular prism of approximate dimensions 0.25 × 0.30 × 0.50 mm was mounted on a glass fiber with epoxy. Initial crystal examination at room temperature on a diffractometer using a random SEARCH routine suggested a triclinic cell of volume 3301 Å^3 in which the *b* axis was roughly 33 Å in length. Examination of intensities revealed that reflections were observed only when $h - k$ was divisible by 3, suggesting the true cell to be of $^1/_3$ the volume. Further examination with SEARCH in different parts of space revealed the correct cell. The space group is *P*1 with a = 11.370 (3) Å, b = 11.705 (4) Å, c = 9.058 (6) Å, α = 94.56 (7)°, β = 111.50 (5)°, γ = 73.32 (2)°, V = 1073.3 (9) Å^3, and Z = 1 at 173 K. These cell constants are similar to those reported previously.[3] Data were measured on an Enraf-Nonius CAD-4 diffractometer equipped with graphite monochromator and Mo Kα radiation (λ = 0.71073 Å). The ω–2θ scan technique was used with a variable scan rate up to 7 deg min^{-1}, 2θ max = 56°, and index ranges $0 \le h \le 15$, $-15 \le k \le 15$, $-11 \le l \le 11$. Intensities (5419 collected) were corrected for Lorentz and polarization effects and for a small (2.9%) decline in intensity of three standards which had been measured every 3 h of exposure time. Correction factors were 1.000 min. and 1.015 max.

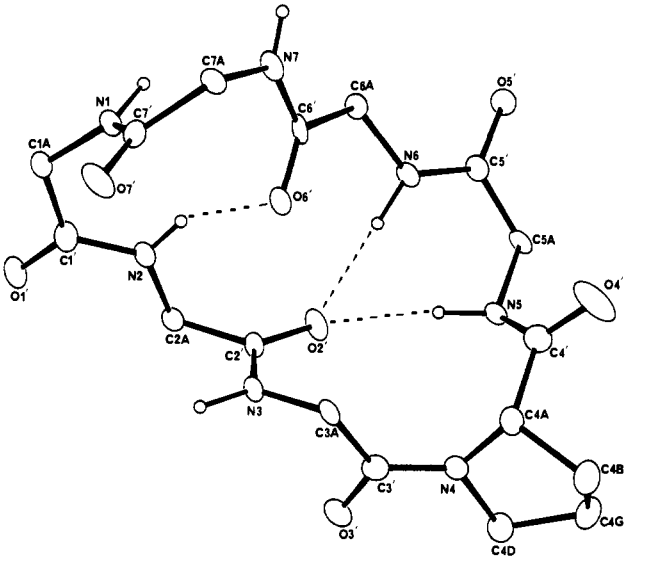

Figure 1. Conformation of the evolidine backbone as determined by X-ray crystallography. Non-hydrogen atoms are drawn as principal ellipses at the 50% probability level and H atoms as spheres of arbitrary size. Side chain atoms have been omitted. Only amide hydrogens are included. Dashed lines indicate hydrogen bonds. Atom labeling is as defined in ref 18.

A unique set of data (5161) was obtained by averaging, R_{int} = 0.027. The calculated crystal density, based on the molecular formula $C_{38}H_{58}N_8O_9 \cdot 4H_2O$ and M_r = 843.0, is 1.304 g/cm^3, $F(000)$ = 454, μ = 0.922 cm^{-1}.

The structure was solved with SHELXS.[11] Coordinates of atom O1′ were fixed to define the origin. Full-matrix least-squares refinement of the non-hydrogen atoms with anisotropic thermal parameters was performed. The function minimized was $\sum w(|F_o| - |F_c|)^2$ with the weights, w, defined as $4F_o^2/\sigma(F_o^2)$ and $\sigma(F_o^2) = [\sigma^2(I_c) + (0.04F_o^2)^2]^{1/2}$. Four well-behaved water molecules were located from a difference Fourier map. Hydrogen atom positions were located from difference maps as well and were included in the model at the suggested positions with fixed isotropic temperature factors calculated as $1.3B_{eq}$ of the atom to which they were attached. Final crystallographic residuals were R = 0.054, wR = 0.061, G.O.F. = 1.459 for a refinement of 530 variables and 3483 observations with $I \ge 3\sigma(I)$. An extinction coefficient[12] was refined in the latter stages to 1.027 (1) × 10^{-6}. In the final cycle the maximum Δ/σ = 0.01. Maximum excursions in a final difference map were within ±0.348 e Å^{-3}. Neutral atom scattering factors from *International Tables for X-ray Crystallography*[13] were used. Other computer programs were from the locally modified SDP/VAX software package.[14] Fractional coordinates for the non-hydrogen atoms are listed in Table I. Bond lengths and angles are included as Table II, while Table III presents torsion angles.

Results and Discussion

Description of the Crystal Structure. In the crystal, evolidine (see Figure 1) contains all-trans, undistorted, peptide bonds except for a cis bond at proline. A type VI(a)[15] β-turn occurs at Leu_3-Pro_4 in addition to a type I β-turn at the Leu_7-Ser_1 juncture. Three transannular backbone hydrogen bonds occur. (See Table IV for metrical details.) These bonds are found between the Phe N–H and Asn carbonyl and between the Phe carbonyl and both Val N–H and Asn N–H, as illustrated by the dotted lines in Figure 1.

Backbone bond distances and angles are presented in Table II. Averaged over the seven residues they are 1.461 Å for N_i–C_i^α,

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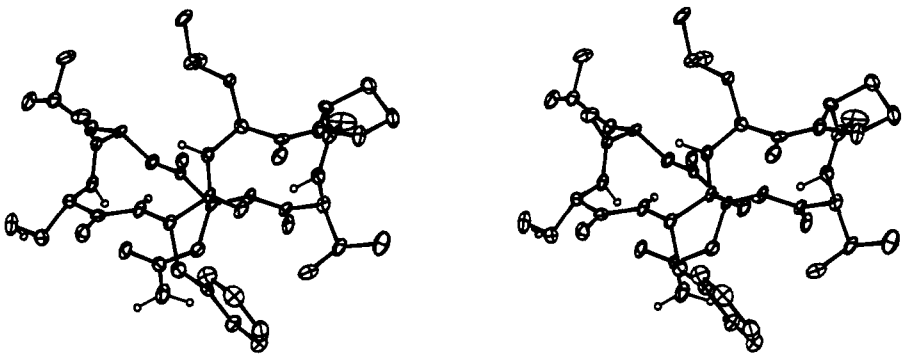

Figure 2. Stereo view of the evolidine structure including side chain orientations.

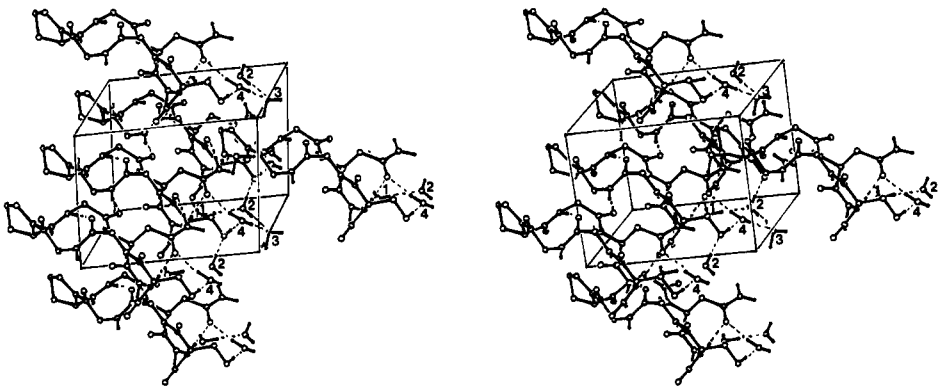

Figure 3. Stereo view of the crystal packing for evolidine. The *a* and *b* axes are approximately vertical and horizontal, respectively. Hydrogen bonds illustrated include those designated a, b, d–g, l–k, n–o in Table IV. Numbers correspond to water molecules in Table IV.

1.535 Å for C_i^{α}–C_i, 1.229 Å for C_i–O_i; 1.337 Å for C_i–N_{i+1} and 122.2° for C_{i-1}–N_i–C_i^{α}; and 110.8° for N_i–C_i^{α}–C_i, 119.8° for C_i^{α}–C_i–O_i, 122.9° for N_{i+1}–C_i–O_i, 117.2° for C_i^{α}–C_i–N_{i+1}. These values agree very well with averages reported for cyclic peptides[16] and suggest that the observed conformation induces no undue strain on the molecular framework. Principal torsion angles are found in Table III. For the type I β-turn the observed torsion angles are ϕ_{i+1} (−54°), ψ_{i+1} (−31°), ϕ_{i+2} (−100°), and ψ_{i+2} (2°). These values are very close to the theoretical values of $(\phi/\psi)_{i+1}$ (−60/−30) and $(\phi/\psi)_{i+2}$ (−90, 0) for type I β-turns.[2c] For the type VI(a) turn the $(\phi/\psi)_{i+1}$ of −65/151 and $(\phi/\psi)_{i+2}$ of −93/13 also are in good agreement with the theoretical values of −60/120 for the former and −90/0 for the latter. Side chain orientations, which are illustrated in Figure 2, likewise are normal, with the exception of the Phe ring twist which has $\chi_2^{2,1}$ and $\chi_2^{2,2}$ 60° away from the usual perpendicular orientation. The prolyl conformation is of the C_2–C^{γ} endo form as defined by the scheme of Ashida and Kakudo.[17] The valyl side chain adopts the least favorable (g^-g^+) orientation.

Hydrogen bonding interactions are multitudinous, as outlined in Table IV. However, not all possible donors participate in H bonds. One hydrogen on O3W and one on the asparagine nitrogen, N6D, appear not to be engaged in H bonding as there are no interatomic distances less than 3.3 Å which would be consistent with other H bonds involving these centers. In addition, all carbonyl oxygens, except O4′, and all side chain oxygens are hydrogen bond acceptors. This includes the three intramolecular transannular H bonds (vide supra) as well as numerous peptide–water interactions. All of these "hydrophilic" interactions are clustered near the Leu_7-Ser_1 β-turn and include the Asn_6 side chain, which is extended toward the water channel, as may be seen in Figure 3. The Asn side chain is further held by an intramolecular H bond from peptide nitrogen N1 which donates to the side chain oxygen O6D. Presumably the intramolecular H bonding of Ser_1 NH to the Asn_6 side chain facilitates adoption of the otherwise unfavorable Ser_1 ψ value near 0°.

Only two direct H bond interactions between peptide molecules are observed: one from the Asn_6 side chain nitrogen to O1′ and the other from the peptide nitrogen N3 to the Val_5 carbonyl. Both of these interactions occur between molecules translated along the *c* axis. The dearth of interpeptide hydrogen bonds suggests that such interactions have little effect on the observed backbone conformation.

Comparison with NMR Observations and Proposed Solution Conformation. Evolidine in solution has been studied by proton magnetic resonance at 220[4a,b] and 500 MHz.[4c] In dimethyl sulfoxide (DMSO), formamide, methanol, or hexafluoro-2-propanol it gives proton NMR spectra corresponding to a single component, indicating that the Leu-Pro peptide bond is entirely either cis or trans. The spectra show considerable dispersion in chemical shifts, backbone H–N–C–H coupling constants, and N–H proton chemical shift temperature coefficients, suggesting limited conformational averaging. Physical model building based on coupling constant, chemical shift, and solvent exposure data led to the suggestion of a preferred conformation in several solvents[4a,b] similar to that now found in the crystal in possessing a cis Leu-Pro bond and a backbone conformation containing a type I β-turn at Leu-Ser. Recently, more precise model building incorporating quantitative nuclear Overhauser measurements at 500 MHz into distance geometry calculations[4c] has yielded two closely related most probable solution conformations that are both very similar to the crystal structure (see Table III).

In large part, the reported experimental measures of peptide proton solvent exposure also agree with expectations from the crystal structure. In DMSO high chemical shift temperature coefficients show both Leu N–H protons to be exposed to solvent; low coefficients indicate the Phe and Asn N–H protons to be sequestered. The coefficients of the Ser and Val protons are intermediate. In methanol, the paramagnetic line broadening effects produced by a nitroxyl cosolute clearly distinguish the Leu N–H protons as exposed and the Phe, Ser, Asn, and Val N–H

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Table I. Positional Parameters and Equivalent Isotropic Temperature Factors for Evolidine[a]

atom	x	y	z	B, Å²
O1′	0.477	0.540	0.012	1.75 (7)
O1G	0.1393 (4)	0.8035 (3)	0.0181 (4)	2.35 (8)
O1W	0.0623 (4)	0.5599 (3)	0.6081 (4)	2.70 (9)
O2′	0.6130 (3)	0.1960 (3)	0.4163 (4)	1.74 (7)
O2W	0.0536 (5)	0.7927 (4)	0.6932 (5)	3.8 (1)
O3′	0.7188 (3)	−0.0423 (3)	0.2414 (4)	1.66 (7)
O3W	0.9499 (4)	0.9788 (4)	0.4585 (5)	3.3 (1)
O4′	0.4483 (4)	−0.0827 (4)	0.6449 (4)	3.53 (9)
O4W	0.1233 (7)	0.8547 (6)	0.3027 (6)	9.7 (2)
O5′	0.4481 (3)	0.2564 (3)	0.8569 (4)	1.87 (8)
O6D	0.2888 (4)	0.6370 (3)	0.4433 (4)	2.29 (8)
O6′	0.2988 (3)	0.3207 (3)	0.2942 (4)	1.47 (7)
O7′	0.0757 (3)	0.4652 (3)	−0.0771 (4)	2.07 (8)
N1	0.1949 (4)	0.5603 (4)	0.1229 (4)	1.36 (8)
N2	0.4452 (4)	0.4233 (4)	0.1732 (4)	1.36 (8)
N3	0.5130 (4)	0.1590 (3)	0.1592 (4)	1.23 (8)
N4	0.6330 (4)	−0.1191 (4)	0.3840 (4)	1.46 (8)
N5	0.5821 (4)	0.0214 (3)	0.6280 (4)	1.42 (8)
N6	0.4208 (4)	0.2547 (4)	0.5968 (4)	1.39 (8)
N6D	0.3646 (5)	0.6598 (4)	0.7075 (5)	2.4 (1)
N7	0.1309 (4)	0.4558 (4)	0.3401 (4)	1.39 (8)
C1′	0.4053 (4)	0.5161 (4)	0.0720 (5)	1.5 (1)
C1B	0.2676 (5)	0.7248 (4)	0.0695 (6)	1.9 (1)
C1A	0.2643 (4)	0.5958 (4)	0.0362 (5)	1.21 (9)
C2Z	1.0902 (5)	0.2112 (5)	0.4811 (8)	3.0 (1)
C2′	0.5716 (4)	0.2252 (4)	0.2738 (5)	1.19 (9)
C2G	0.8201 (4)	0.3282 (4)	0.3891 (6)	1.5 (1)
C2A	0.5783 (4)	0.3425 (4)	0.2174 (5)	1.27 (9)
C2B	0.6770 (5)	0.3961 (4)	0.3434 (5)	1.7 (1)
C3A	0.4918 (4)	0.0519 (4)	0.1988 (5)	1.34 (9)
C3′	0.6247 (4)	−0.0399 (4)	0.2790 (5)	1.35 (9)
C3B	0.4122 (5)	−0.0039 (4)	0.0488 (5)	1.4 (1)
C3G	0.2753 (5)	0.0741 (4)	−0.0443 (6)	2.0 (1)
C4D	0.7536 (5)	−0.2196 (5)	0.4470 (6)	2.0 (1)
C4A	0.5260 (5)	−0.1322 (4)	0.4322 (5)	1.5 (1)
C4′	0.5149 (4)	−0.0617 (4)	0.5798 (6)	1.6 (1)
C4B	0.5695 (5)	−0.2665 (5)	0.4725 (6)	2.1 (1)
C4G	0.7188 (5)	−0.2936 (5)	0.5478 (6)	2.1 (1)
C5′	0.4778 (4)	0.2053 (4)	0.7438 (5)	1.28 (9)
C5A	0.5815 (4)	0.0858 (4)	0.7730 (5)	1.5 (1)
C5B	0.7195 (5)	0.0993 (5)	0.8765 (6)	1.9 (1)
C6A	0.3338 (4)	0.3744 (4)	0.5640 (5)	1.18 (9)
C6B	0.4222 (4)	0.4621 (4)	0.6078 (6)	1.7 (1)
C6G	0.3505 (5)	0.5936 (4)	0.5802 (5)	1.6 (1)
C6′	0.2507 (4)	0.3814 (4)	0.3859 (5)	1.10 (9)
C7B	−0.0830 (4)	0.5668 (4)	0.1577 (5)	1.4 (1)
C7G	−0.1861 (5)	0.6000 (4)	−0.0106 (5)	1.7 (1)
C7′	0.1084 (4)	0.4979 (4)	0.0608 (5)	1.21 (9)
C7A	0.0427 (4)	0.4686 (4)	0.1706 (5)	1.19 (9)
C2E1	1.0056 (5)	0.2065 (5)	0.3233 (7)	2.7 (1)
C2D1	0.8720 (5)	0.2648 (5)	0.2791 (6)	2.3 (1)
C2D2	0.9067 (5)	0.3320 (5)	0.5442 (6)	2.2 (1)
C2E2	1.0382 (6)	0.2746 (5)	0.5881 (7)	2.9 (1)
C3D1	0.1875 (5)	0.1071 (5)	0.0549 (7)	2.8 (1)
C3D2	0.2124 (5)	0.0064 (5)	−0.1893 (6)	2.4 (1)
C5G1	0.8209 (5)	−0.0220 (6)	0.9244 (7)	3.0 (1)
C5G2	0.7651 (5)	0.1816 (5)	0.7986 (7)	2.9 (1)
C7D1	−0.2313 (5)	0.4948 (5)	−0.0945 (6)	2.4 (1)
C7D2	−0.3038 (5)	0.7015 (5)	−0.0016 (6)	2.3 (1)

[a] Esd's are in parentheses. $B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*a_ia_j$.

protons as sequestered. The distinction is preserved in hexafluoro-2-propanol. A methanol → hexafluoro-2-propanol solvent perturbation study yielded the same clear distinction.[4b] In the crystal, the Leu N–H protons, belonging to $i + 1$ residues of turns, are highly exposed, while Phe, Asn, and Val N–H protons, directed into the peptide ring, are to some degree buried and the Ser N–H is hydrogen bonded to the Asn side chain carbonyl. This latter interaction differs from the solution NMR prediction for the Asn side chain which has χ^1 of Asn predominantly near +60° [4a] (in contrast to the value near 180° observed in the crystal) and intramolecularly H bonded through the carboxamido proton to the carbonyl of the Leu_3-Pro_4 peptide bond. The experimental NMR data therefore are consistent with a stable solution backbone conformation close to that observed in the crystal.

Comparison to Other Cyclic Heptapeptides and to the Classical Protein β-Bulge. A comparison of the structurally characterized cycloheptapeptides reveals several conformational motifs. The evolidine structure is representative of the most populated of these. Cycloheptasarcosine, because of complete N-methylation, cannot adopt the evolidine conformation. Similarly, the ability of rhizonin A, which has the sequence *cyclo*((D-*allo*-isoleucyl)-D-valyl-L-valyl-(*N*-methyl-3-(3-furyl)-L-alanyl)-L-leucyl-(*N*-methyl-D-alanyl)-(*N*-methyl-3-(3-furyl)-D-alanine)), to adopt the β-turn, β-bulge motif is self-limited. Methylation of the L-furylalanine amide, which would otherwise function as the second of two hydrogen bond donors in the β-bulge (analogous to N–H of Leu_3 in evolidine), as well as the presence of a D-Ile residue, which appears sterically to block formation of a second β-turn about the L-Val-L-furylalanine section (in analogy to the Pro_4-Val_5 turn in evolidine), combine to exclude adoption of such a conformation. Ilamycin B_1 and evolidine adopt similar backbone conformations, as illustrated in Figure 4. Table V shows the close parallel of many backbone torsion angles for the two structures. Despite the principal difference in type for one of the two turns, the transannular hydrogen bonding pattern is identical in the two heptapeptides: Tyr NH to aminohexenoic CO in ilamycin corresponds to Phe NH to Asn CO in evolidine and Tyr CO bonded to both aminohexenoic NH and Leu NH in ilamycin corresponds to Phe CO bonded to both Asn NH and Val NH in evolidine. As with evolidine, there is definitive evidence that the crystal peptide backbone conformation of ilamycin β_1 is preserved in DMSO.[5b] Good dispersion in both H–N–C–H coupling constants and chemical shifts (3 to 9 Hz and 1.6 ppm) is observed, suggesting a relatively rigid backbone. The Tyr and aminohexenoic acid amide protons, corresponding to the Phe and Asn protons in the analogous evolidine conformation, have analogous low chemical shift temperature coefficients.

There are, of course, some significant differences in portions of the evolidine and ilamycin B_1 backbone conformations. Overall, the ilamycin backbone is flatter than that of evolidine, for which the conformation may roughly be approximated by orthogonal halves. (See Figure 2 for an appreciation of the twist in the evolidine structure.) The trans peptide bond of the evolidine type 1 turn may dictate this twist, in contrast to the cis bond in ilamycin B_1 which appears to flatten the peptide backbone through this turn.

Despite the differences mentioned, in a gross overview both the evolidine and ilamycin structures correspond to the minimal version of the classical β-bulge identified in proteins by Richardson et al.[10] The analogy is maintained on closer examination. Not only is the hydrogen bonding pattern analogous but the side chain orientations relative to the ring also are as expected. Corresponding to residues 1, 2, and 3 in the β-bulge model, the Val_5, Asn_6, and Phe_2 side chains is evolidine are on the same side of the cyclic heptapeptide ring as are those of Leu_5, aminohexenoic $acid_6$, and 3-nitrotyrosine$_2$ in ilamycin B_1. Furthermore, the ϕ/ψ torsion angles for these residues, as observed in the crystal (see Table V), are in reasonably close agreement to the averages cited for β-bulges. In particular, the extended region through residue 6 in these structures preserves the β-sheet character of the protein models as do the ϕ angles of residue 5 (position 1 in the bulge). In the model peptides, the ϕ angles of residue 2 (position 3 in the bulge) are more extended than the protein averages.

Possibility of Similar Structure with No Cis Peptide Bonds. The resemblance of evolidine and related structures to protein β-bulges is itself interesting, but the incorporation of a structure adopting all-trans peptide bonds into such a cyclic heptapeptide backbone would more closely parallel naturally occurring protein bulges. The question of whether a β-bulge like structure in cyclic heptapeptide systems is peculiar to peptides containing *N*-alkyl residues naturally arises. From the solution NMR work of Kessler and Bernd[8] on *cyclo*(Ala-Ile-Val-Ser(Bzl)-Aib-Phe-Gly), which incorporates no prolines or other N-alkylated residues, there is reason to presume that cyclic heptapeptides with all-trans bonds could form such structures. Here, a very clear distinction in

Table II. Bond Lengths (Å) and Angles (deg)[a] for Evolidine

	Ser_1	Phe_2	Leu_3	Pro_4	Val_5	Asn_6	Leu_7
				Bonds			
N_i–C_i^α	1.451	1.461	1.438	1.483	1.463	1.443	1.485
C_i^α–C_i'	1.532	1.534	1.532	1.540	1.515	1.537	1.553
C_i'–O_i	1.230	1.239	1.229	1.200	1.249	1.228	1.229
C_i'–N_{i+1}	1.340	1.336	1.341	1.355	1.342	1.321	1.327
C_i^α–C_i^β	1.525	1.520	1.546	1.537	1.551	1.568	1.526
C_i^β–$C\gamma$		1.504	1.520	1.525	1.523	1.512	1.539
					1.536		
$C\gamma$–C_i^δ		1.397	1.527	1.526			1.513
		1.396	1.529				1.535
C_i^δ–C_i^ϵ		1.390					
		1.367					
C_i^ϵ–$C\gamma$		1.408					
		1.376					
N_i–C_i^δ				1.481			
$C\gamma$–O_i^δ						1.243	
$C\gamma$–N_i^δ						1.323	
C_i^β–$O\gamma$	1.410						
				Angles			
$C_iN_iC_i^\alpha$	123.7	121.1	120.3	127.7	119.4	122.1	121.2
$N_iC_i^\alpha C_i$	113.9	106.1	109.6	114.0	113.8	106.1	112.2
$C_i^\alpha C_iO_i$	123.3	122.3	121.8	123.9	121.4	123.6	124.3
$C_i^\alpha C_iN_{i+1}$	116.5	115.4	118.5	117.1	119.1	117.1	116.7
$C_i'C_i^\alpha C_i^\beta$	109.0	112.7	108.2	109.2	111.0	111.9	110.9
$N_iC_i^\alpha C_i^\beta$	110.5	111.1	111.2	102.0	112.2	107.3	107.6
$C_i^\alpha C_i^\beta C\gamma$		117.0	115.5	102.7	111.2	116.3	116.4
					112.9		
$C_i^\beta C\gamma C_i^\delta$		122.2	112.2	104.0			112.8
		119.8	108.5				109.4
$C\gamma C_i^\delta C_i^\epsilon$		121.2					
		121.0					
$C_i^\delta C_i^\epsilon C\gamma$		119.7					
$C\gamma C_i^\beta C\gamma$					111.7		
$C_i^\delta C\gamma C_i^\delta$		117.9	109.3				110.6
$C_i^\epsilon C\gamma C_i^\epsilon$			118.8				
$C\gamma C_i^\delta N_i$				103.7			
$C_i^\delta N_iC_i^\alpha$				111.5			
$C_i^\delta CN_iC_{i-1}$				120.1			
$C_i^\alpha C_i^\beta O\gamma$	111.1						
$C_i^\beta C\gamma C_i^\delta$							120.7
$C_i^\beta C\gamma N_i^\delta$							116.6
$N_i^\delta C\gamma O_i^\delta$							122.5

[a] Esd's are of the order of 0.005–0.007 for bond lengths and 0.3–4° for bond angles.

Table III. Torsion Angles[a] (deg) for Evolidine and Computational Models[b]

	Ser_1	Phe_2	Leu_3	Pro_4	Val_5[c]	Asn_6	Leu_7
φ_i (N_i–C_i^α) crystal	−100	−157	−65	−93	−95	−159	−54
NMR[d]	−62	−142	−76	−85	−77	−138	−90
cyclo(Ala_7) one cis	−84	−163	−60	−92	−71	−150	−53
cyclo(Ala_7) all-trans	−51	−105	35	−60	−67	−173	−23
ψ_i (C_i^α–C_i') crystal	2	74	151	13	−16	151	−31
NMR[d]	−40	109	156	4	−34	170	−39
cyclo(Ala_7) one cis	15	62	140	4	−43	170	−50
cyclo(Ala_7) all-trans	−48	49	−98	−39	−48	150	−60
ω_i (C_i'–N_{i+1}) crystal	180	−179	−175	2	−176	−172	180
cyclo(Ala_7) like crystal	−180	−179	−175	1	−175	178	−179
cyclo(Ala_7) all-trans	−179	172	179	−172	180	173	−170
χ_{i1} crystal	−62	−174	−61	37	68	179	179
χ_{i2} crystal		35	−60	−40		−69	58
		−148	179			115	−179
χ_{i3} crystal				27			
χ_{i4} crystal				−4			

[a] Torsion angles are defined in ref 18; esd's are ~1° for the crystal structure. [b] Torsion angles are listed for the minimized models, see Discussion. [c] $\chi_5^{1,1}$ and $\chi_5^{1,2}$ from the crystal structure are listed for valine. [d] Reference 4c, for a representative probable solution structure; Leu_3Pro_4 peptide bond is cis.

temperature coefficients, low for Ile, Val, and Gly and high for the other residues, is taken with other indications to suggest a preferred backbone conformation with type I turns at Aib-Phe and Ala-Ile.

To explore computationally the possibility of an all-trans backbone otherwise similar to the evolidine conformation, we constructed an all-trans, all-alanine model using both the evolidine backbone coordinates and those of the β carbons, but with ϕ_3/

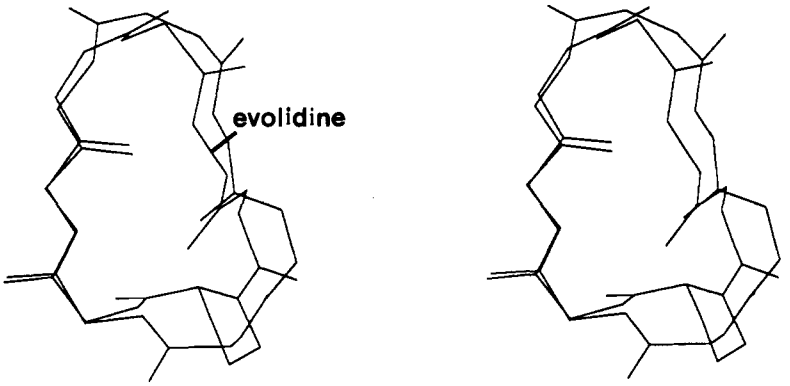

Figure 4. Overlay of the peptide backbones of evolidine with ilamycin B_1 in stereo. The view highlights both the corresponding transannular H bond features and the extended β-sheet structure through the residues preceding the turns as well as some of the differences in backbone conformation.

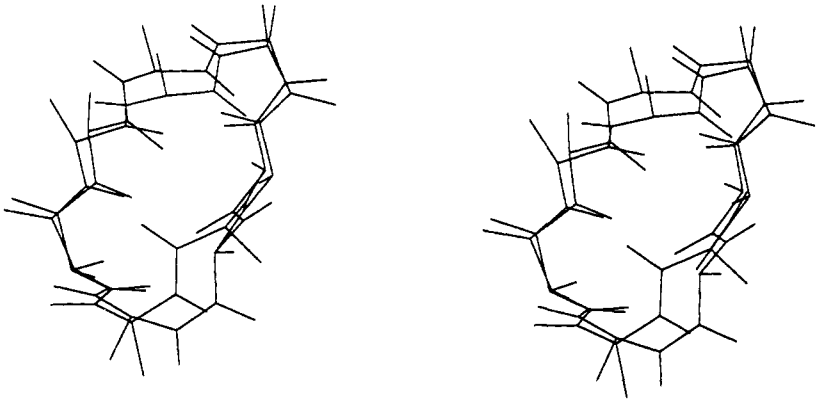

Figure 5. Stereo plot of the superimposed all-trans and cis bond containing *cyclo*(Ala_7) structures after energy minimization.

Table IV. Hydrogen Bonds for Evolidine Tetrahydrate

donor	acceptor	distance (esd), Å	angle,[a] deg	translation[b]
a. N1	O6D	2.870 (5)	164	000
b. O1G	O4W	2.660 (7)	179	000
c. N2	O6′	2.848 (6)	152	000
d. N3	O5′	2.806 (5)	167	001
e. N5	O2′	3.084 (6)	173	000
f. N6	O2′	3.081 (6)	178	000
g. N6D	O1′	2.864 (4)	174	001
h. N7	O1W	2.891 (6)	148	000
i. O1W	O2W	2.757 (6)	179	001
J. O1W	O7′	3.077 (6)	179	001
k. O2W	O3W	2.842 (6)	175	$\bar{1}$00
l. O2W	O1G	2.746 (6)	175	00$\bar{1}$
m. O3W	O3′	2.709 (5)	168	0$\bar{1}$0
n. O4W	O3W	2.838 (9)	178	100
o. O4W	O6D	2.765 (7)	161	000

[a] Angle at hydrogen. [b] Translations are along *x*, *y*, and *z*, respectively.

$\psi_3/\phi_4/\psi_4$ changed to accommodate a trans peptide bond. This molecule and, for comparison, the *cyclo*(Ala_7), corresponding precisely to evolidine with its cis peptide bond, were subjected to energy minimization with use of AMBER 3.0[19] with the all-atom model. To ensure that transannular hydrogen bonds were maintained, a dielectric of 1 was used with a 99 Å nonbond cutoff and the structures were minimized such that the norm of the gradient of the energy was ≤0.01 kcal/mol Å.

The resulting backbone dihedral angles are presented in Table III for comparison to the NMR and crystal structure results. Transannular hydrogen bond distances (N→O distances) and relative energies for the models are given in Table VI. Minimization of the all-alanine peptide in the crystal structure conformation led to a backbone very similar to the starting point with no dihedral differing by more than 25° from the crystal and the transannular hydrogen bond distances within 0.3 Å of those in the crystal structure. Minimization of the all-trans, all-alanine model led to a structure closely superimposable to the crystal except in the region of the cis-to-trans conversion. The short transannular distances at the turns are retained, although the Phe

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Table V. Torsion Angle Comparison for Evolidine and Ilamycin B_1 Crystal Structures[a]

	ϕ	ψ	ω	turn location
Ser	−100	2	180	*i*′ + 2 (I)
(*N*-MeLeu)	−128	99	1	*i*′ + 2 (VI)
Phe	−157	74	−179	
(3-NO_2-Tyr)	−156	120	180	
Leu	−65	151	−175	*i*′ + 1 (VI)
(Ala)	−61	126	−175	*i*′ + 1 (VI)
Pro	−93	13	2	*i*′ + 2 (VI)
(*N*-MeLeu)	−121	38	−11	*i*′ + 2 (VI)
Val	−95	−16	−176	
(Leu)	−123	2	−168	
Asn	−159	151	−172	
(aminohexenoic)	−163	168	165	
Leu	−54	−31	180	*i*′ + 1 (I)
(Trp)	−86	117	171	*i*′ + 1 (VI)

[a] Sequence alignment was done to juxtapose turns in the two peptides. Values for ilamycin B_1 are in parentheses. Estimated standard deviations on angles are ±1°.

Table VI. Transannular Hydrogen Bond Distances (Å) and Relative Energies (kcal/mol)

	hydrogen bond distances			
	N_2---O_6	N_5---O_2	N_6---O_2	energy
crystal	2.848	3.085	3.081	0.0
cyclo(Ala_7) like crystal	2.863	2.776	2.935	−184.1
cyclo(Ala_7) all-trans	2.701	2.987	3.806	−191.8

O-Asn N distance is lengthened by about 0.8 Å. Figure 5 shows a stereo plot of the superimposed all-trans and cis bond containing structures after minimization. While the all-trans cyclic heptapeptide *cyclo*(Ala_7) conformation so generated may not be optimal, it illustrates that the two-turn β-bulge backbone is also accessible to cyclic heptapeptides in the absence of an *N*-alkyl residue.

Investigations of cyclic heptapeptide structures in three "phases" (solid, liquid, computer) suggest that the two-turn β-bulge motif is a stable, accessible one for several constitutional and conformational variants. This backbone may represent a useful addition to the growing library of conformationally defined cyclic peptide backbones and thus be of utility in modeling interesting protein features as well as in the design of conformationally restricted pharmacological tools.

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Supplementary Material Available: Tables of anisotropic thermal parameters for non-hydrogen atoms and hydrogen atom coordinates (7 pages); listing of structure factors (28 pages). Ordering information is given on any current masthead page.

Conformation of a Cyclic Heptapeptide in Solution: An NMR Constrained Distance Geometry Search Procedure

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Abstract: A constrained distance geometry search procedure was used in conjunction with proton NMR data to determine the conformation of the cyclic heptapeptide evolidine, *cyclo*(Ser-Phe-Leu-Pro-Val-Asn-Leu), in dimethyl sulfoxide solution. Key features of the search procedure included more even sampling of dihedral angle space, generation of many conformations, exclusion of electrostatic interactions in energy minimization steps, and evaluation of possible conformations by their prediction of experimental data. With use of this procedure, two classes of closely related backbone conformations were identified for the cyclic heptapeptide. Both are similar to the recently determined crystal structure which can be described as containing a β bulge flanked by two β turns. Inclusion of electrostatic interactions in the energy minimization steps did not result in different backbone conformations provided that the NMR derived distance constraints were included. This latter result confirms that incorporation of NMR derived distance constraints can be an effective means of including solvent and electrostatic interactions in the conformational calculations for compounds in this size range.

If cyclic oligopeptides are to be used as conformationally constrained molecules for identifying the biologically active conformations of peptides, the range of conformations accessible to them must be understood. A crystal structure, if obtained, indicates a possible, but not necessarily the only, conformation. In solution, multiple conformations are often possible and the conformation-determining information is limited to what is experimentally observable. In the past, suggested solution conformations have been based on consistency with the limited data, but a search for multiple conformations consistent with the observations has not been carried out. Molecular dynamics has been used to refine structures generated from NMR data, but molecular dynamics refinement will not generally result in a broad-ranging search for additional conformations consistent with the observations unless multiple, conformationally distinct starting points are tried.

We have recently reported a procedure using distance geometry to search conformation space of cyclic oligopeptides in the absence of experimental data.[1] Important features of this procedure include use of torsion angle sampling for 1,4 distances (introduced to maximize the breadth of the search) and exclusion of electrostatic interactions from energy minimization steps. With the inclusion of constraints derived from NMR observations, the same distance geometry search can be used to find the conformational possibilities that agree with experiment. This paper describes the incorporation of experimental data into that procedure as applied to a cyclic heptapeptide and also compares conformational results obtained by varying the procedure to include electrostatic effect as a way of ascertaining their importance to this type of search.

A crystal structure of the cyclic heptapeptide evolidine, *cyclo*(Ser-Phe-Leu-Pro-Val-Asn-Leu), was recently solved in this laboratory.[2] Some time ago, a probable solution conformation of evolidine was proposed on the basis of limited NMR observations (coupling constants, solvent, and temperature effects on chemical shifts).[3] Although that proposed NMR structure has now turned out to be close to the crystal structure, it is based on more limited data than would be available today and other possibilities may not have been well considered. We have therefore reinvestigated the NMR spectra of evolidine to obtain quantitative nuclear Overhauser effect (NOE) data, in accord with current practice, and we have applied these NMR data to constraining a distance geometry based conformational search. The probable conformations identified by the search were further screened on the basis of predicted Overhauser interactions additional to the incorporated data.

Two main conclusions are reached from these studies, one in regard to the evolidine backbone and the other in regard to the search methodology: Incorporation of NMR constraints can be an effective means of including solvent and electrostatic interactions in a conformation search of molecules of this size. The probable cyclic heptapeptide backbone conformations in solution, two classes of which are identified, are very close to the conformation in the crystal. This supports the β-turn/β-bulge backbone as a generally probable cyclic heptapeptide conformation.[2]

Methods

A flowchart indicating the procedure used for generation and evaluation of evolidine solution conformations is given in Figure 1. Details

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