Structure of Cyclic Hexa-Pseudopeptide Constructed from *N, N'*-Ethylene-Bridged-(*S*)-Alanyl-(*S*)-Alanine and Glycine

Yoshitane Kojima,* Tetsushi Yamashita, and Hiroyuki Miyake
Department of Chemistry, Faculty of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558

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The crystal structure of an 18-membered cyclic pseudopeptide containing *N,N'*-ethylene-bridged-(*S*)-alanyl-(*S*)-alanine and glycine was determined by X-ray diffraction analysis. Moreover, the structure of this pseudopeptide was examined by ^{1}H NMR measurement in CD_3CN, and by molecular mechanics calculations.

The conformation analyses of cyclic peptides are important for understanding the peptide loop structures and for designing novel functional molecules.[1] The host molecules of cyclic peptides containing natural amino acid residues are generally hydrophilic and have intramolecular hydrogen bonds which shut their cavity, so that are not adequate to include guest molecules. To overcome these problems, we have designed various *N,N'*-ethylene bridged dipeptides and used them as the units of cyclic peptides for the study of the Host-Guest Chemistry.[2] These cyclic pseudopeptides have no significant intramolecular hydrogen bonds and are hydrophobic.[3] Their structures have been studied extensively in solution, but not in solid. This paper describes the structure of an 18-membered cyclic pseudopeptide, cyclo(Gly-eAA)$_2$(**1**), containing (2*S*,3'*S*)-2-(3'-methyl-2'-oxo-piperazin-1'-yl)-propanoic acid (*N,N'*-ethylene bridged (*S*)-alanyl-(*S*)-alanine; eAA)[4] and glycine. Among a series of cyclic pseudopeptides containing *N,N'*-ethylene bridged dipeptides, **1** is the first one whose structure was studied by X-ray diffraction method.

A cyclic peptide **1** was obtained from the active ester of linear hexa-pseudopeptide, HCl·(Gly-eAA)$_2$-OSu,[5] according to our previous method[4] and recystallized from methanol (46.5% cyclization yield).[6]

X-ray structural analyses[7] of **1** recrystallized from CH_3CN revealed that there are two independent molecules of **1** (**1a** and **1b**), a H_2O and a CH_3CN per asymmetric unit. Disordered H_2O and CH_3CN molecules are incorporated in the extramolecular spaces. The bond lengths and torsion angles of **1a** are similar to those of **1b**, suggesting that the overall structure of **1a** is the same as that of **1b**. Figure 1 shows the structure of **1a** drawn with ORTEP II.[8] A peptide bond, N3-C12(=O13), is cis and the other ones are trans.[9] No intramolecular hydrogen bond was observed. On the other hand, the observed intermolecular hydrogen bonds (O13(**1a**)---H-N31(**1a**), O13(**1b**)---H-N31(**1b**) and O29(**1a**)---H-N15(**1b**)) are apparent to stabilize the crystal packing. Two piperazin-2-one rings, C1-C2-N3-C4-C5-N6-C1

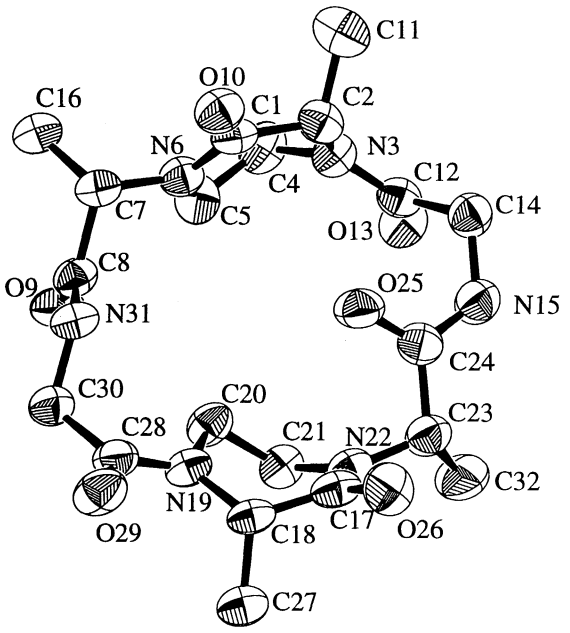

Figure 1. ORTEP drawing of **1a** showing 50% probability ellipsoids. Selected angles(deg): C24-C15-N14-C12, -66.6(7); N15-C14-C12-N3, 118.2(5); C12-N3-C2-C1, -135.6(5); N3-C2-C1-N6, 1.5(7); C1-N6-C7-C8, -131.4(5); N6-C7-C8-N31, 106.3(5) ; C8-N31-C30-C28, 127.6(6) ; N31-C30-C28-N19, -67.1(7); C28-N19-C18-C17, -151.4(5) ; N19-C18-C17-N22, -40.1(6); C17-N22-C23-C24, -99.4(6) ; N22-C23-C24-N15, -166.6(5); C1-C2-N3-C4, 35.3(6); C2-N3-C4-C5, -65.1(6); N3-C4-C5-N6, 55.5(6); C4-C5-N6-C1, -20.0(7); C5-N6-C1-C2, -8.9(7); C17-C18-N19-C20, 22.5(7); C18-N19-C20-C21, 24.4(7); N19-C20-C21-N22, -57.4(6); C20-C21-N22-C17, 43.1(6); C21-N22-C17-C18, 6.4(6).

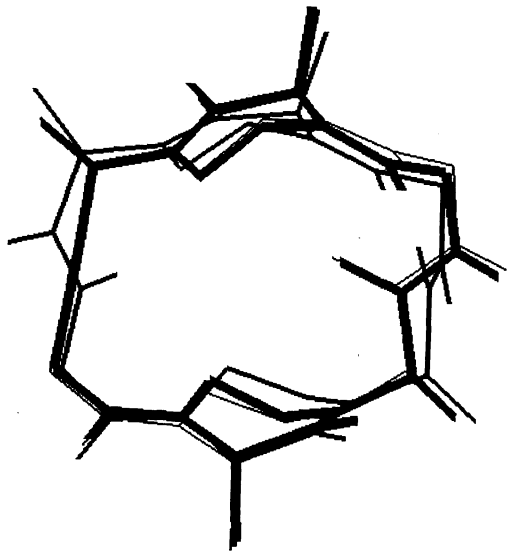

Figure 2. Overlap structure of **1a** with backbone and amide protons (━━ : crystal structure, ——— : MMPEP calculation, _____ : AMBER calculation).

and C17-C18-N19-C20-C21-N22-C17, are in distorted[10] (1,2-diplaner[11]) and pseudo-boat forms,[11] respectively (see torsion angles in the caption of Figure 1). All other torsion angles in the peptide backbone are within the allowed regions of the Ramachandran plot.[12]

Molecular mechanics calculations were carried out on the basis of the X-ray structure (Figure 2).[13] The structure from the AMBER calculation is similar to the one from X-ray analyses, but the structure from MMPEP is different in the glycine residue in comparison with the one from X-ray. This fact shows that the AMBER calculation reproduce the crystal structure of **1** in preference to the MMPEP one.

On the other hand, ^{1}H NMR measurements in CD_3CN revealed that the signal of **1** is very broad at room temperature, while the one is sharp at -40° C, and suggests the presence of a few conformers. The major conformer (abundance; ca. 67 %) of **1** exists in C_2-conformation and all peptide bonds of this conformer are trans, referring our previous data.[3] Also, the temperature coefficient (ppm deg^{-1}) of the amide proton signal of the major conformer is -6.5×10^{-3} at -40 to 0° C, indicating no presence of intramolecular hydrogen bond.[3]

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References and Notes

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5 Gly: glycine, Boc: *t*-butoxycarbonyl, OSu: active ester with *N*-hydroxysuccinimide.

6 Characterization of **1**: Anal. Found: C, 50.69; H, 6.83; N, 17.62%. Calcd for $C_{20}H_{30}N_6O_6\cdot3/2H_2O$: C, 50.31; H, 6.97; N, 17.60%. Dec = 270-280° C. $[\alpha]_D$ = +136.7 deg dm^{-1} g^{-1} cm^3 (in H_2O). IR($CDCl_3$): 1640(C=O) and 3250(NH) cm^{-1}. ^{1}H NMR(CD_3CN at -40° C referring CH_3CN signal, 1.93 ppm), δ=7.34ppm(bd, *J*=6.7Hz, 1H, H15), 4.57(dd, 14.0, 8.6, 1H, H14A), 3.19(dd, 13.1, 2.1, 1H, H14B), 4.64(q, 7.3, 1H, H2), 1.27(d, 7.3, 3H, H11), 3.88(dt, 12.8, 4.3, 1H, bridged ethylene), 3.49(ddd, 13.1, 8.9, 4.0, 1H, bridged ethylene), 3.29(m, 2H, bridged ethylene), 5.19(q, 7.3, 1H, H7), 1.20(d, 7.3, 3H, H16).

7 Crystallographic data for **1**: $C_{20}H_{30}N_6O_6\cdot1/2H_2O\cdot1/2CH_3CN$, Mw=480.03, monoclinic, space group $P2_1$(#4), a=11.339(3), b=10.527(3), c=20.235(2) Å, β=99.02(1)°, V=2385.5(8) Å^3, Z=4, D_{calc}=1.336 g cm^{-3}, μ(CuKα)=8.42 cm^{-1}, $2\theta_{max}$=120°. Intensity data were collected on a Rigaku AFC7R diffractometer. The final cycle of full-matrix least-squares refinement was based on 3318 observed reflections (I>3σ(I)) and 851 variable parameters, and converged to R=0.057 and R_w=0.083. Maximum peak in final diff. map is 0.64e Å^{-3}. All calculations were performed using the TEXSAN crystallographic software package (Molecular Structure Corporation).

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13 The MMPEP calculation was performed using MM2L program (F. Imashiro and T. Uegaki, CPLOTM Ver.2, *Japan Chemistry Program Exchange* P051, *JCPE Newslett.*, **5**, 60(1994)) with peptide parameters(S. Wolfe, D. F. Weaver, and K. Yang, *Can. J. Chem.*, **66**, 2687 (1988)). The AMBER calculation was done with the Discover program and the graphical displays were printed out from the InsightII molecular modeling system(Biosym Technologies of San Diego) on a IRIS work station(Silicon Graphics, Inc.).