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NOTES

Macrocyclic Peptides VII. Solution Conformation and Cation-Binding Properties of an Ionophorous Cyclic Octapeptide Containing *N,N'*-Ethylene-Bridged (*S*)-Valyl-(*S*)-Valine and Glycine

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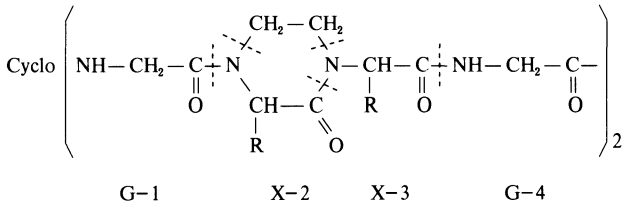
The preparations and conformations of various cyclic peptides, and their interactions with metal ions have been investigated widely by many workers.[1]

The authors studied the preparations and conformations of several synthetic, cyclic peptides including *N,N'*-ethylene-bridged dipeptides (eXX) and their interactions with organic and inorganic substrates.[2,3] Detailed, structural studies of cyclic peptides are necessary in order to examine their functionalities. In the preceding paper,[3] the structure of Ba^{2+}/cyclo[G-eLL-G]$_2$ {**1**; G=glycine and X=L=(*S*)-leucine} complex ion was clarified, though that of **1** itself have not yet been established enough. This is the first paper in which the structure of a cyclic peptide{cyclo-[G-eVV-G]$_2$=**2**; X=V=(*S*)-valine} including eXX is determined clearly in acetonitrile/1,4-dioxane(DOX) (*v/v*=4/1) by NMR and FTIR measurements, and molecular mechanic calculations (MM2).[4] The conformations and cation-binding properties of **2** with alkaline earth metal ions (Mg^{2+}, Ca^{2+}, and Ba^{2+}) were examined by NMR and CD spectra, and then CPK model.

EXPERIMENTAL

Reagents grade perchlorates dried *in vacuo*



Cyclo [G−eLL−G]$_2$=**1**; R=iso-butyl group.
Cyclo [G−eVV−G]$_2$=**2**; R=iso-propyl group.

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at 120°C were used as alkaline earth metal salts.[5] **2** was prepared according to a method similar to that of **1**.[2] **2**: mp >300°C. $[\alpha]_D^{20}$ −103° ($c=1$ in methanol). MS: m/z 676 (M^+). A cyclization yield calculated from the free carboxylic acid of Boc-octapeptide: 41%. *Anal.* Calcd for $C_{32}H_{52}N_8O_8 \cdot 3/2H_2O$ (703.8): C, 54.61%; H, 7.88%; N, 15.92%. Found: C, 54.76%; H, 7.99%; N, 15.95%.

CD data obtained in CH_3CN/DOX ($v/v=4/1$) using a quartz cell (0.005 dm) over the wavelength region from 210 to 250 nm at 23°C were represented as mean residue ellipticities. NMR spectra were obtained in CD_3CN/DOX-d_8 ($v/v=4/1$) at 30—60°C using DOX (3.50 ppm for ^1H and 66.5 ppm for ^{13}C) as internal standards. The concentrations were 6 mmol dm^{-3}. All signals were assigned by two-dimensional and selective decoupling methods. FTIR spectra were measured with concentrations of 0.5—6.6 mmol dm^{-3} in CH_3CN/DOX ($v/v=4/1$) at room temperature in an NaCl cell.

A Jeol GX-400 (NMR spectra), a Jasco DIP-320 (optical rotation), a Nicolet 5ZDX-FTIR (FTIR spectra), a Jeol JMS-HX-100 (mass spectra), and a Jasco J-500A with a DP-500 data processor (CD spectra) were used for the measurements.

The molecular structure deduced from ^1H NMR data of **2** was optimized by MM2, using the parameters of Wolfe *et al.*[6]

RESULTS AND DISCUSSION

As shown in Figure 1, CD spectra resulted in 1.3—1.5 fold increase of negative ellipticities, and the extreme shifted from 229 nm of free **2** to 226 and 227 nm for Mg^{2+} and Ca^{2+}, respectively, when 10-fold alkaline earth metal salts were added to a solution of **2**.

The CD titration curves of **2**/Mg^{2+}, Ca^{2+}, and Ba^{2+} complex ions were negative hyperbolas, and only one plateau was observed for each metal complex ion within less than 10 mol equiv. of cation, suggesting the formation of a 1 : 1 complex. Of course, the existence of other species cannot be excluded in the presence of larger amounts of metal ions. K_1 Values of the complexes of **2** with Mg^{2+}, Ca^{2+}, and Ba^{2+}, calculated by the methods similar to those of Bergeron *et al.*,[7] are 4.7, 5.7, and 1.3×10^4 $mol^{-1}\,dm^3$, respectively.

Table I shows ^1H NMR chemical shifts and coupling constants of **2**, **2Mg** ([Mg^{2+}]/[**2**]=5) and **2Ca** ([Ca^{2+}]/[**2**]=1). Tables I and II indicate that **2**, **2Mg** ([Mg^{2+}]/[**2**]=5), **2Ca** ([Ca^{2+}]/[**2**]=1 and 5) and **2Ba**([Ba^{2+}]/[**2**]=5) each have different C_2-symmetry structures from their simple NMR data. The assignments of their signals were established with NOE observed between asymmetric (H7) and amide (H21) protons.

Table I reveals that G-eVV(C14-N3) peptide bonds of **2**, **2Mg** and **2Ca** are all *trans* from their chemical shifts (4.85, 4.38, and 4.60 for H2 and 3.63, 3.79, and 3.71 ppm for H4e, respectively), based on previous data on a unit of **2**, Boc-G-eVV-OH(**3**).[8] The coupling constants (7.3, 10.4, and 9.8 for H2 and 11.3,

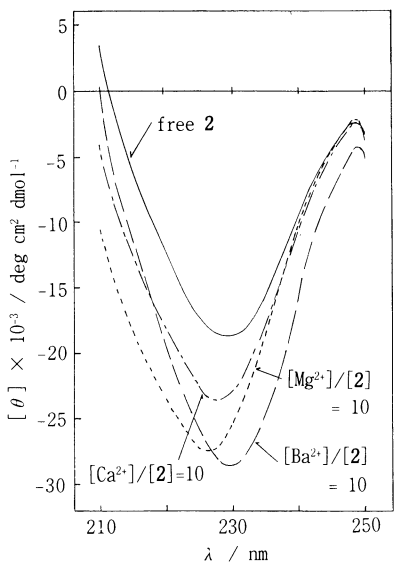
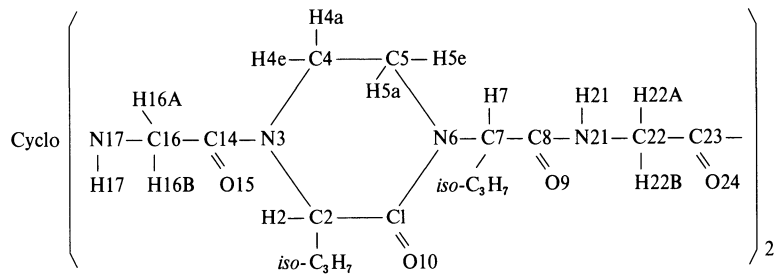


Figure 1. CD Spectra of free **2** and **2** in the presence of Mg^{2+}, Ca^{2+}, and Ba^{2+} in acetonitrile/1,4-dioxane($v/v=4/1$). Mole ratios of salts to **2** are shown in Figure. [**2**] $\simeq 2.3\times10^{-4}$ mol dm^{-3}.

Table I. ^{1}H NMR Chemical shifts and coupling constants of **2**[a], **2Mg** ([Mg^{2+}]/[**2**] = 5)[a] and **2Ca** ([Ca^{2+}]/[**2**] = 1)[a] in DOX-d_8/CD_3CN (v/v = 1/4) at 35°C

	Chemical shift, δ/ppm (Coupling constants/J, Hz)										
	H17	H16A and B		H2	H4e	H4a	H5e	H5a	H7	H21	H22A and B	
2	6.71 (s[b])	3.93 (ABqd,[c] 17.4,4.6)		4.85 (d, 7.3)	3.63 (m)		3.3—3.4 (m)		4.63 (d, 11.3)	7.14 (dd, 7.6, 4.9)	4.05 (dd, 16.5, 7.6)	3.31 (dd, 16.5, 4.9)
2Mg	7.16 (t, 5.7)	4.09 (dd, 17.5, 7.2)	3.69 (dd, 17.4, 4.9)	4.38 (d, 10.4)	3.79 (bd, 14.3)		3.3—3.6[d]		4.42 (d, 11.3)	6.90 (bs)	4.25 (dd, 17,1, 8.6)	3.4[d]
2Ca	7.32[b]	4.22 (dd, 17.1, 7.2)	3.77 (dd, 17.1, 4.9)	4.60 (d, 9.8)	3.71 (dt, 12.2, 4.7)		3.4—3.6[d]		4.68 (d, 11.6)	7.60[b]	4.21 (dd, 16.8, 7.6)	3.54 (dd, 16.8, 4.6)

[a] The numbering of **2**, **2Mg** and **2Ca**: iso-C_3H_7: iso-propyl.



[b] Triplet-like.
[c] Quartet-like.
[d] Overlapped signals.

11.3, and 11.6 Hz for H7, respectively) of **2**, **2Mg**, and **2Ca** show that α- and β-methine protons of two V residues of each eVV situate in *trans* each other, though the side chains on the piperazin-2-one (MKP) ring vibrate in a manner similar to those of **3**.[8] The MKP ring of **2** cannot take a boat form because of steric hindrance of the iso-propyl group on MKP ring. Accordingly, the multiplet signals of the ethylenic protons suggest that the MKP ring of **2** exists in equilibrium, between a pseudo-chair form and distorted one deviating a little from a pseudo-chair form, compared with the MKP ring of **3**.[8] On the other hand, the MKP ring of **2Ca** may be a pseudo-chair form from the coupling constants (dt, 12.2 and 4.7 Hz) of H4e observed clearly. Moreover, changes (from 7.3 to 9.8 Hz) of the coupling constants of H2 indicate that the vibration of the side chain on MKP ring is reduced because of the fixation to the pseudo-chair form. On the basis of torsional angles (ϕ and ψ)[9] of **2** estimated from ^{1}H NMR data of Table I by the method of Bystrov,[10] steric energies were obtained by MM2. In MM2, ϕ of G-1 changed in the range of 360°, and minimized steric energies (45.7,

Table II. ^{13}C NMR data of amide carbons of **2**, **2Mg**, **2Ca**, and **2Ba** in DOX-d_8/CD_3CN ($v/v=1/4$) at 35°C

	$[M^{2+}]/[2]$	Chemical shift, δ/ppm ($\Delta\delta$[a])			
		G-1 (C14)	V-2 (C1)	V-3 (C8)	G-4 (C23)
2[b]		168.03	169.29	170.26	169.38
2Mg[b]	5	170.52	171.64	170.61	173.21
		(2.49)	(2.35)	(0.35)	(3.83)
	1	170.46	170.94	170.70	173.47
		(2.43)	(1.65)	(0.44)	(4.09)
2Ca[b]	5	170.43	170.91	170.59	173.03
		(2.40)	(1.62)	(0.33)	(3.65)
2Ba[b]	5	170.45	171.32	170.54	174.33
		(2.42)	(2.03)	(0.28)	(4.95)

[a] $\Delta\delta=\delta$(complex ion)$-\delta$(**2**).
[b] Refer[a] of Table I for the numbering of **2**, **2Mg**, **2Ca**, and **2Ba**.

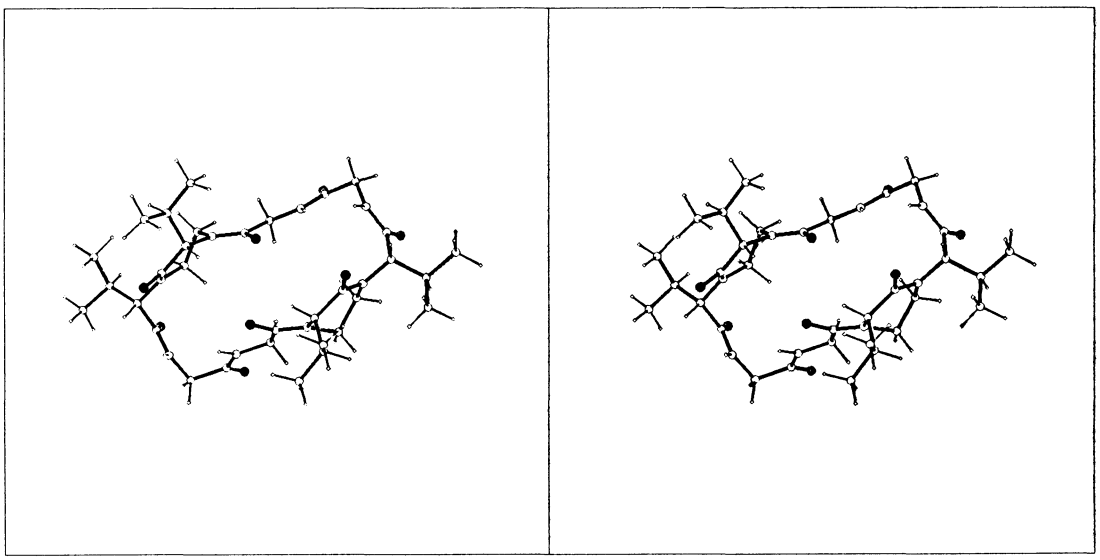


Figure 2. A structure of **2** with a pseudo-chair form for MKP ring was optimized by molecular mechanics calculations. ●, oxygen atoms.

Table III. Torsional angles ($\pm 20°$) for a proposed conformation of **2**, **2Mg**, and **2Ca** in DOX-d_8/CD_3CN ($v/v=1/4$)

	2		**2Mg**		**2Ca**	
	G-1	G-4	G-1	G-4	G-1	G-4
ϕ	−173.8	86.5	60	—[a]	60	60
ψ	162.4	43.3	170	165	165	165

[a] Not estimated owing to overlapping signals.

44.4, and 46.3 kcal mol^{-1}, respectively) were obtained for the ϕ (−173.8, −75.2, and 90.6°). Table III shows the most reasonable calculated values of ϕ and ψ of G-1 and -4 of **2**, and those estimated from NMR data for **2Mg** and **2Ca**.

The temperature coefficients[11] (ppm deg^{-1}) of the amide protons obtained in CD_3CN/DOX-d_8 ($v/v=4/1$) by ^1H NMR measurements are -1.4 and -2.5×10^{-3} for H17 and H21 of **2**, respectively, and -3.4×10^{-3} for *N*-

methylacetamide without intramolecular hydrogen bond. Small temperature dependence observed for H17 of **2** indicates that, in spite of the increasing temperature, the conformation of **2** does not change. Also, the temperature coefficients in dimethyl sulfoxide-d_6 were -3.7 and -7.1×10^{-3} for two amide protons of **2**, and almost identical with those of the other amide compounds[12] without intra-molecular hydrogen bonds. These results and the CPK model speculation suggest that no intra- and inter-molecular hydrogen bonds of **2** exist.[13] Moreover, the NH absorption (3388 cm^{-1}) of **2** in IR spectra varied scarcely in CH_3CN/DOX($v/v = 4/1$) at various concentrations, thus excluding the existence of inter-molecular hydrogen bonds. From the above results and MM2, the structure of **2** with a pseudo-chair form is proposed in Figure 2.

As shown in Table II, the signals of three amide carbons (G-1, V-2, and G-4) of **2Mg**, **2Ca**, and **2Ba** each shifted down markedly from those of **2**, while that of V-3, a little. When the alkaline earth metal salts were added to the solution of **2**, the oxygen (O24) of the most flexible amide group of **2** moved into the cavity. As a result, Mg^{2+}, Ca^{2+}, and Ba^{2+} were in the cavity surrounded by the six amide oxygens of G-1, V-2, and G-4.

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