SHORT COMMUNICATIONS

Interactions of Organic Substrates with 30- and 36-Membered Ring Peptides Containing (2*S*,3'*S*)-2-(2'-Oxo-3'-methylpiperazin-1'-yl)-propanoic Acid and Sarcosine

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Several workers¹ have previously investigated interactions of synthetic cyclic peptides with organic substrates such as α-amino acid esters. In these studies, they used a cyclic compound (proline) as a unit of cyclic peptides effectively.

The authors used a new cyclic compound, (2*S*,3'*S*)-2-(2'-oxo-3'-methylpiperazin-1'-yl)-propanoic acid² (EAA), which is expected to play a role similar to that of proline, and prepared 30- and 36-membered ring peptides (CP-1 and -2, respectively) consisting of EAA and sarcosine (Sar) in order to include organic substrates more effectively.

In these cyclic peptides (CP) having no amide protons, there are no intramolecular hydrogen bonds and their cavities are larger and deeper than those of the cyclic peptides reported earlier by the other workers.¹

A synthetic route of CP-2 is outlined as follows (Boc, *t*-butyloxycarbonyl; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; HOSu, *N*-hydroxysuccinimide).

Table I. ^1H NMR Spectra^a in CD_3OD at $30.0 \pm 0.2^\circ\text{C}$

Compound ^c	^1H Chemical shift, δ ($\Delta\delta^b$)								
	Cation						Anion		
	α	$\beta+\gamma$	δ	2	3	4	CH_3	2	3
S-1	—	—	—	—	—	—	2.366	7.713	7.222
CP-1/S-1	—	—	—	—	—	—	2.366 (0.000)	7.709 (-0.004)	7.228 (0.006)
CP-2/S-1	—	—	—	—	—	—	2.368 (0.002)	7.714 (0.001)	7.226 (0.004)
S-2	4.095	—	—	—	7.430 ^d	—	2.365	7.710	7.218
CP-1/S-2	4.112 (0.017)	—	—	—	7.434 (0.004)	—	2.336 (0.001)	7.707 (-0.003)	7.225 (0.007)
CP-2/S-2	4.111 (0.016)	—	—	—	7.436 (0.006)	—	2.365 (0.000)	7.705 (-0.005)	7.224 (0.006)
S-3 ^e	2.907	1.673 ^d	2.662	7.184	7.260	7.176	2.362	7.708	7.220
CP-1/S-3	— ^f	1.686 (0.013)	2.662 (0.000)	7.187 (0.003)	7.261 (0.001)	7.178 (0.002)	2.363 (0.001)	7.707 (-0.001)	7.226 (0.006)
CP-2/S-3	— ^f	1.692 (0.019)	2.665 (0.003)	7.187 (0.003)	7.262 (0.002)	7.178 (0.002)	2.365 (0.003)	7.711 (0.003)	7.225 (0.005)

^a ^1H -NMR data were obtained by a JEOL GX-400 (estimated uncertainty ± 0.001 ppm).^b $\Delta\delta = \delta$ (cyclic peptide + substrate) $-\delta$ (substrate); ppm.^c The molal ratio of cyclic peptides to substrates $\approx 2:1$.^d The β - and γ -methylene protons of S-3 and the aromatic protons of S-2 were observed as overlapped signals. ^f The signal of α -methylene protons overlapped

that of N-methyl protons.

The cyclization was carried out in pyridine-dichloromethane (1:3) at high dilution (0.1 weight%) for 4~5 days at room temperature, giving a crude **CP-2** which was purified by a silica gel and a successive Sephadex LH-20 column chromatography. A yield of pure **CP-2** was 24%. mp 205—215°C. $[\alpha]_D^{25} + 37.0^\circ$ (*c* 0.37, methanol). IR(cm^{-1}): 1640—1660. Mass spectrum m/e 956 (M^+). *Anal.* Calcd for $\text{C}_{44}\text{H}_{68}\text{N}_{12}\text{O}_{12} \cdot 3.5\text{H}_2\text{O}$: C, 51.80%; H, 7.41%; N, 16.48%. Found: C, 51.90%; H, 7.26%; N, 16.39%.

CP-1 [cyclo(Sar-Sar-Sar-EAA-)]₂ was also prepared in a 25% yield in a manner similar to that of **CP-2**. mp 240—250°C. $[\alpha]_D^{15} - 38.2^\circ$ (*c* 1.12, ethanol). IR (cm^{-1}): 1640—1660. Mass spectrum m/e 762 (M^+). *Anal.* Calcd for $\text{C}_{34}\text{H}_{54}\text{N}_{10}\text{O}_{10} \cdot 4\text{H}_2\text{O}$: C, 48.91%; H, 7.48%;

N, 16.78%. Found: C, 49.08%; H, 7.32%; N, 16.50%.

In this experiment, *p*-toluenesulfonic acid salts of sodium, benzylamine and 4-phenylbutylamine (**S-1**, **-2** and **-3**, respectively) were used as organic substrates. The interactions of **CP-1** and **-2** with **S-1**, **-2**, and **-3** were examined in CD_3OD at $30 \pm 0.2^\circ\text{C}$ by ^1H NMR spectroscopy.

The results obtained for **S-1**, **-2**, and **-3** in the presence of **CP-1** and **-2** are shown in Table I. Table I indicates the distinct down field shifts (0.017 and 0.016 ppm, respectively) of methylene protons of **S-2** for **CP-1** and **-2**, and the smaller down shifts (0.004 and 0.006 ppm, respectively) of aromatic protons of **S-2** for **CP-1** and **-2**. These results suggest that the ammonium group of **S-2**

forms hydrogen bonds with the amide carbonyl groups of **CP-1** and **-2**, and the methylene protons adjacent to the ammonium group are strongly affected. On the other hand, the aromatic ring of **S-2** may not be included enough in the cavities of **CP-1** and **-2** as supposed from the smaller shifts of aromatic protons.

As shown in Table I, the α -methylene proton signals of **S-3** were not observed due to signal overlapping with one of the N-methyl protons of sarcosine. However, Table I also shows that the $(\beta + \gamma)$ -methylene protons of **S-3** for **CP-2** shift distinctly to a low field (0.019 ppm) while those of **S-3** for **CP-1** move less (0.013 ppm). Furthermore, the down field shifts of δ -methylene protons of **S-3**, far from the ammonium group, were 0.003 ppm for **CP-2** and 0.000 ppm for **CP-1**. These results suggest the possibility that the interactions of **CP-1** and **-2** with **S-3** are mainly attributable to the hydrogen bonds between the amide carbonyl groups of cyclic peptides and the ammonium groups of the substrates, and the methylene groups of **S-3** are more effectively included

into **CP-2**. The more effective inclusion of the methylene groups with **CP-2** may be due to the fact that the cavity of **CP-2** is surrounded more densely by higher walls (EAA) than those of **CP-1** because the ratio (1:1) of EAA to sarcosine in **CP-2** is higher than that (1:3) in **CP-1**.

The *p*-toluenesulfonate ions of substrates indicated only smaller up or down field shifts (within 0.007 ppm), showing weak or no interaction of these anions with cyclic peptides.

The conformation of **CP-1** and **-2** has not still been clarified because of the complex and broad signals observed by NMR measurements.

REFERENCES

1. C. M. Deber and E. R. Blout, *J. Am. Chem. Soc.*, **96**, 7566 (1974); B. Bartman, C. M. Deber, and E. R. Blout, *ibid.*, **99**, 1028 (1977); V. Madison, C. M. Deber, and E. R. Blout, *ibid.*, **99**, 4788 (1977).
2. S. Kubo, Y. Kojima, T. Yamashita, and A. Ohsuka, presented at the 1st Symposium on Biofunctional Chemistry, Osaka, June 4, 1986, A206.