Characterization of Crystalline L-Carnosine Zn(II) Complex (Z-103), a Novel Anti-gastric Ulcer Agent: Tautomeric Change of Imidazole Moiety upon Complexation

Takefumi MATSUKURA,*[,a] Tamiko TAKAHASHI,[a] Yasuhiro NISHIMURA,[a] Takuzo OHTANI,[a] Michikazu SAWADA,[a] and Kozo SHIBATA[b]

Research Laboratories, Hamari Chemicals Ltd.,[a] 1-4-29, Kunijima, Higashiyodogawa-ku, Osaka 533, Japan and Faculty of Science, Osaka City University,[b] 3-3-138, Sugimoto, Sumiyoshi-ku, Osaka 558, Japan. *Received April 11, 1990*

A novel crystalline Zn(II) complex of L-carnosine was prepared and evaluated for inhibitory activity against gastric ulceration in rats. The complex was found to be much more active than various other Zn(II) compounds. It was characterized by means of infrared (IR) spectroscopy, solid-state carbon-13 (^{13}C) and nitrogen-15 (^{15}N) ^{1}H-cross-polarization (CP) magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopy and X-ray photoelectron spectroscopy (XPS). The spectroscopic data indicated that L-carnosine coordinates to Zn(II) as a quadridentate ligand. A comparison of the ^{13}C-NMR signals of the imidazole carbons of the complex with those of several histidine derivatives revealed that a tautomeric change of imidazole moiety had occurred upon complexation. The binding mode of the complex was considered to be analogous to that of the corresponding Cu(II) complex.

Keywords L-carnosine; Zn(II) complex; anti gastric ulcer agent; solid NMR; X-ray photoelectron spectrum; tautomeric change

L-Carnosine (β-Ala–L-His) (**1**), discovered in Liebig's meat extract,[1] is present in the muscle tissues of human and other vertebrates in relatively high concentration. Although its physiological role has not been fully established yet, some interesting biological activities were reported recently, which suggested the potential medical use of the compound to promote the healing of surgical wounds, and to treat gastric ulcers, arthritis, inflammation and diseases caused by active oxygens.[2] In the past two decades, much attention has also been focused on zinc compounds. It is well-known that Zn(II) ion is an indispensable component of certain enzymes such as alkaline phosphatase, carbonic anhydrase, deoxyribonucleic acid (DNA) polymerase and alcohol dehydrogenase.[3] In these metalloenzymes, the metal is located at the active site and participates in the actual catalytic processes. Some inorganic or simple organic zinc compounds such as zinc sulfate or zinc acetate have been used therapeutically for sickle cell anemia, rheumatoid arthritis, wound healing, zinc deficiency, hepatic disorder and gastric ulcer.[4] It is generally considered that the toxicity of zinc compounds is very low, if the ligands are properly chosen.[4] However, no effort has been made to select the most effective non-toxic ligand, working presumably as a carrier of Zn(II) ion into the body. In the present work, we chose L-carnosine as the ligand, because an enhanced pharmacological effect for gastric ulcerations can be expected by a synergistic interaction between the two components.

The structure of L-carnosine Zn(II) complex in acidic solution was initially discussed on the basis of potentiometric pH titration data,[5] but the complex formed under neutral or basic conditions could not be characterized owing to precipitation. In this paper, we report the synthesis of crystalline L-carnosine Zn(II) complex (**2**), and compared its ability to inhibit gastric ulcerations in rats with those of other zinc compounds. The characterization of the complex was carried out by physicochemical approaches such as infrared absorption (IR) and far-IR spectroscopy, solid-state carbon-13 (^{13}C) and nitrogen-15 (^{15}N) ^{1}H-cross-polarization (CP) magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopy, and X-ray photoelectron spectroscopy (XPS) in the solid state because of the poor solubility of the complex in every solvents examined.

Results and Discussion

Preparation and Composition of 2 L-Carnosine (**1**) reacted with Zn(II) in methanol in the presence of sodium methoxide to afford the complex (**2**), which gave a sharp X-ray powder diffraction pattern as shown in Fig. 1, whereas a different hydrated complex (**3**), obtained by Weitzel's method using water as a solvent,[6] gave a very diffused and featureless pattern. These results suggest the former has high crystallinity but the latter is nearly amorphous. The amorphous complex **3** gave broad signals in the IR and solid-state ^{13}C-CP/MAS NMR spectra, whose patterns were completely different from that of **2**. Attempts to obtain

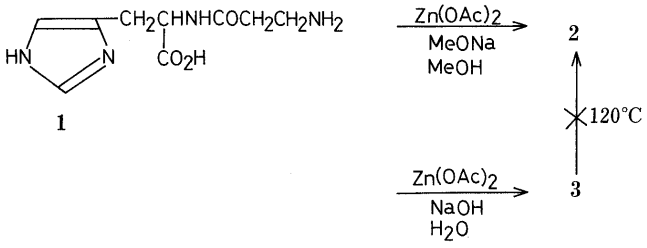

Chart 1

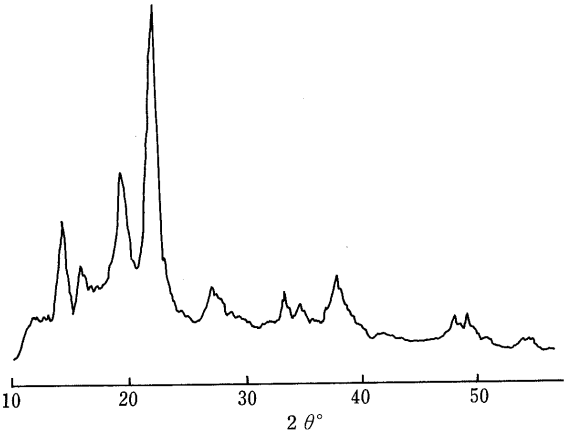

Fig. 1. X-Ray Powder Diffraction Pattern of **2**

TABLE I. Inhibitory Effects of Zn(II) Complexes on Gastric Ulcers Induced by Water-Immersion Stress in Rats

Compound	Dose[a] (mg/kg)	Inhibition (%)	Compound	Dose[a] (mg/kg)	Inhibition (%)
1	310	−2	(L-Val–L-His)Zn·H_2O	100	26
2	100	96[b]	(β-Ala–L-Ala)Zn·H_2O	100	72[c]
4	100	26	(β-Ala–Gly)Zn·$2H_2O$	100	86[c]
9	100	9	(Gly–Gly)Zn·$2H_2O$	100	31
$(Gly)_2Zn·3H_2O$ (**15**)	100	52[d]	(Gly–Gly–Gly))Zn·H_2O	100	67[c]
(Hcar)Zn·$2H_2O$[e]	100	62	(Gly–L-His)Zn·H_2O	100	28
(L-Glu)Zn·$2H_2O$	100	65[c]	$(Undec)_2Zn$[f]	100	−23
(L-Asp)Zn·$3H_2O$	100	59	$(Stea)_2Zn$[g]	100	8
$ZnSO_4$	100	13	Cimetidine	100	76

a) Oral administration (*p.o.*). Student's *t* tests were carried out. Significantly different from control: *b*) $p<0.001$, *c*) $p<0.01$, *d*) $p<0.05$. *e*) Homocarnosine complex. *f*) Undecylic acid salt. *g*) Stearic acid salt.

TABLE II. Inhibitory Effects of **2** and Cimetidine on Gastric Ulcers Induced by Water Immersion Stress in Rats

Compound	Dose (mg/kg, *p.o.*)	Ulcer index Mean±S.E. (mm)	Inhibition (%)
Control	—	13.4±2.8	—
2	30	8.6±3.3	36
	100	1.1±1.0[a]	92
	300	0.1±0.1[a]	99
Cimetidine	30	4.8±1.8[c]	65
	100	2.1±0.5[b]	84

Student's *t* tests were carried out. Significantly different from control: *a*) $p<0.001$, *b*) $p<0.01$, *c*) $p<0.05$.

TABLE III. Inhibitory Effects of **2** and Cetraxate Hydrochloride (CET) on Gastric Ulcers Induced by HCl–EtOH in Rats

Compound	Dose (mg/kg, *p.o.*)	Ulcer index Mean±S.E. (mm)	Inhibition (%)
Control	—	78.5±10.0	—
2	3	45.9± 8.3[a]	42
	10	16.4± 7.3[b]	79
	30	1.9± 0.9[b]	98
CET	30	58.0± 9.9	26
	100	17.8± 6.6[b]	77

Student's *t* tests were carried out. Significantly different from control: *a*) $p<0.05$, *b*) $p<0.001$.

a single crystal of **2** of suitable size for X-ray crystallography were unsuccessful. Molecular weight determination using fast atom bombardment mass spectrometry also failed, because no mass peak was observed below 1200 mass units. Elemental analysis of **2** was satisfactory for the formula $C_9H_{12}N_4O_3Zn$ (Calcd: C, 37.33; H, 4.18; N, 19.35; Zn, 22.58. Found: C, 37.07; H, 4.27; N, 19.06; Zn, 22.23). Dissociation of **2** by 3 N HCl followed by treatment with cation exchange resin gave an almost equimolar amount of **1**. From these observations, **2** is proved to be composed of doubly deprotonated L-carnosine and Zn(II) with 1 : 1 molar ratio. The elemental analysis of **3** was not consistent with any simple composition.

Biological Activity Tables I and II show the efficacy of **2** against water-immersion stress ulceration in rats. The L-carnosine complex (**2**) dose-dependently inhibited the formation of gastric ulcers. The inhibitory effect of **2** was greater than that of cimetidine or zinc acexamate ($(CH_3CONH(CH_2)_5CO_2)_2Zn$) (**4**); cimetidine is known to be a strong anti-gastric ulcer agent and the latter compound is being studied clinically in Europe.[7] We have also investigated the inhibitory effect of **1** and the K, Na, Ca and Mg salts of **1**, but no significant effect was observed with those compounds. From the results, the pharmacologically active component seems to be Zn(II) ion though the mechanism is not clear yet. The refinement of the structure–activity relationship is in progress. Table III shows that **2** is also very effective against HCl–EtOH ulceration in rats, indicating that the complex acts on the ulcer cytoprotectively.[8] In this case, cimetidine was not effective at all.

TABLE IV. IR Spectral Data for **1** and **2**[a]

1	**2**	Assignment
3240—2100 (br)[b]	3280 (s)[b]	NH_2 stretching
1656 (s)	1628 (vs)	N(H)CO stretching
	1600 (s)[b]	NH_2 bending
1582 (s)	1560 (s)	COO^- asym. stretching
1408 (s)	1388 (s)	COO^- sym. stretching
1270 (m)[b]	1260 (m)[b]	NH_2 twisting
1162 (m)	1115 (m)	C–N stretching
	999 (m)[b]	
	700 (m)[b]	

a) Spectra were taken in a KBr pellet. Characteristic absorption bands are given in wave number units (cm^{-1}). *b*) These absorption bands disappeared or were diminished in intensity in the deuterated compounds.

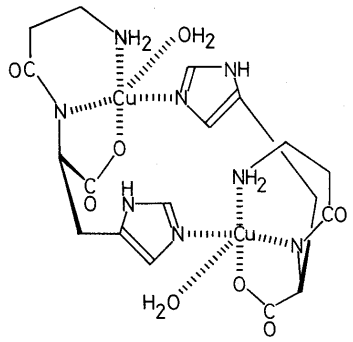

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Fig. 2

Characterization of 2 IR Spectra: The major IR absorption bands of **2** are shown in Table IV. A remarkable low-frequency shift of the amide carbonyl absorption in **2** by 28 cm^{-1} from that of **1** was observed. Lukton and Sisti

pointed out that the low-frequency shift (38 cm^{-1}) of the amide carbonyl absorption in the Cu(II) complex (**5**) of L-carnosine could be attributed to the decreased C=O bond order caused by the deprotonation from amide nitrogen.[9] The X-ray geometry of **5**, shown in Fig. 2,[10] is consistent with their hypothesis. The low-frequency shift of the absorption in **2** is considered to occur because the deprotonated amide nitrogen coordinates to Zn(II) as in the case of **5**. Such deprotonation from amide nitrogen by Zn(II) ion was reported recently for the Zn(II) complexes of Gly–L-His and L-Ala–L-His (an isomer of L-carnosine) by Rabenstein and others.[11]

The two absorption peaks of **2** at 1560 and 1388 cm^{-1} were assigned to antisymmetric and symmetric stretching vibrations of the carboxylate group, shifted to the lower frequency region by 22 and 20 cm^{-1} from those of **1** respectively. A strong single absorption at 3280 cm^{-1}, located in the lower frequency region compared to those of primary amines, indicates that the amino group is coordinated to the metal.[12] We prepared the corresponding deuterated **2** in order to assign the absorption bands related to the amino group, which were shifted to a lower frequency region in the deuterated compound. Of the four potential coordination sites of a carnosine ligand in **2**, *i.e.*, i) pyridine-type nitrogen of the imidazole ring,[13] ii) carboxylate oxygen, iii) amino nitrogen and iv) deprotonated amide nitrogen, the IR spectra revealed at least three to be present, *i.e.*, ii), iii) and iv).

Solid-State ^{13}C-CP/MAS NMR: Recently, solid-state CP/MAS NMR spectra of some amino acids and peptides have been measured in order to investigate the conformation of those molecules in the solid state.[14] However, little work has yet been done in this field, and no attempt to characterize metal complexes by means of this method has been made. Metal complexes usually undergo dynamic ligand change in solution, and the imidazole moiety in a carnosine molecule is considered also to be in rapid tautomeric equilibrium. For such flexible compounds, we can observe only the time-averaged NMR signals in solution. In the solid state, on the other hand, the structure of those compounds is frozen to give predominantly one chemical species, and the spectrum is expected to be simple and easy to understand.[15] We measured the solid-state ^{13}C-CP/MAS NMR spectra of **2** in addition to those of structurally related compounds whose structures had been confirmed by means of X-ray crystallography.

Figure 3 shows the solid-state ^{13}C-NMR signals of the imidazole moiety. In the case of imidazole (**6**) and its Zn(II) complex (**7**),[16] the resonance signal of C(4) in **7** is shifted downfield by 4 ppm due to complexation, whereas those of C(2) and C(5) are almost unaffected. The difference of the chemical shifts between C(2) and C(5) in L-histidine (**8**) was very small (*ca.* 1.7 ppm), and the signals overlapped in the case of the corresponding complex (**9**). The signal of C(4) in **9** is shifted downfield slightly upon complexation as in the case of the imidazole complex (**7**). From X-ray crystallography, it is known that the imidazole moiety in **8** and the complex (**9**) has N(3)-H tautomeric form, and Zn(II) ion binds to the pyridine-type nitrogen N(1).[17,18]

From these observations, no marked change in the ^{13}C chemical shift upon complexation would be expected except for the slight downfield shift (*ca.* 4 ppm) of the C(4) signal. However, in the case of **1** and the complex (**2**), as shown in

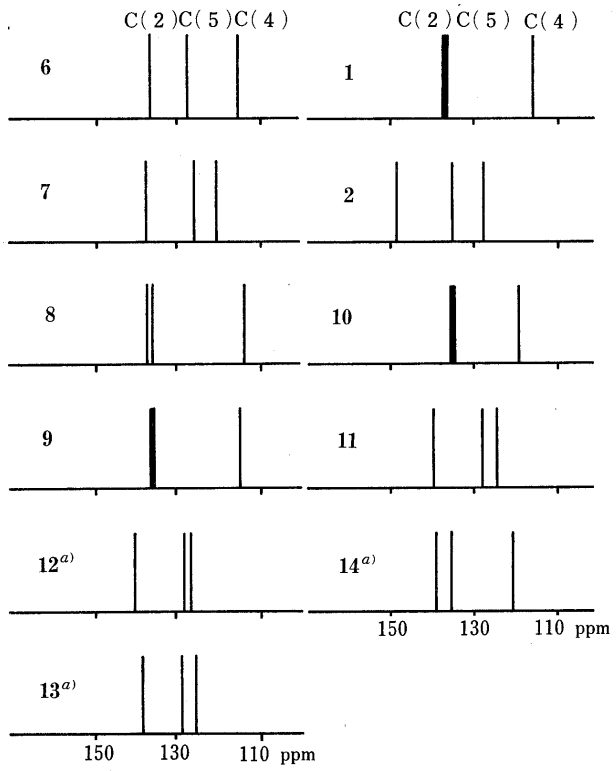

Fig. 3. Solid-State ^{13}C-NMR Chemical Shifts of Imidazole Carbons
a) Measured in D_2O.

TABLE V. Solid-State ^{13}C-NMR Chemical Shifts (δ, ppm)

Compd.	CO_2^-	N(H)CO	C(2)	C(5)	C(4)	C_α	$C_{\beta'}$	$C_{\alpha'}$	C_β	$C_{\gamma'}$	Me
1[a]	178.4	169.6	136.2[b]	136.2[b]	115.6	56.8	37.9	34.2	32.3		
2	177.2	172.5	149.4	135.8	128.0	57.3	38.7[b]	38.7[b]	32.3		
6			136.9	127.3	115.8						
7			137.6	126.1	119.9						
8	176.2		137.9	136.2	114.3	57.6			27.2		
9	178.5		137.2[b]	137.2[b]	115.8	55.2			30.0		
10		170.5[c]	135.3[b]	135.3[b]	119.5	55.8	32.5[b]	53.3	32.5[b]	25.8	16.1
11		168.3	139.6	128.6	124.3	55.3	39.2[d], 37.1[d]	55.3	34.7	29.1	15.1
12[e]	174.8		140.1	127.8	126.8	54.4	32.2	26.0			
13[e]	177.0	171.3	138.4	128.9	125.5	53.7	35.7	32.3	31.1		26.1
14[e]	174.7		139.5	135.5	120.4	55.9	34.0	29.6			

a) Assignments have been established in ref. 23. *b*) Two signals overlapped. *c*) With a shoulder. *d*) Split into two signals. *e*) Measured in D_2O.

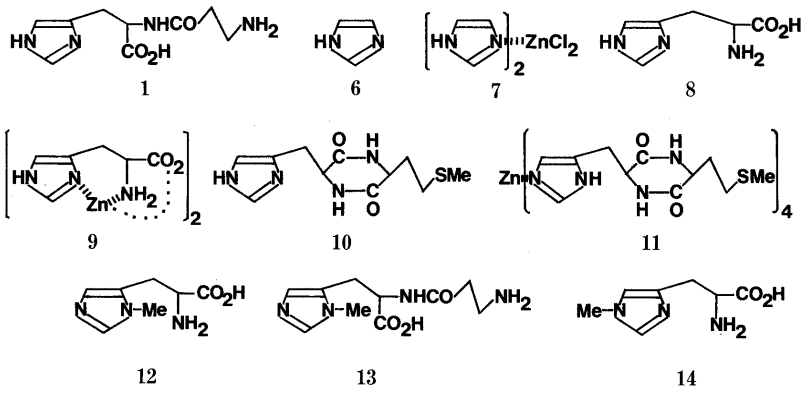

Chart 2

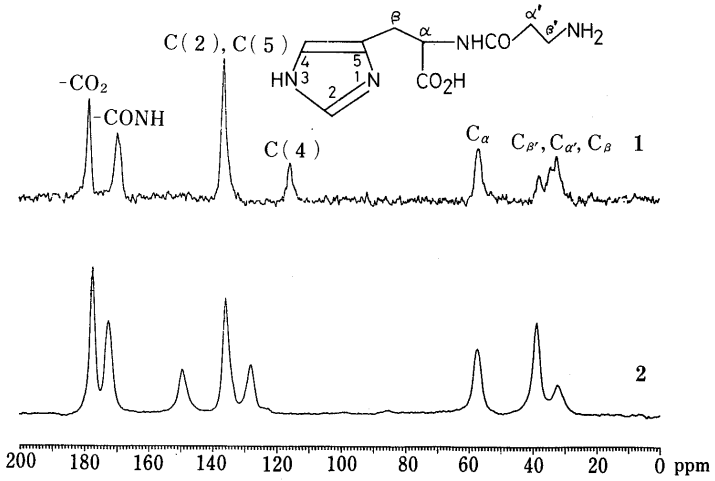

Fig. 4. Solid-State ^{13}C-NMR Spectra of **1** and **2**

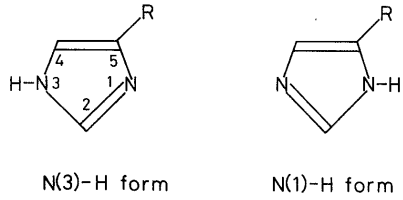

Fig. 5. Valence Tautomers of the Imidazole Ring

Fig. 3 and Fig. 4, the overlapped resonance signals of C(2) and C(5) at δ 136.2 in **1** are separated to δ 135.8 and δ 149.4, respectively, upon complexation. Moreover, the signal of C(4) at δ 115.6 in **1** is shifted downfield by 12.4 ppm in **2**. These shift changes of C(2) and C(4) are extraordinarily large compared with those observed in **7** or **9**, suggesting a drastic geometrical change of the ligand upon complexation. L-Carnosine (**1**) has an N(3)-H tautomeric imidazole moiety in the solid state.[19] In order to rationalize the NMR spectra in terms of N(3)-H/N(1)-H valence tautomerism of the imidazole ring, we measured the solid-state ^{13}C-NMR spectra of some reference compounds. It is known that cyclo-(L-Met–L-His) (**10**) has an N(3)-H tautomeric form,[20] whereas the Zn(II) complex (**11**) has an N(1)-H form in the solid state.[21] The histidine derivatives, N(1)-Me–L-His (**12**), N(1)-Me–L-carnosine (L-anserine) (**13**),[22] and N(3)-Me–L-His (**14**) have fixed tautomeric form even in solution, and the ^{13}C-NMR measurements for these compounds were carried out in D_2O. Friedlich and Wasylishen mentioned that signals of C(2) and C(5) in the N(3)-H tautomer almost overlapped, whereas the C(4) signal was located separately in the upper field region.[23] For the N(1)-H tautomer, they suggested that the signals of C(2) and C(5) would be shifted down- and upfield to give *ca.* 8 ppm separation, and that of C(4) would move downfield. Different patterns of ^{13}C-NMR signals are expected for the two tautomeric forms from their prediction. This discussion is consistent with the results for the histidine derivatives examined in Fig. 3. The resonance pattern of **2** is thus classified as that of the N(1)-H tautomer, and the tautomeric form must have changed from the N(3)-H one upon complexation. The same tautomeric changes upon complexation were observed in the case of **5** and **11**.[10,21] Besides the imidazole carbons, ^{13}C-NMR signals of the amide carbonyl and an α'-carbon in **2** are shifted by 2.9 and 4.5 ppm, respectively, and the shifts suggest the participation of the amide group in coordination.

Solid-State ^{15}N-CP/MAS NMR: As shown in Fig. 6, the solid-state ^{15}N-NMR signals of **2** were observed clearly at δ 0.2 (–NH_2), δ 106.8 (–NCO–) and δ 177.1 (two imidazole N overlapped). Attempts to detect ^{15}N resonance signals of solid **1** failed, since no signal was observed under the conditions investigated. Gattegno *et al.* tried to detect the ^{15}N-NMR signals of **1** in solution, and signals of all ^{15}N were observed only under very strongly acidic conditions.[24] They attributed this phenomenon to contaminating paramagnetic metal ions and the long relaxation time of ^{15}N nuclei. Blomberg and others reported the ^{15}N resonance

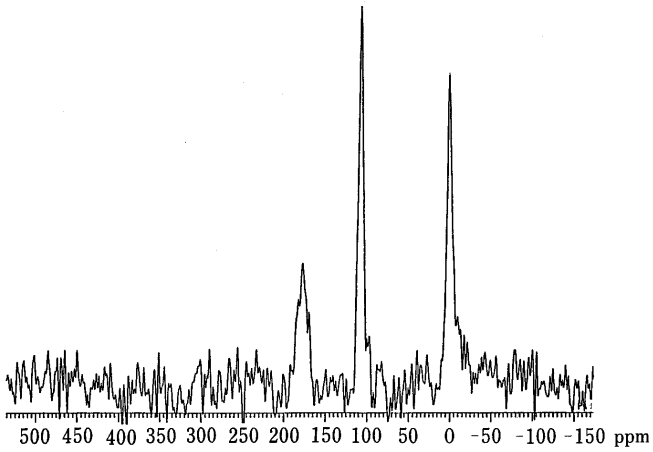

Fig. 6. Solid-State ^{15}N-NMR Spectrum of **2**

TABLE VI. The N_{1s} Electron Binding Energies (eV) of the Imidazole, Amino, and Amide Groups[a]

Compd.	>NH	≥N	$-NH_{2(3)}$	N(H)CO
1[b]	400.0	398.8	400.9	399.5
2	401.1	400.1	400.1	399.2
5	401.3	400.1	400.4	399.3
6	402.3	400.7		
7	401.4	400.1		
8[b]	400.8	398.7	401.8	
9	401.3	399.5	400.4	
Gly[b]			401.6	
15			400.3	

a) Clark *et al.* reported typical N_{1s} binding energies for –NH– and =N– in a histidine residue as 401.0 and 399.3 eV, respectively. He also reported values of 402.0 eV for $-\overset{+}{N}H_3$ in dipeptides and 400.1 eV for $-NH_2$.[27] *b*) These compounds are zwitterionic in the solid state and the amino group is protonated.

TABLE VII. The O_{1s} Electron Binding Energies (eV) of the Carboxylate, Amide and Crystal Water[a]

Compd.	$C^{=O}_{O^-_*}$	$C^{=O^*}_{O^-}$	N(H)CO	H_2O
1	531.8[b]		531.6	
2	533.3	532.2	531.9	
5	533.3	532.2	531.9	531.0
8	531.7[b]			
9	532.9	531.9		531.1
Gly	531.4[b]			
15	532.4	531.5		530.6
Stea-Zn[c]	533.1	532.0		
Stea-Cu[d]	532.5	531.9		
Benzanilide			531.7	

a) Typical O_{1s} binding energy of the carboxylate oxygens (equivalent) is 532.4 eV whereas those of the protonated carboxylate (nonequivalent) are 534.3 eV for –OH and 532.8 eV for C=O.[27] The largest binding energy in the complexes was assigned to the coordinated anionic oxygen. *b*) The two oxygens are equivalent. *c*) Zinc (II) stearate. *d*) Copper (II) stearate.

signals of **8** under neutral conditions. They assigned the signal at δ 211.1 to the pyridine-type nitrogen in the imidazole ring, and that at δ 157.7 to the pyrrole-type one, respectively.[25] The difference of the chemical shifts of these signals was some 50 ppm. Alei *et al.* found a very large upfield shift of the signal corresponding to the pyridine-type nitrogen by *ca.* 40—50 ppm when an imidazole moiety was

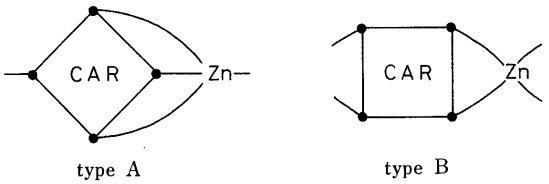

Fig. 7. Possible Binding Modes

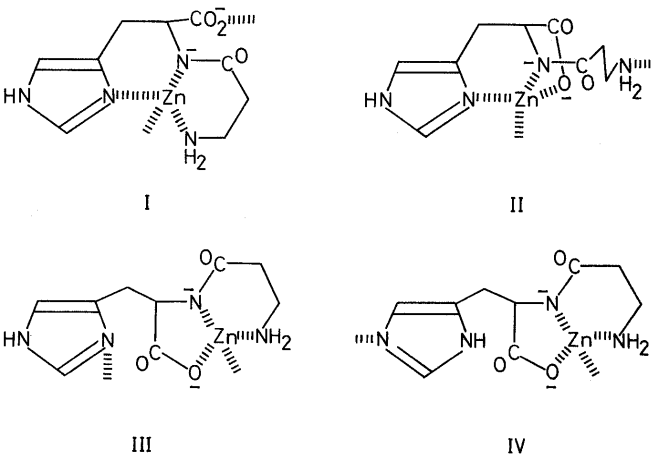

Fig. 8. Candidate Structures for **2**

complexed with Zn(II) ion.[26] In accordance with these results, we consider that the overlapped signals of **2** at δ 177.1 are derived from the pyridine-type nitrogen shifted up by *ca.* 40 ppm from the over δ 200 region upon complexation and the pyrrole-type one is almost unaffected or moved slightly downfield. The solid-state ^{15}N-NMR indicates that the pyridine-type nitrogen in the carnosine molecule is one of the coordinating sites in **2**. The resonance signal of the amino nitrogen at δ 0.2 located upfield compared with that of **1** at δ 10.7,[24] suggests that coordination occurred at this site.

XPS: Tables VI and VII show the N_{1s} and O_{1s} binding energies of **2** and some related compounds determined by XPS. As a result of the coordination to Zn(II) ion, the N_{1s} binding energies of both nitrogen atoms in the imidazole moiety increased, whereas those of the amino group decreased in the three complexes. The O_{1s} binding energies of carboxylate oxygens and amide oxygen are also increased by complexation. Interestingly, **2** has almost the same N_{1s} and O_{1s} binding energies for these atoms as those in the corresponding Cu(II) complex (**5**), reflecting the very close geometrical relationship between these complexes. The copper complex (**5**) has N(1)-H tautomeric form, which coincides with our conclusion for **2** from the solid ^{13}C-NMR.

Structure Elucidation From the spectroscopic data discussed above, L-carnosine is considered to coordinate as a quadridentate ligand to the Zn(II) ion. The simultaneous binding of all the coordination sites with one Zn(II) should be impossible in a four-coordinate complex, because of high chelate ring strain. Therefore, a plausible structure of **2** is a polymeric one containing some stable chelate rings. Figure 7 represents two possible binding modes, type A and type B, where three or two coordination sites of the four in L-carnosine bind to the same zinc ion, respectively. Taking account of the thermodynamical stability of five- and

six-membered chelate rings,[28] we can postulate four possible structures, I, II, III and IV for the type A mode, as shown in Fig. 8. For the type B mode, no combination of coordinating sites with stable chelate rings is available. Of the structures I—IV, I, II and III with N(3)-H tautomeric form are precluded on account of the ^{13}C-NMR results as discussed above. We concluded that **2** has the structure IV,[29] the analogue of the Cu(II) complex (**5**).[11] However, the IR and the far-IR spectra of **2** differ completely from those of **5**, indicating that **2** is not dimeric like the copper complex. With regard to the driving force of the tautomeric change upon complexation, we can consider the situation as follows. Among three structures with N(3)-H form, structure I with two six-membered rings would be more flexible and less stable compared to those having five- and six-membered rings. The ring strain of the structure II would be too large to form a stable four-coordinate complex. Structure III also seems to be unfavorable because of the steric hindrance when a Zn(II) ion of another unit approaches the pyridine-type nitrogen of the N(3)-H tautomeric moiety. In contrast to those disadvantages in I—III with N(3)-H tautomeric form, structure IV is less hindered and energetically favored.

Conclusion

Among many Zn(II) complexes of amino acids or peptides, **2** which has the L-carnosine molecule as a ligand, was the most promising candidate for new anti gastric ulcer drug. Characterization of **2** was achieved by spectroscopic methods: IR, solid-state ^{13}C- and ^{15}N-CP/MAS NMR and XPS. It was concluded that the structure of **2** is analogous to that of the corresponding Cu(II) complex. The tautomeric change of the imidazole moiety due to complexation was observed by solid-state ^{13}C-CP/MAS NMR, which proved to be a potent new method to investigate "frozen" flexible molecules in the solid state.

Experimental

Apparatus The solid-state ^{13}C- and ^{15}N-NMR spectra were recorded at 67.80 and 27.26 MHz, respectively, on a JEOL JNM-GX270 using a ^{1}H-CP and MAS unit. The solid-state ^{13}C chemical shifts were calibrated indirectly through external adamantane (methine carbon signal at 29.5 ppm relative to Me_4Si). The solid-state ^{15}N chemical shifts were calibrated indirectly through external glycine-^{15}N (11.59 ppm) relative to saturated $^{15}NH_4NO_3$ solution in H_2O.[30] Spectra were usually accumulated *ca.* 500—10000 times to achieve reasonable signal-to-noise ratios for natural abundance samples. ^{13}C-NMR spectra in D_2O solution were obtained on a JEOL JNM-GX400. IR and far-IR measurements were carried out on JASCO IR-810 and Bruker IFS-113V FT-IR instruments, respectively. X-Ray powder diffraction patterns were obtained on a Rigaku RU-200B over the range of 5—90°. XPS were obtained on a Shimadzu 750 electron spectrometer using monochromated Mg K_α radiation at room temperature. The normal operating vacuum pressure was less than 3×10^{-5} Pa. A microcomputer was used for deconvolution of overlapping peaks.[27] Binding energies were determined relative to the C_{1s} peak (285.0 eV) which gradually increased in intensity as residual hydrocarbons were deposited on the sample surface.

Materials Commercial grade L-carnosine (Hamari Chemicals Ltd.) (**1**) was purified by recrystallization from methanol–water to 99.9% purity (high performance liquid chromatography (HPLC)). Cyclo-(L-Met–L-His) (**10**) was prepared as described previously.[20] Cimetidine, N(1)-Me–L-His (**12**), L-anserine (**13**) and N(3)-Me–L-His (**14**) were obtained from Sigma Chemical Co. L-Homocarnosine, Gly–Gly and Gly–Gly–Gly were of commercial grade (>99% purity) made by Hamari Chemicals Ltd. Other peptides were used as received from Kokusan Chemical Works, Ltd. Cetraxate hydrochloride was obtained from capsules (Daiichi Seiyaku Co., Ltd.). All other chemicals were of reagent grade. $(Imidazole)_2ZnCl_2\cdot4H_2O$ (**6**),[16] $(L\text{-His})_2Zn\cdot2H_2O$ (**9**),[18] $(Gly)_2Zn\cdot2H_2O$ (**15**),[31] $(Gly\text{–}Gly)_2Zn\cdot2H_2O$,[32] [cyclo-(L-Met–L-His)]$_4ZnSO_4\cdot10H_2O$ (**11**)[21] and [(L-carnosine)-Cu]$_2\cdot2H_2O$ (**5**)[10] were prepared according to the methods described previously. All other zinc complexes were prepared by the same method as used for **2**. Elemental analyses were satisfactory for these complexes.

Preparation of 2 Zinc acetate dihydrate (21.95 g, 0.1 mol) in methanol (300 ml) was dropped slowly into a stirred solution of **1** (22.6 g, 0.1 mol) and sodium methoxide (10.8 g, 0.2 mol) in methanol (200 ml) at room temperature. After a half volume had been added, white precipitates appeared gradually. Stirring was continued for 2 h after completion of the addition, then the product was collected by filtration, washed with water (300 ml) and dried at 80 °C *in vacuo* for 5 h to afford 28.1 g (97.1%) of **2** as a white fine crystalline powder: mp>300 °C. Far-IR (Nujol): 694, 678, 658, 614, 579, 568, 448, 429, 403, 375, 340, 291, 231, 214, 183, 150 cm^{-1}. When the reaction was carried out in water, a hydrated amorphous complex **3** was obtained, which was not converted to **2** by drying at 120 °C for 8 h over P_2O_5 *in vacuo*: mp>300 °C. IR (KBr)/far-IR (Nujol): 3270, 1620, 1120, 1045, 980, 666, 615, 488, 461, 386, 289 cm^{-1}. Solid-state ^{13}C-NMR δ: 177.8 (with a shoulder), 143.4, 136.0, 124.8, 55.5, 40—25 (unresolved signals overlapped).

***In Vivo* Assay** Water-Immersion Stress Ulcer[33]: Male Slc: SD rats aged seven weeks were used in groups of 6—8 animals. The animals were fasted for 24 h, then the test compounds were administered orally. After 0.5 h, the animals were immobilized in a water immersion stress cage and immersed in water at 23 °C up to the xiphoid for stress loading. After 7 h, the stomach was isolated, perfused with 10 ml of 2% formalin and fixed in the same solution. After 15 min, the stomach was incised along the greater curvature. The major lengths of ulcers induced in the glandular portion of the stomach were measured under a stereoscopic microscope (×10). The summed value for each animal was taken as the ulcer index.

HCl–EtOH Ulcer[9]: Male Slc: SD rats aged seven weeks were used in groups of 6—8 animals. The animals were fasted for 24 h, then the test compounds were administered orally. After 0.5 h, 1 ml/rat of 150 mM HCl in 60% EtOH was further administered orally. The stomach was enucleated after 1 h. The subsequent procedure was identical with that used in the water-immersion stress ulcer experiment.

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