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Mugineic Acid–Iron(III) Complex and Its Structurally Analogous Cobalt(III) Complex: Characterization and Implication for Absorption and Transport of Iron in Gramineous Plants

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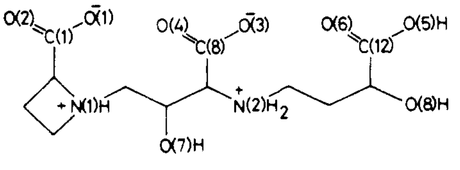
Abstract: Mugineic acid, a typical phytosiderophore, shows a remarkably stimulating effect on ^{59}Fe-uptake and chlorophyll synthesis in the rice plant. The ESR (g = 9.4, 4.51, 4.44, and 4.31) and Mössbauer (ΔE_Q = 0.24 and δ_{Fe} = +0.39 mm/s) parameters for the mugineic acid–Fe(III) complex are typical of a high-spin ferric type. A salient feature is the higher reduction potential ($E_{1/2}$ = −102 mV vs. NHE) of the mugineic acid–Fe(III) complex than those of bacterial siderophores. The X-ray diffraction study for the structurally analogous Co(III) complex of the mugineic acid–Fe(III) complex demonstrates that the azetidine nitrogen and secondary amine nitrogen, and both terminal carboxylate oxygens, coordinate as basal planar donors, and the hydroxyl oxygen, intermediate carboxylate oxygen bind as axial donors in a nearly octahedral configuration. The iron-transport mechanism in gramineous plants appears to involve the excretion of mugineic acid from the roots which aids Fe(III)-solubilization and reduction of Fe(III) to Fe(II).

Aerobic microorganisms have produced powerful and low molecular weight chelating agents known as siderophores in order to facilitate the uptake of ferric ion, because ferric ion, the major source of iron available in biological systems, is quantitatively insoluble as the hydroxide at physiological pH.[1] The most common functional groups in the siderophores are the hydroxamate group as shown in the ferrichromes and ferrioxamines and the catecholate group as in enterobactin.[2,3] Microbial siderophores have been actively studied by Neilands's and Raymond's groups.[4]

Plants also require a continuing supply of iron to maintain proper growth, and absorb iron from the soil in spite of practical insolubility of ferric ions around neutral pH values. Nevertheless, a phytosiderophore which promotes absorpton and transport of iron in plants had never been isolated. Takagi found the presence of amphoteric iron-chelating substances in the root washing of water-cultured oats under iron-deficient conditions.[5] Subsequently, we demonstrated the isolation and structure of mugineic acid (MA), an amino acid possessing an iron-chelating activity from roots washings of *Hordeum vulgare* L.[6] This novel amino acid is capable of solubilizing ferric hydroxide effectively in the pH range, 4 to 9. Similar iron-chelating amino acids were also obtained from other gramineous plants such as wheat and oats.[7] We previously communicated that MA may be a phytosiderophore.[8]

In order to establish the role of MA, herein, we have examined ^{59}Fe-uptake in rice plants, iron-solubilizing ability of MA, and physicochemical properties of the MA–Fe(III) complex and its structurally analogous Co(III) complex and have obtained some valuable information. In this paper, the coordination donor atoms of MA ligand are numbered as follows:



Mugineic acid

Experimental Section

Materials. MA and 2′-deoxymugineic acid were isolated and purified according to our reported procedures.[6,7] Nicotianamine[9] was synthesized by the method of Kristensen et al.[10] and checked by elemental analysis and ^{1}H NMR spectrum. The radioisotope ^{59}FeCl$_3$ in 0.5 M HCl was purchased from New England Nuclear Corp., Boston. The stable isotope ^{57}Fe (Fe$_2$O$_3$, 90.42%) was obtained from Oak Ridge National Laboratory and converted to reduced metal iron or ferric nitrate. Desferrioxamine was kindly provided by Ciba-Geigy, Basel. All other reagents were the highest quality available and deionized water was used throughout the experiments.

The 1:1 MA–Fe(III) complex was easily prepared by mixng MA and Fe(III) ion in aqueous solution (pH 7.0) and also obtained by the oxidation of the corresponding Fe(II) complex with oxygen. An equimolar amount of Na$_3$[Co(CO$_3$)$_3$] was added to an aqueous solution of 1 mM MA, and the reaction solution was stirred for 10 min at room temperature. After the 1:1 MA–Co(III) complex was purified by gel filtration of Sephadex G-10, the single crystals of the complex were obtained as a violet plate upon recrystallization from dioxane–water.

Determination of Biological Activity. The plants were cultured in a glass house during early springtime. Young seedlings of rice (*Oriza sativa* L. var Koshihikari) were transplanted into holes in a plastic plate placed on the top of a 13-L polyethylene pot. The pot contained 12 L of a nutrient solution, pH 5.0, with the following composition: NH$_4$NO$_3$,

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[†] Osaka College of Pharmacy.
[‡] Suntory Institute for Bioorganic Research.
[§] Kyoto University.

(1) Raymond, K. N.; Carrano, C. J. *Acc. Chem. Res.* **1979**, *12*, 183–190.

(2) Snow, G. A. *Bacteriological Rev.* **1970**, *34*, 99–125.

(3) Neilands, J. B. In. "Bioinorganic Chemistry-II"; Raymond, K. N., Ed.; American Chemical Society: Washington, DC, 1977; pp 3–32.

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(9) It has been postulated that nicitianamine plays an important role for intercellular movement of iron in *Lycopersicon esculentum* which contains excessively high levels of iron in the leaves: Budesinsky, M.; Budzikiewicz, H.; Prochazka, A.; Pipperger, H.; Romer, A.; Scholz, G.; Schreiber, K. *Phytochemistry* **1980**, *19*, 2295–2297.

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Table I. Iron-Solubilizing Abilities of Mugineic Acid and Its Related Compounds

	iron concentration (ppm) of filtered solutions						
chelators	buffer only	+ all nutrients	+ $CaCl_2$ (0.5 mM)	+ $MgSO_4$ (0.8 mM)	+ NaH_2PO_4 (0.5 mM)	three nutrients	the other
control	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
mugineic acid	3.9	2.0	3.0	2.9	3.0	2.3	3.0
2'-deoxymugineic acid	3.7	1.6	*a*				
nicotianamine	0.4	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
HEDTA	4.8	<0.3	0.5	1.3	3.1	<0.3	4.7
EDTA	7.5	<0.3	<0.3	0.4	3,3	<0.3	6.0
desferrioxamine	9.3	7.9	8.1	8.2	8.4	7.9	9.3
citric acid	0.4	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3

[a] The experiment was not done.

2.0; K_2SO_4, 0.5; $CaCl_2$, 0.5; $MgSO_4$, 0.8; and NaH_2PO_4, 0.5 mM. The micronutrient concentration was as follows: B, 6; Mn, 10; Zn 1; Cu, 1; Mo, 0.1; and Fe, 20 μM as $FeCl_3$. The nutrient solutions were aerated continuously and the pH was maintained at 7.0 (± 0.5) by daily addition of 0.5 M NaOH throughout this experiment. When the rice plants moderately became chlorosis, after a week of more, they were transported to glass pots containing 200 mL of the freshly prepared nutrient solution and 0.4 mg of each chelator tested. After 2 weeks, the newly developed leaves for this period were collected, and the chlorophyll content was determined by the method of A.O.A.C..[11] For iron-uptake experiments, the plants grown in an air-conditioned chamber (light: 8000 lux, 28 °C, 14 h; dark: 23 °C, 10 h) were used and followed by similar procedures as above. After the chlorotic plants were cultured in the ^{59}Fe- and chelator-containing nutrient solution for a week, the radioactivity of ^{59}Fe in the leaves was measured by a well-type scintillaton counter.

Determination of Iron-Solubilizing Ability. One milliliter of a 5 mM aqueous suspension of $Fe(OH)_3$ was added to 0.5 M sodium borate buffer (pH 7.0) containing 1.8 μmol of each chelator. The solution was shaken at 50 °C for 3 h. The incubation mixture was filtered into a test tube containing 0.2 mL of 6 M HCl. Finally, the iron concentration of this solution was determined by atomic absorption spectrometry (248.33 nm).

Crystal Structure Determination of Mugineic Acid–Co(III) Complex by X-ray Diffraction. A single crystal of the MA–Co(III) complex with dimensions of ca. 0.1 × 0.1 × 0.1 mm was used for the present X-ray study. The diffraction angles and the intensities were measured on a Rigaku-Denki automatic four-circle diffractometer with graphite monochromatized Cu Kα radiation ($\lambda = 1.5404$ Å) with use of the ω–2θ scan technique. The crystal data were as follows: crystal system, monoclinic; space group, $P2_1$; $a = 9.425$ (2) Å; $b = 17.350$ (4) Å; $c = 9.428$ (2) Å; $\beta = 91.08$ (2)°; V, 1541.4 (5) Å^3; $Z = 4$; $D_m = 1.770$ g cm^{-1}; $D_x = 1.785$; and μ (Cu Kα) = 91.5 cm^{-1}. The intensities of 2718 ($F_o \geq 3\sigma(F_o)$) indepedent reflections were measured up to a limit of $\sin \theta/\lambda = 0.588$ Å ($\theta = 64.99°$). The position of the cobaltic ion was indicated on a three-dimensional Patterson function. The positions for all other non-hydrogen atoms were found by successive cycles of Fourier and difference Fourier syntheses coupled with structure-factor calculations. The coordinates and anisotropic thermal parameters were refined by the block-diagonal least-squares method. The positions of all hydrogen atoms were calculated. The further least-squares refinement, including hydrogen atoms with isotropic temperature factors, was performed with a weighting sheme as follows: $\omega = 0.068$ for $F_o = 0$, $\omega = 1/(\sigma|F_o|^2 - 0.104|F_o| \times 0.008|F_o|^2)$. The final R value was 0.066, including $F_o = 0$.

Results and Discussion

^{59}Iron-Uptake in a Gramineous Plant. The chlorophyll content of leaves is sensitive to the iron stress in plants, because chlorophyll synthesis requires a continuing supply of biologically available iron. Figure 1 shows the effect of MA and its related compounds on the ^{59}Fe-uptake and chlorophyll synthesis in rice plants which are susceptible to iron chlorosis. Among the iron-chelating agents tested, MA and 2'-deoxymugineic acid significantly stimulated both the iron-uptake and chlorophyll synthesis. This results indicates that the chelators of plant origin facilitate not only the iron-uptake but also iron-utilizaton by the plant. In contrast, the effect of nicotianamine, EDTA, desferrioxamine (DS), and citric acid was remarkably small and comparable to the control. The large difference in the biological activity between 2'-deoxymugineic acid and nicotianamine strongly suggests the importance of the terminal alcoholic hydroxyl group in the MA ligands. Of interest is the fact that the chlorophyll content was evidently increased by the addition of *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*,*N'*-triacetic acid (HEDTA). Indeed, the formation constants of the HEDTA complexes with ferric ($\log K_{ML}{}^{M} = 19.8$) and ferrous ions (11.6)[12,13] are close to those (18.1 and 8.1)[8] of the corresponding MA–iron complexes, and also the HEDTA ligand shows similar hydroxyl coordination for Fe(III). The structural similarity of HEDTA to MA probably contributes to the positive effect on ^{59}Fe-uptake and chlorophyll synthesis. Herein, it should be noted that similar iron-chelating amino acids isolated from other gramineous plants all have similar structure with six coordination groups (see Chart I). The addition of MA to the iron-free medium only slightly increased the chlorophyll content, but the slight effect can be accounted for by the iron contamination of the roots arising from the pre-cultivation in normal nutrient so-

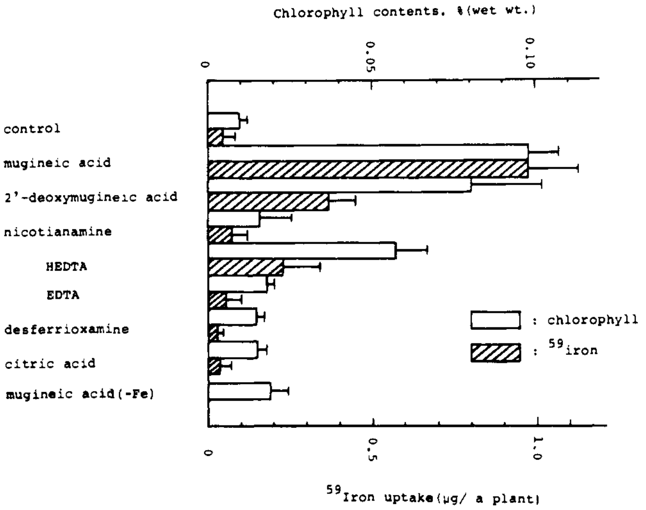
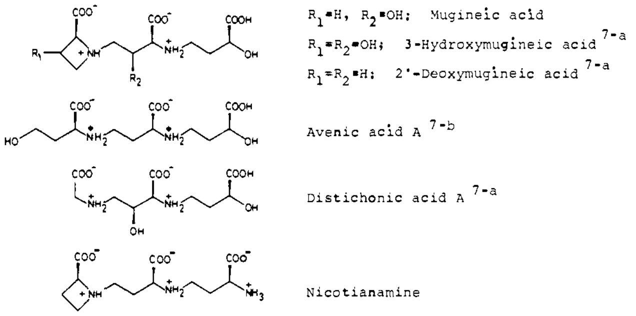


Figure 1. Effect of some chelators on iron-uptake and chlorophyll synthesis in a water-cultured rice plant.

Chart I



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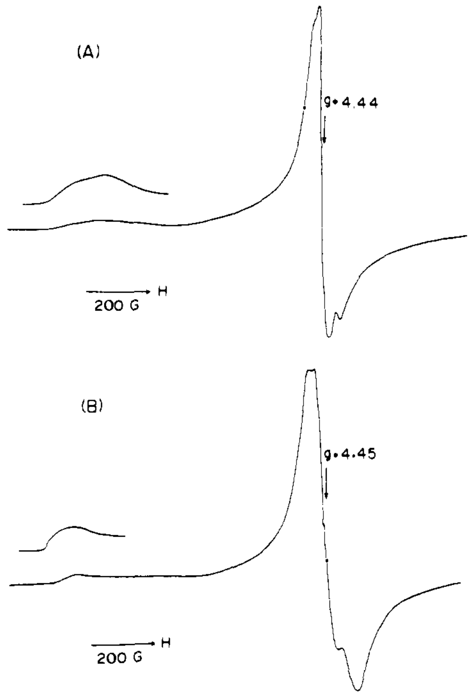

Figure 2. ESR for Fe(III) complexes of mugineic acid (A) and nicotianamine (B) at pH 7.0 and 77 K.

lution. Since iron-solubilizing action is a prerequisite to the iron-uptake by plant, the iron-solubilizing ability of MA was compared with those of other chelators, As shown in Table I, the order of iron-solubilizing abilities in buffer solution (pH 7), DS > EDTA > HEDTA > MA, is consistent with that of the stabilities of their iron(III) complexes, i.e., 30.6, (DS)[14] > 25.1 (EDTA)[15] > 19.8 (HEDTA) > 18.1 (MA). In contrast, the order in the nutrient solution (pH 7) is DS > MA > HEDTA = EDTA = control. The result indicates that the excretion of MA from the root is favorable in order to solubilize the iron in natural soils. Furthermore, Table I demonstrates that Ca^{2+}, Mg^{2+}, and PO_4^{3-} strongly inhibit the iron-solubilizing action of EDTA and HEDTA, but do not prevent that of MA. This is mainly due to the difference in affinity for Ca^{2+} and Mg^{2+} between MA and two synthetic chelators. Calcium ion is usually contained at a high level in alkaline soils where iron stress occurs frequently. Even under such conditions, MA is able to solubilize the iron effectively. The inhibitory effect of a phosphate anion can be explained by the formation of insoluble ferric phosphate. Indeed, high phosphate levels of soil result in iron deficiency.[16] In spite of the high solubilizing ability, DS did not stimulate the iron-uptake by the rice plant, The phenomenon is presumably attributable to the small permeability of the DS–Fe(III) complex into biomembranes of the root.

Spectroscopic and Electrochemical Properties of Mugineic Acid–Iron(III) Complex. The Mössbauer parameters (ΔE_Q = 0.24 and δ_{Fe} = +0.39 mm/s) of the MA–Fe(III) complex are characteristic of those for high-spin ferric (S = 5/2) complexes. The high-spin ferric assignment was also supported by the large magnetic hyperfine interaction (H_{hf} = ca. -500 KOe) induced by the applied magnetic field (600-G) at low temperature. Indeed, the ESR spectrum of the MA–Fe(III) complex is typical of the high-spin type. Figure 2 shows the ESR spectra for the 1:1 ferric complexes of MA (A) and nicotianamine (B) at pH 7.0 and 77 K. The g tensor anisotropy of the MA–Fe(III) complex (g = 9.4, 4.51, 4.44, and 4.31) is smaller than that of the nicotanamine–Fe(III) complex (g = 9.5, 4.56, 4,45, and 4.02). The difference in these spectra is probably due to the coordination of the terminal amino nitrogen in nicotianamine ligand instead of the alcoholic hydroxyl oxygen in MA toward Fe(III). The same g values of the Fe(III) complexes between MA and 2′-deoxymugineic acid strongly indicate that the intermediate alcoholic O(7)H group is not participating in the complexation with ferric ion. The MA–Fe(III) complex exhibited a qausi-reversible one-electron oxidation–reduction wave with an $E_{1/2}$ value of −102 mV vs. the normal hydrogen electrode (NHE), whereas the nicotanamine–Fe(III) complex showed an irreversible one-electron redox wave with an $E_{1/2}$ = −181 mV vs. NHE. The reduction potential of the MA–Fe(III) complex is clearly higher than those of the microbial hydroxamates (−350 to −450 mV vs. NHE) and the ferric enterobactin chelates (~−750 mV),[17] which indicate that the complexes are not readily reducible by physiologically available reductants such as NAD(P)H (ca. −0.32 V) and gultathione (−0.23 V). Indeed, the spectrophotometrical measurement for reductive transfer of iron from the MA–Fe(III) complex to the ferrous-chelating agent ferrozine[18] revealed that the MA–Fe(III) complex was reduced in the presence of NADPH and thereby the iron moved from the considerable labile MA–Fe(II) complex to the stable ferrozine–Fe(II) complex.

Table II. Bond Lengths[a] (Å)

	molecule A	molecule B
	Metal-Ligand Bonds	
Co-O(1)	1.918 (5)	1.912 (5)
Co-O(3)	1.896 (5)	1.915 (6)
Co-O(5)	1.895 (6)	1.885 (5)
Co-O(8)	1.939 (5)	1.941 (5)
Co-N(1)	1.973 (7)	1.970 (6)
Co-N(2)	1.921 (6)	1.947 (6)
	Ligand Molecules	
C(1)-C(2)	1.533 (11)	1.524 (11)
C(1)-O(1)	1.270 (9)	1.279 (9)
C(1)-O(2)	1.228 (10)	1.225 (10)
C(2)-C(3)	1.543 (12)	1.560 (12)
C(2)-N(1)	1.495 (10)	1.490 (10)
C(3)-C(4)	1.547 (12)	1.568 (12)
C(4)-N(1)	1.511 (11)	1.535 (10)
C(5)-C(6)	1.516 (13)	1.551 (13)
C(5)-N(1)	1.504 (11)	1.470 (11)
C(6)-C(7)	1.516 (12)	1.528 (13)
C(6)-O(7)	1.412 (12)	1.386 (12)
C(7)-C(8)	1.493 (12)	1.549 (13)
C(7)-N(2)	1.508 (10)	1.503 (11)
C(8)-O(3)	1.318 (10)	1.304 (11)
C(8)-O(4)	1.211 (12)	1.199 (13)
C(9)-C(10)	1.521 (11)	1.530 (11)
C(9)-N(2)	1.471 (10)	1.453 (10)
C(10)-C(11)	1.502 (11)	1.513 (11)
C(11)-C(12)	1.518 (11)	1.526 (10)
C(11)-O(8)	1.453 (9)	1.423 (9)
C(12)-O(5)	1.270 (10)	1.254 (9)
C(12)-O(6)	1.221 (10)	1.227 (10)

[a] Standard deviations are given in parentheses.

Crystal Structure of Mugineic Acid–Co(III) Complex. The two molecules (molecules A and B) of the 1:1 MA–Co(III) complex exist in an asymmetric unit, and the structures of both the molecules are remarkably similar to each other. The X-ray crystallographic results clarified a nearly octahedral configuration, in which the acetidine nitrogen, N(1), secondary amine nitrogen, N(2), and both terminal carboxylate oxygens, O(1) and O(5), coordinate to the Co(III) ion as basal planar donor atoms, and the hydroxyl oxygen, O(8), and intermediate carboxylate oxygen, O(3), coordinate as axial donors. The coordination of the Co(III)

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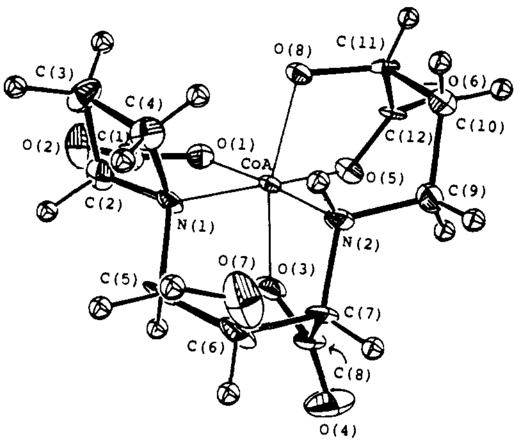

Figure 3. Molecular structure of the mugineic acid-Co(III) complex (molecule A).

ion in molecule A is illustrated in Figure 3. The bond lengths and valence angels for the A and B molecules are listed in Tables II and III. The coordination environment in the present Co(III) complex is substantially similar to that in the corresponding Cu(II) complex.[19] The main differences between both metal complexes were as follows: (1) the axial bond lengths, Co–O(8) (1.90–1.93 Å) and Co–O(3) (1.99–2.05 Å), are considerably shorter than those (2.477 and 2.557 Å) in the Cu(II) complex, (2) the deviations of the donor atoms, N(1) (molecules A = -0.010 and B = -0.018 Å), O(5) (-0.010 and -0.016 Å), N(2) (0.010 and 0.016 Å), and O(1) (0.009 and 0.018 Å), from the basal plane are smaller than those (0.101, 0.184, -0.110, and -0.160 Å for N(1), O(5), N(2), and O(1)) in the MA-Cu(II) complex in which the coordination geometry around the copper atom somewhat distorts from square-planar toward tetrahedral, (3) the intermediate carboxylate oxygen, O(3), coordinates to the Co(III) ion in place of the carboxylate oxygen, O(4), in the Cu(II) complex, and (4) the deprotonation occurs at the alcoholic hydroxyl oxygen, O(8), which is one axial donor atom toward the Co(III) ion, as reflected by the crystal packing arrangement.

In the conformation of each chelate ring denoted by the endocyclic torsion angles, the equatorial five-membered ring (Co–O(1)–C(1)–C(2), molecules A = 5 and B = 6°; O(1)–C(1)–C(2)–N(1), -9 and -11°; C(1)–C(2)–N(1)–Co, 9 and 11°; C(2)–N(1)–Co–O(1), -5 and -6°; and N(1)–Co–O(1)–C(1), 1 and 0°) adopts a nearly planar conformation, whereas the axial rings (Co–O(3)–C(8)–C(7), 20 and 20°; O(3)–C(8)–C(7)–N(2), -39 and -39°; C(8)–C(7)–N(2)–Co, 37 and 36°; C(7)–N(2)–Co–O(3), -22 and -22°; N(2)–Co–O(3)–C(8), 2 and 1°; Co–O(5)–C(12)–C(11), 8 and 9°; O(5)–C(12)–C(11–O(8), -33 and -33°; C(12)–C(11)–O(8)–Co, 40 and 39°; C(11)–O(8)–Co–O(5), -30, and -28°; and O(8)–Co–O(5)–C(12), 13 and 11°) distort, as similarly seen in the Cu(II) complex. The deviations of each atom from the five-membered chelate rings (Co molecules A = 0.027 and B = 0.032 Å), O(1) (-0.017 and 0.010 Å), C(1) (-0.050 and -0.046 Å), C(2) (0.062 and 0.064 Å), and N(1) (-0.031 and -0.057 Å); Co (-0.021 and -0.065 Å) , O(3) (0,057 and -0.021 Å), C(8) (0.145 and 0.135 Å), C(7) (-0.307 and -0.251 Å), and N(2) (0.156 and 0.218 Å); and Co (0.129 and 0.006 Å), O(5) (-0.091 and -0.039 Å), C(12) (-0.097 and -0.016 Å), C(11) (0.266 and 0.329 Å), and O(8) (-0.0229 and -0.259 Å)) and the azetidine ring (C(2) (-0.59 and -0.071 Å), C(3) (0.071 and 0.065 Å), C(4) (-0.082 and -0.065 Å), and N1) (0.074 and 0.073 Å)) are smaller than those in the corresponding Cu(II) complex. The results of the X-ray structural determination and atomic absorption spectrometry revealed that there are one sodium ion and four water molecules (the occupancy of two water molecules is 1/2) together with the two complex molecules in an asymmetric unit. Figure 4 shows the arrangement of the hydrated sodium ion, and coordination geometry around the sodium ion is summarized in Table IV. As seen in Figure 4 and Table IV, six binding sites of the sodium ion are coordinated by two carboxyl oxygens, two hydroxyl oxygens, and two water oxygen atoms. Several hydrogen bonds between the polyhedra and the complex stabilize the crystal structure. We also note that the hydrogen bond is formed between both the alcoholic hydroxyl oxygens, O(8), of the A and B molecules and that no heteroatoms exist in the neighborhood of the oxygen, O(8), except for the oxygen, O(8), of another molecule. The result suggests that a proton on the hydroxyl group, O(8)H, of either the A or B molecule is released, and thereby the plus charge arising from sodium ion is neutralized. Indeed, the previous potentiometric titration exhibited that the terminal alcoholic hydroxyl group is deprotonated by Fe(III) coordination, but not by the Cu(II) coordination.[8] The similarity in the coordination chemistries between Fe(III) and Co(III) makes the present Co(III)

Table III. Bond Angles[a] (deg)

	molecule A	molecule B
Metal-Ligand Bonds		
O(1)-Co-O(3)	91.4 (2)	90.8 (2)
O(1)-Co-O(5)	88.0 (2)	88.6 (2)
O(1)-Co-O(8)	96.9 (2)	97.0 (2)
O(1)-Co-N(1)	86.4 (3)	86.5 (2)
O(1)-Co-N(2)	174.1 (3)	174.5 (3)
O(3)-Co-O(5)	84.6 (2)	84.5 (2)
O(3)-Co-O(8)	165.9 (2)	166.0 (2)
O(3)-Co-N(1)	92.9 (3)	92.0 (3)
O(3)-Co-N(2)	87.5 (3)	87.8 (3)
O(5)-Co-O(8)	84.3 (2)	84.2 (2)
O(5)-Co-N(1)	173.8 (3)	173.8 (3)
O(5)-Co-N(2)	97.6 (3)	96.7 (3)
O(8)-Co-N(1)	99.0 (2)	100.0 (2)
O(8)-Co-N(2)	85.4 (2)	85.4 (2)
N(1)-Co-N(2)	87.9 (3)	88.2 (3)
Ligand Molecules		
C(2)-C(1)-O(1)	115.7 (6)	115.0 (6)
C(2)-C(1)-O(2)	118.1 (7)	118.8 (7)
O(1)-C(1)-O(2)	126.2 (7)	125.8 (7)
C(1)-C(2)-C(3)	115.1 (7)	114.8 (7)
C(1)-C(2)-N(1)	112.0 (7)	112.6 (6)
C(3)-C(2)-N(1)	90.1 (6)	91.6 (6)
C(2)-C(3)-C(4)	87.8 (6)	86.6 (6)
C(3)-C(4)-N(1)	89.3 (6)	89.6 (6)
C(6)-C(5)-N(1)	115.1 (7)	113.7 (7)
C(5)-C(6)-C(7)	113.4 (7)	114.7 (8)
C(5)-C(6)-O(7)	110.3 (7)	111.8 (8)
C(7)-C(6)-O(7)	108.3 (7)	107.9 (7)
C(6)-C(7)-C(8)	108.1 (7)	107.3 (7)
C(6)-C(7)-N(2)	109.0 (7)	108.3 (7)
C(8)-C(7)-N(2)	109.2 (7)	107.6 (7)
C(7)-C(8)-O(3)	112.0 (7)	113.4 (8)
C(7)-C(8)-O(4)	125.4 (8)	120.7 (9)
O(3)-C(8)-O(4)	122.5 (8)	125.8 (9)
C(10)-C(9)-N(2)	110.0 (6)	110.2 (6)
C(9)-C(10)-C(11)	112.9 (6)	113.0 (7)
C(10)-C(11)-C(12)	109.4 (6)	108.3 (6)
C(10)-C(11)-O(8)	110.9 (6)	111.2 (6)
C(12)-C(11)-O(8)	107.7 (6)	109.0 (6)
C(11)-C(12)-O(5)	113.2 (6)	111.9 (6)
C(11)-C(12)-O(6)	123.0 (7)	122.5 (7)
O(5)-C(12)-O(6)	123.8 (7)	125.5 (7)
Co-O(1)-C(1)	116.9 (5)	116.9 (5)
Co-O(3)-C(8)	113.6 (5)	112.9 (6)
Co-O(5)-C(12)	115.3 (5)	116.3 (5)
Co-O(8)-C(11)	104.8 (4)	104.7 (4)
Co-N(1)-C(2)	108.3 (5)	107.9 (5)
Co-N(1)-C(4)	117.3 (5)	116.0 (5)
Co-N(1)-C(5)	111.7 (5)	114.0 (5)
C(2)-N(1)-C(4)	90.9 (6)	90.3 (6)
C(2)-N(1)-C(5)	111.5 (6)	111.3 (6)
C(4)-N(1)-C(5)	115.1 (6)	114.6 (6)
Co-N(2)-C(7)	104.0 (5)	104.6 (5)
Co-N(2)-C(9)	116.1 (5)	115.8 (5)
C(7)-N(2)-C(9)	113.6 (6)	113.8 (6)

[a] Standard deviations are given in parentheses.

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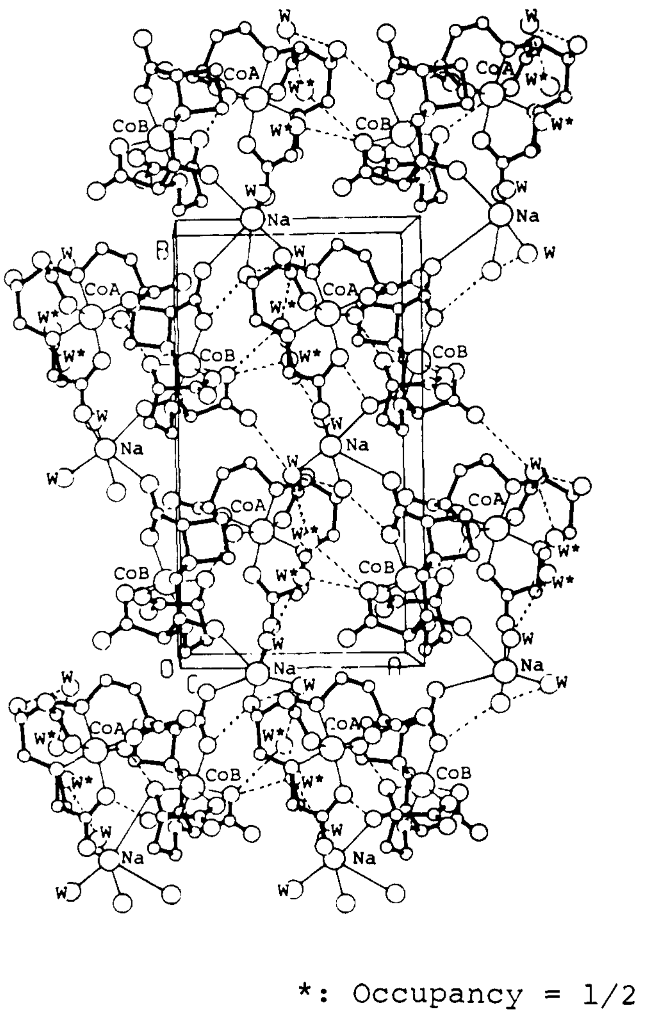

Figure 4. Arrangement of molecules in the crystal. Hydrogen bonds are shown by broken lines. Because of the disorder of the water molecules, W(3) and W(4) were assigned as occupancy factor of 1/2.

Table IV. Hydrogen Bond Distances and Coordination Distances around the Sodium Ion

bond	distance, Å
O(8A)---O(8B)	2.458 (7)
O(7A)---O(1B)	2.996 (9)
O(7B)---O(1A)	3.012 (9)
O(1W)---O(7B)	3.06 (1)
O(1W)---O(4A)	2.87 (1)
O(2W)---O(7B)	3.06 (1)
O(2W)---O(4B)	2.86 (1)
O(3W)[a]---O(3B)	2.82 (1)
O(3W)[a]---O(1W)	2.90 (2)
O(4W)[a]---O(3B)	3.04 (2)
O(4W)[a]---O(2W)	2.94 (2)
O(2A)-Na	2.350 (8)
O(7B)-Na	2.361 (8)
O(1W)-Na	2.440 (8)
O(2B)-Na	2.440 (8)
O(7A)-Na	2.368 (7)
O(2W)-Na	2.457 (9)

[a] A reduced occupancy factor of 1/2 was assumed.

complex a useful vehicle for probing the structure of the biologically important MA–Fe(III) complex.

^{1}H NMR Spectra of Co(III) and Fe(III) Complexes of Mugineic Acid. Figure 5 shows 360-MHz FT ^{1}H NMR spectra of metal-free MA and its complexes with Fe(III) and Co(III) ions at pD 4.5. The MA–Fe(III) complex gave no detectable proton signals in the regions of ±100 ppm because of remarkable line broadening, which is probably due to the paramagnetism of high-spin Fe(III) ion ($S = 5/2$), while the Co(III) complex of MA exhibited sharp and numerous proton signals, indicating the presence of diamagnetic ion ($S = 0$) in this complex. The spin decoupling and pD titration experiments of the respective proton signals established the signal assignments as shown in Figure 5. Careful analysis revealed that the structure of the Co(III) complex in aqueous medium is evidently similar to that in the above-mentioned crystal structure, and that the complex molecule undergoes no significant structural alteration in the pD range 4–10. This information is a prerequisite to the understanding of the conformation of the MA–Fe(III) complex in aqueous medium. Full details of the NMR study for MA–metal complexes will be published elsewhere.

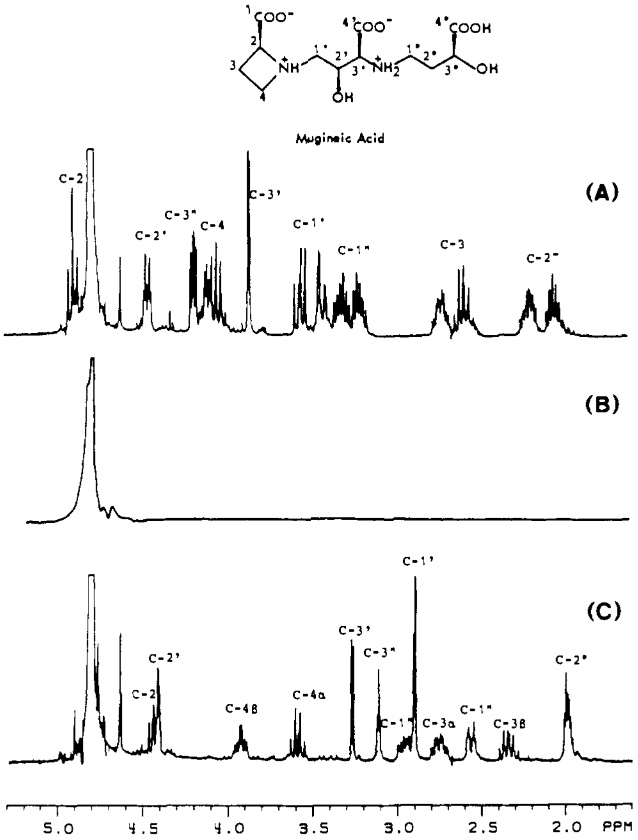

Figure 5. 360-MHz FT ^{1}H NMR spectra of mugineic acid only (A), mugineic acid–Fe(III) complex (B), and mugineic acid–Co(III) complex (C) at pD 4.5.

Most microbial siderophores have the hydroxamate or the phenolate group as Fe(III)–ligand donors. In microbial siderophores the liganding atoms are oxygen except for mycobactin, agrobactin, and parabactin, where the single tertiary nitrogen atom of the oxazoline ring participates in bonding to the iron.[20] All of the hydroxamate siderophores show a characteristic broad absorption band at near 25 000 cm^{-1} (ϵ 3000), and the iron(III) in these complexes has been demonstrated to be high-spin d^5 by magnetic susceptibility, ESR, and Mössbauer spectroscopies.[1] The present iron-chelating agent of plant origin includes carboxyl, amine, and hydroxyl groups as ligand functional groups. The subsequent reaction with ferric ion forms a water-soluble and octahedral high-spin ferric ($S = 5/2$) complex. Although the crystal structure of the MA–Fe(III) complex has not as yet been established by diffraction techniques, the coordination structure and the bonding of the ferric complex should be sufficiently like to those of the corresponding cobaltic complex, which consisted of the regular octahedral configuration with N_2O_4 donor sets. The crystal field stabilization for d^6 cobalt complex (24 Dq) is con-

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siderably greater than that for the high-spin ferric complex (0 Dq).[21] Thus, the Co(III)-substituted complex of MA should be kinetically inert. The present result of ^{59}Fe-uptake and chlorophyll synthesis clearly shows that MA is a typical phytosiderophore. In gramineous plants, the mechanism for absorption and transport of iron appears to involve the excretion of MA from the roots which aids Fe(III) solubilization and reduction of Fe(III) to Fe(II).

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Registry No. MA-Fe(III), 85956-49-6; MA-Co(III), 85994-19-0; MA, 69199-37-7; ^{59}Fe, 14596-12-4; Fe^{III}, 20074-52-6; Fe(OH)$_3$, 1309-33-7; nicotianamine iron(III), 82678-55-5.

Supplementary Material Available: Crystal structure data for the mugineic acid–Co(III) complex; fractional atomic coordinates (Table S-I), thermal parameters with estimated standard deviations (Table S-II), fractional atomic coordinates and thermal parameters for hydrogen atoms (Table S-III), deviations of atoms from the least-squares plane (Table S-IV), endocyclic torsion angles calculated for various chelate rings (Table S-V), and a listing of observed and calculated structure factors (23 pages). Ordering information is given on any current masthead page.

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Protonation and Alkylation of Ambident (9-Anthryl)arylmethyl Anions

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Abstract: We have investigated the protonation of ambident (9-anthryl)arylmethylsodiums (Na-**2**) and -lithiums (Li-**2**) (substituent = *p*-OMe, *p*-Me, H, *m*-F) with various oxygen and carbon acids in tetrahydrofuran to give a mixture of the anthracene derivatives **3a–d** and the 9,10-dihydroanthracenes **4a–d**. Product compositions were dramatically influenced by the substituent electronic effects of the carbanions **2** and the acidities of the proton donors. In the protonation with the oxygen proton donors the change of the countercation **2** and addition of hexamethylphosphoramide also exerted remarkable effects on the **3**:**4** ratio, whereas these two factors were not important in the case of the carbon acids. This fact would be interpreted as the extent of coordination of oxygen acids to metal cations being important in determining protonation regiochemistry. The reaction of (9-anthryl)arylmethyl anions (**2a–d**) with a series of aliphatic and benzylic halides gave a mixture of two cross-coupling products, **7** (coupling at C-α) and **8** (coupling at C-10), and/or dimers, **5** (C_α–C_{10} coupling) and **6** (C_{10}–C_{10} coupling), the composition being influenced by the substituent electronic effects of the carbanions **2** and the structure of the alkyl groups of the halides. The leaving group effects also have a significant influence on the product composition. Using dependence (or independence) of the product composition on the electronic effects of the substituent of **2** as a tool to differentiate the reaction mechanisms, we have attempted to rationalize the apparently complicated alkylation of the carbanions **2**.

With the realization that the allyl moiety is an integral feature of many natural products and biosynthetic intermediates,[1] it has become important to develop methods that would lead to controlled C–C bond formation at either the α- or γ-position of an allyl substrate. For this purpose the use of allylic organometallics seems to be promising,[2] but the regiochemistry of the products has been found to be a function of various factors[3] including substituents (electronic[4] and steric[5] effects), electrophiles,[6] countercations,[7] solvents,[8] and the presence of strongly coordinating additives toward metal cations (e,g., tetramethylethylenediamine, hexamethylphosphoramide, crown ether, etc.),[8] To develop more sophisticated methods, it must be, therefore, important to understand the detailed mechanism of the reaction. When stereochemistry and CIDNP are used as the most definitive indicators of the mechanisms, it has been confirmed that in the case of allylic alkali and alkaline-earth metallics, at least two different mechanisms, i.e., single electron transfer (SET) (eq 1) and nucleophilic substitution (eq 2), are possible. However, a delicate balance

$$R^1X + R^2M \xrightarrow[\text{transfer}]{\text{electron}} [R^1X^{-\cdot}, R^2M^{+\cdot}] \rightarrow R^1\text{–}R^2 \tag{1}$$

$$R^1X + R^2M \xrightarrow[\text{substitution}]{\text{nucleophilic}} R^1\text{–}R^2 \tag{2}$$

must exist between these mechanistic alternatives, and a minor

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