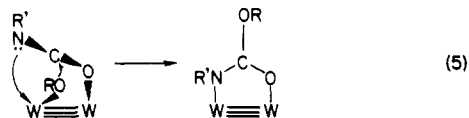


one in which both the μ -PhNC(OCMe₃)O ligand and the PMe₃ ligand are coordinated.

We thus feel reasonably secure in proposing that the overall process represented in eq 1 begins with the formation of 1 followed by eq 4. Beyond this we are again reduced to sheer speculation, but it does not seem unlikely that a simple unimolecular rearrangement, as represented schematically by (5), might be the way



in which the process is completed. It is possible, though perhaps unlikely, that further mechanistic information may be obtained about these reactions and some additional experiments are to be

carried out. We believe, however, that our present understanding is reasonably sound and that a report giving the results now in hand may be of value to others investigating the chemistry of metal-metal multiple bonds.

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Registry No. 1, 96791-13-8; 2, 93110-45-3; 3, 96760-84-8; W₂(OCMe₃)₆, 57125-20-9; PhNCO, 103-71-9; PMe₃, 594-09-2.

Supplementary Material Available: Table of observed and calculated structure factors, anisotropic thermal parameters, and bond lengths and angles and a more detailed account of the crystallographic work (52 pages). Ordering information is given on any current masthead page.

Unusual Configuration and Low Iron-Uptake Ability of Isomugineic Acid Produced from Chlorotic Gramineous Plants

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Abstract: The two-dimensional ¹³C/¹H NMR and CD data showed that isomugineic acid is the isomer of mugineic acid, (2*S*,2'*S*,3'*S*,3''*S*)-*N*-[3-carboxyl-3-[(3-carboxy-3-hydroxypropyl)amino]-2-hydroxypropyl]azetidino-2-carboxylic acid, with respect to the configuration at the 3'-carbon position. The ESR *g* tensors and reduction potential of the isomugineic acid-Fe(III) complex were clearly different from those of the corresponding mugineic acid and 2'-deoxymugineic acid complexes, but they were remarkably close to those of the 2'-deoxy-3'-decarboxylmugineic acid-Fe(III) complex. The present results revealed that the 3'-carboxyl group of isomugineic acid does not participate in the metal binding and that the ⁵⁹Fe-uptake activity of isomugineic acid is significantly lower than that of normal mugineic acid.

The biochemistry of iron transport is becoming an extremely popular subject. Although microorganisms are often in danger of iron starvation, the mechanism of siderophore-mediated iron uptake has now been elucidated and indeed many different siderophores have been identified from microorganisms.^{2,3} The general feature of all siderophores is that they are capable of forming a "molecular cage" at the center of which ferric ion is held as part of a six-coordinate, octahedral complex. On the other hand, certain plants also excrete phytosiderophores such as mugineic acid in order to absorb and utilize iron in soil.⁴ It is well-known that iron-sufficient (green) plants often become chlorotic. Heavy metals and arsenate induce iron stress and cause iron chlorosis which turns the young leaves to yellow or white.⁵ As a result, the plants cease to grow and eventually they wither up. For the purpose of effective incorporation of iron in soil, gramineous plants such as barley, wheat, and oats have been found to excrete novel amino acids from their roots.⁴ Mugineic acid, (2*S*,2'*S*,3'*S*,3''*S*)-*N*-[3-carboxyl-3-[(3-carboxy-3-hydroxypropyl)amino]-2-hydroxypropyl]azetidino-2-carboxylic acid, is a typical phytosiderophore isolated from the water-cultured barley of *Hordeum vulgare* L. var. Minorimugi, and in fact this chelator

significantly stimulates ⁵⁹Fe uptake in the rice root.⁶

Herein, we found that the ligand configuration and metal complexation of isomugineic acid (1) obtained from growth-depressed barley are clearly different from those of normal mugineic acid (2). Of special interest is the fact that isomugineic acid showed lower biological activity for iron uptake than mugineic acid. The structure of mugineic acids is given in Table I.

Experimental Sections

The isolation of mugineic acid from chlorotic barley was performed according to the procedure reported previously for the isolation of mugineic acid.^{4,7} When barley plants expanded their second and third leaves, they were transported to iron-deficient solution and then the culture was continued for 1 week or more. After strong development of the yellowing of the leaves, the root washings were collected by the soaking of the root in deionized water once a day for 2 weeks. The root washings were first chromatographed on an Amberlite IR-120 B(H⁺ form). For the separation of isomugineic acid and mugineic acid, the technique of gradient elution with ammonia-formate buffer (pH 2.5-3.1) on a Dowex 50W × 4 resin was suitable. Isomugineic acid was eluted with the buffer of pH 2.6-2.7 and mugineic acid with the buffer of pH 2.8-2.9. After the gel filtration (Sephadex G-10) of the former fractions containing the iron-chelating activity which was determined by the *o*-phenanthroline method, amorphous powder of isomugineic acid was obtained. The gel filtration of the latter fractions yielded mugineic acid as crystals.

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Table I. ESR Parameters of Metal Complexes, Redox Potential of the Iron Complex, and Biological Activity for Mugineic Acid Ligands

ligand	Cu(II) complex			Fe(III) complex				$E_{1/2}$ (mV vs. NHE)	biological activity ^{59}Fe uptake (%)
	g_{\parallel}	g_{\perp}	$A(\times 10^{-4} \text{ cm}^{-1})$	g					
(1)	2.254	2.059	179.0	9.5	4.95	4.41	4.22	-82	17
(2)	2.277	2.060	175.9	9.4	4.51	4.44	4.31	-102	100
(3)	2.277	2.065	172.9	9.4	4.51	4.44	4.31	-100	38
(4)	2.255	2.059	180.1	9.5	4.91	4.40	4.19	-78	25
(5)	2.236	2.054	190.4	9.5	4.56	4.45	4.02	-181	8

The ^1H and ^{13}C NMR spectra of isomugineic acid and mugineic acid were measured at pD 4.5 and 360 MHz on a Nicolet NT-360 NMR spectrometer equipped with the NIC 1280/293 B data system. The proton chemical shifts were given with respect to sodium 3-(trimethylsilyl)[2,2,3,3- $^2\text{H}_4$]propionate (TSP) as internal standards, and the ^{13}C resonances were referenced to TSP in a separate sample. The measurements of circular dichroism were carried out with a Jasco J-20C spectropolarimeter. The X-band ESR spectra for the 1:1 Cu(II) and Fe(III) complexes of mugineic acid related ligands were obtained at pH 7.0 and 77 K. The redox potential, $E_{1/2}$ value vs. the normal hydrogen electrode (NHE), was estimated from one electron oxidation-reduction wave of a cyclic voltammogram at pH 7.0. The electrochemistry was performed on a hanging mercury drop electrode with 100 mV/s of scan rate.⁸ The determination of biological activity was carried out according to the previous procedures.⁶ After the chlorotic rice plants were cultured in the ^{59}Fe - and chelator-containing nutrient solutions for 1 week, the radioactivity of ^{59}Fe in the leaves was measured by a well-type scintillation counter.

Results and Discussion

Isomugineic acid ($[\alpha]_D -97.0^\circ$ (c, 1.24 in water)) showed a weak positive ninhydrin reaction. The elemental analysis of isomugineic acid revealed the molecular formula $\text{C}_{12}\text{H}_{20}\text{O}_8\text{N}_2$, the same as that of mugineic acid, and its FD mass spectrum gave m/z 303 ($M + 1 - \text{H}_2\text{O}$). The IR spectrum of isomugineic acid also exhibited the characteristic bands at 3450–3200 (OH and NH) and 1605 cm^{-1} (COO^-).

From the homonuclear spin decoupling and J -resolved two-dimensional spectroscopic experiments, all proton resonances of normal mugineic acid were previously assigned by us.⁹ Isomugineic acid also displayed the same three spin systems in the proton spin networks as mugineic acid, and the proton assignment for isomugineic acid was achieved by the same procedures. On the basis of the complete assignment of all proton signals of the two mugineic acids, as shown in Figure 1, the carbon signals of these compounds were determined by two-dimensional $^{13}\text{C}/^1\text{H}$ heteronuclear chemical shift correlated spectroscopy.¹⁰ Of particular importance is the fact that the 3'-carbon (62.8 ppm) of isomugineic acid was evidently shifted to upfield in comparison with that (66.6 ppm) of mugineic acid, although the other carbon signals of the two mugineic acids showed same chemical shifts. Isomugineic acid and mugineic acid have the same molecular formulas. Therefore, the present result strongly indicates that isomugineic acid is the isomer of mugineic acid with respect to the configu-

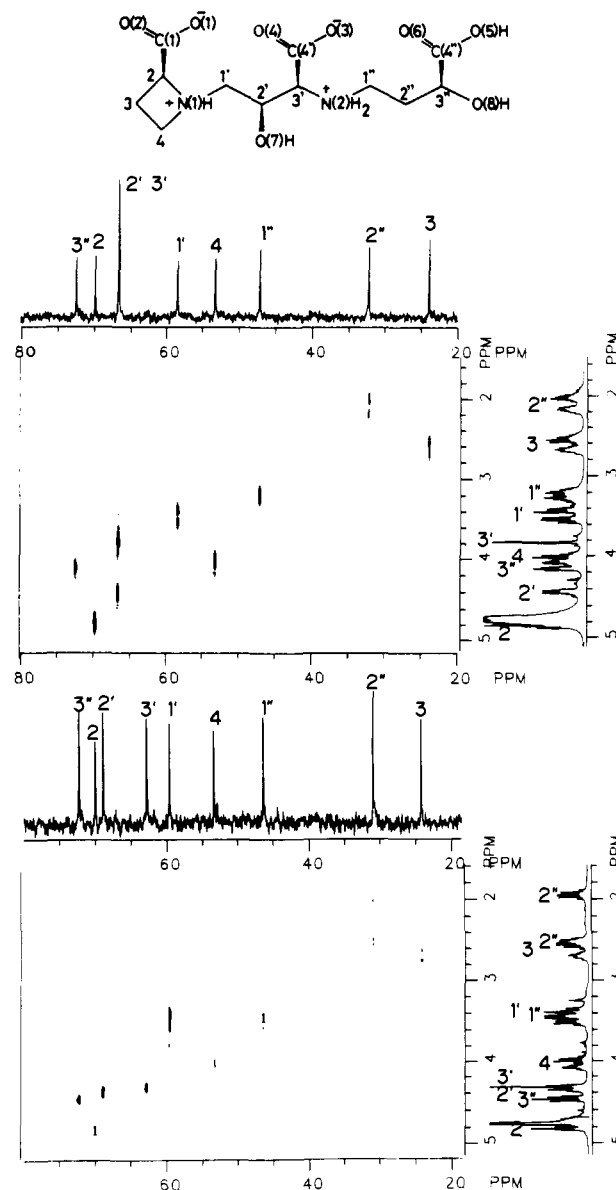


Figure 1. Two-dimensional ^{13}C - ^1H chemical shift correlations of mugineic acid (upper) and isomugineic acid (lower). A $(90^\circ(\text{H})-t_{1/2}-180^\circ(\text{C})-t_{1/2}-\Delta_1-90^\circ(\text{H})-90^\circ(\text{C})-\Delta_2-t_2)$ pulse sequence was used.

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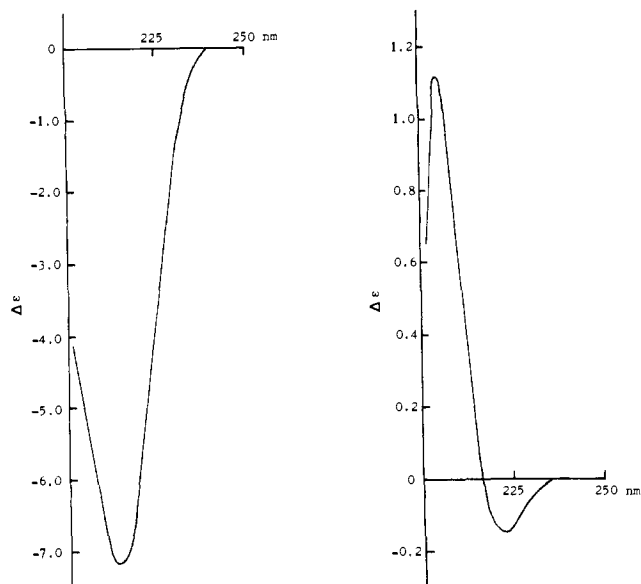


Figure 2. Circular dichroism spectra of isomugineic acid (left) and mugineic acid (right) in aqueous solution.

ration at the 3'-carbon position. Indeed, the circular dichroism feature of isomugineic acid was clearly different from that of mugineic acid (see Figure 2). Mugineic acid gave the positive Cotton effect ($\Delta\epsilon +1.11$) near 205 nm together with the weak CD peak (-0.15) at 223 nm. While isomugineic acid obviously exhibited the negative Cotton effect (-7.23) at 215 nm, its CD sign appears to be dependent on the configuration of ligand. A weak extremum near 225 nm which is expected in the isomugineic acid spectrum may hide in the large broad CD peak centered at 215 nm.

Table I summarizes the ESR parameters for the Cu(II) and Fe(III) complexes, together with the redox potentials of iron complexes and the iron-uptake activities of chelators in the mugineic acid related ligands. The g tensors and $E_{1/2}$ values of the isomugineic acid-Cu(II) and -Fe(III) complexes were clearly different from those of the corresponding mugineic acid, 2'-deoxymugineic acid (3), and nicotianamine (5) complexes but were remarkably close to those of the 2'-deoxy-3'-decarboxylmugineic

acid (4)¹¹-metal complexes. Our recent X-ray diffraction studies for the mugineic acid-Cu(II) complex and the structurally analogous Co(III) complex of the mugineic acid-Fe(III) complex demonstrated that the azetidine nitrogen, secondary amine nitrogen, and both terminal carboxylate oxygens coordinate as basal planar donors, and the terminal hydroxyl oxygen and intermediate carboxyl oxygen are bonded axially in a nearly octahedral conformation.^{6,12} Therefore, the present results strongly suggest that the 3'-carboxyl group in the isomugineic acid ligand does not participate in the metal chelation. When the CD spectra for the Fe(III) complexes of two mugineic acids were measured at pH 7.0, the yellow-colored isomugineic acid-Fe(III) complex gave the Cotton effects at 212 ($\Delta\epsilon -7.23$), 267 ($+0.44$), and 338 nm ($+0.09$). In the mugineic acid-Fe(III) complex, three CD peaks have been observed at 235 ($\Delta\epsilon +9.11$), 270 ($+2.22$), and 380 nm (-2.39).^{4,8} The CD features suggest apparent distinction of the configuration between the Fe(III) complexes of isomugineic acid and mugineic acid. As shown in Table I, the effect of isomugineic acid on the ⁵⁹Fe uptake in rice plants was significantly smaller than that of normal mugineic acid. The large difference in the biological activity between the two mugineic acids indicates the importance of the configuration at the 3'-carboxyl group which in particular is sensitive to the iron binding. Indeed, stereospecificity of the ferric enterobactin receptor of *Escherichia coli* K-12 has been demonstrated by the fact that Δ -cis Fe(III)-enterobactin is active and the Λ -cis isomer inactive in this microorganism.¹³

In conclusion, isomugineic acid isolated from chlorotic barley has a ligand configuration and metal complexation different from normal mugineic acid. The unusual configuration of the 3'-carboxyl group in isomugineic acid contributes to the low biological activity for ⁵⁹Fe uptake, and hence the configuration of phyto-siderophores is one important factor in determining iron absorption by plants. In addition, it seems most probable that a mutation leading to the biosynthesis of isomugineic acid, instead of mugineic acid, is responsible for the depressed growth of some species of barley.

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