ration and purifications of the sample, and that the R factors (Table I) indicate that our experimental data are of a higher quality than those of Berry et al.

In conclusion, our study, like the calculations of Williamson and Hall, indicates that the methyl group geometry in Cl_3TiCH_3 is more or less normal; hopefully the issue will be finally resolved by a successful MW study.

Acknowledgment. We are grateful to the Norwegian Research Council for Science and the Humanities and to the VISTA program for financial support.

Stereospecific Iron Uptake Mediated by Phytosiderophore in Gramineous Plants†

Fumio Oida, Nagayo Ota, and Yoshiki Mino*

Osaka University of Pharmaceutical Sciences Matsubara-city, Osaka 580, Japan

Kyosuke Nomoto*

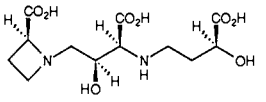
Suntory Institute for Bioorganic Research Shimamoto-cho, Mishima-gun, Osaka 618, Japan

Yukio Sugiura*

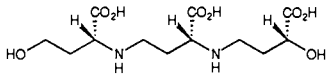
Institute for Chemical Research, Kyoto University Uji, Kyoto 611, Japan

Received November 1, 1988

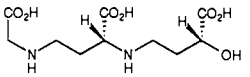
Iron is an essential element for almost all living organisms. To obtain the iron needed for proper growth, gramineous plants such as barley, wheat, and oat excrete low-molecular-weight iron chelators, generally termed phytosiderophores, from the root to solubilize and uptake iron in soil.[1] The phytosiderophores so far isolated are amino acids containing α-hydroxy carboxylate and α-amino carboxylate ligands.[2-4] In contrast, most aerobic and facultative anaerobic bacteria produce catecholate- and/or hydroxamate-type siderophores at low levels of iron(III).[5] The most typical phytosiderophore is mugineic acid (MA) (**1**),



1



2



3

(2*S*,2′*S*,3′*S*,3″*S*)-*N*-[3-carboxy-3-[(3-carboxy-3-hydroxypropyl)amino]-2-hydroxypropyl]azetidine-2-carboxylic acid, excreted from the roots of barley (*Hordeum vulgare* L. var. Minorimugi). The structural, physicochemical, and biochemical properties of MA and its metal complex have been elucidated.[2,6-9]

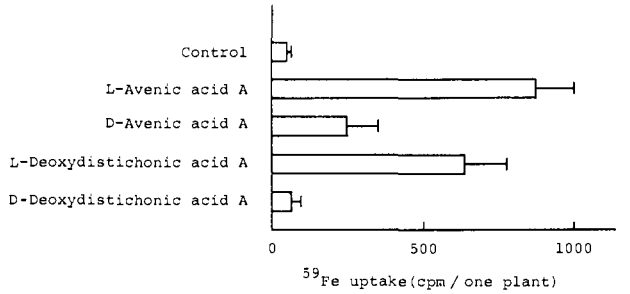


Figure 1. Effect of the optical isomers of phytosiderophore on iron-uptake in water-cultured rice plant. The experiments were performed in the same manner as reported previously.[8] The samples contained 20 μM iron (as $FeCl_3$) and 2 ppm chelator, and the control lacked only chelator from the sample.

The most salient feature among them is that the phytosiderophore facilitates not only the iron uptake but also iron utilization by the plant, whereas desferrioxamine and EDTA, which are capable of solubilizing iron as much as or more than MA, have remarkably small iron-uptake ability relative to the control.[8] This observation seems to suggest the existence of a special (stereospecific) iron-transport system in the membrane of the root.

In order to clarify this point, we synthesized the enantiomers of phytosiderophore, namely enantioavenic acid A (D-AA) (**2**) and enantiodeoxydistichonic acid A (D-DDA) (**3**), and then compared their iron-uptake ability with those of the corresponding natural phytosiderophores, L-AA[3] and L-DDA.[10] In the enantiomeric ligands, the configuration of COOH and H is a mirror relation.

D-AA and D-DDA were synthesized as follows: D-α-Hydroxy-γ-butyrolactone (**4**),[11] available from D-malic acid, was converted into a diastereomeric mixture of tetrahydropyranylated (THP) derivatives. After hydrolysis of the THP derivative, followed by benzylation, the oxidation with pyridinium chlorochromate (PCC) yielded D-malic half-aldehyde (**5**). Coupling of the half-aldehyde with the homoserine moiety (**6**) was carried out via a reductive amination procedure (sodium cyanoborohydride)[12] and resulted in the formation of the desired lactone amine (**7**). After protection of **7** with di-*tert*-butyl dicarbonate, successive treatments (2.5% KOH, $PhCH_2Br$; see Scheme I) produced the dibenzyl ester. In the same manner (oxidation with PCC), the corresponding aldehyde (**8**) was derived. This compound is an important intermediate for the syntheses of MA derivatives because the coupling with one homoserine moiety or glycine, followed by the removal of the protecting groups and hydrolysis with alkali (KOH), gave D-AA or D-DDA, respectively. Finally, treatment with Dowex 50W (H^+ form), elution with 2 N ammonia, and subsequent Sephadex G-10 chromatography yielded optically pure D-AA ($[\alpha]_D$ −16.5°, 2 N HCl, *c* 0.1) or D-DDA ($[\alpha]_D$ +8.95°, H_2O, *c* 0.1). A full report will be published elsewhere.[13]

L-AA was synthesized by the method of Ohfune et al.,[14] and L-DDA was obtained by using glycine instead of L-homoserine in the final coupling step.

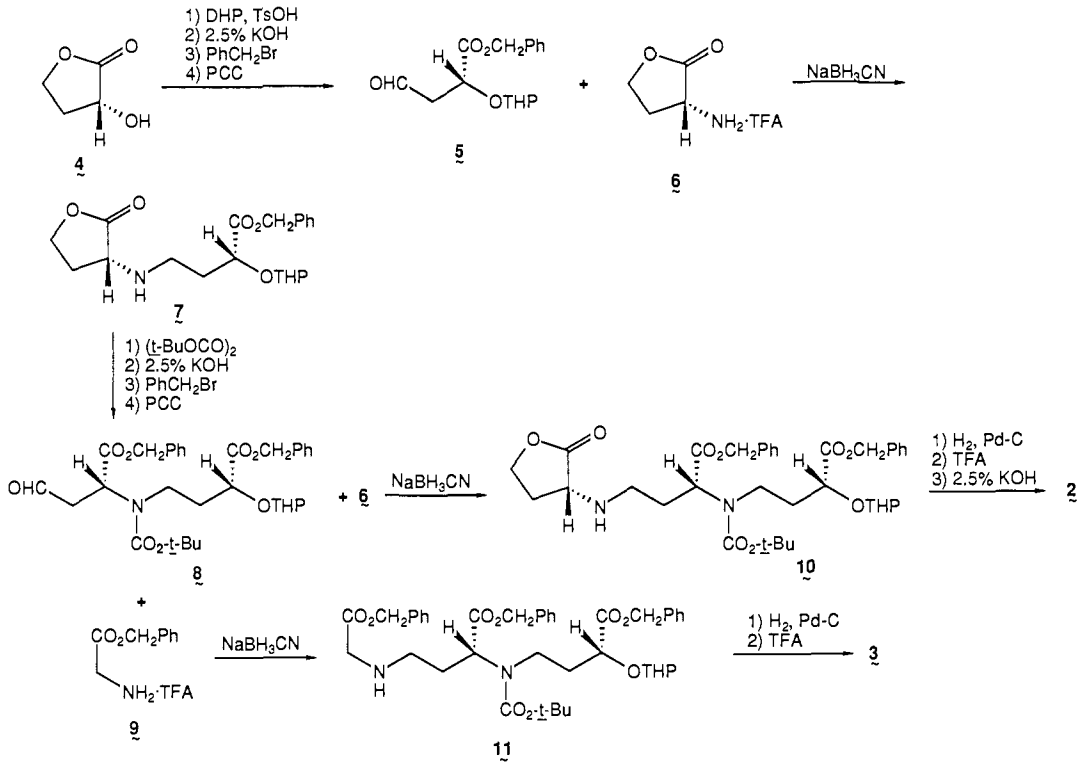
These synthetic compounds were identified by mass, ^1H NMR, and ^{13}C NMR spectra. Each D form gave essentially the same spectral data as the corresponding L form. In addition, the optical rotation ($[\alpha]_D$) showed the same absolute value (16.5°, 2 N HCl, *c* 0.1 for L-AA; −8.95°, H_2O, *c* 0.1 for L-DDA) but opposite sign,

† This paper is dedicated to Prof. Haruaki Yajima on the occasion of his retirement from Kyoto University in March 1989.

(1) Sugiura, Y.; Nomoto, K. *Struct. Bonding (Berlin)* **1984**, *58*, 25-87.
(2) Takemoto, T.; Nomoto, K.; Fushiya, S.; Ouchi, R.; Kusano, G.; Hikino, H.; Takagi, S.; Matsuura, Y.; Kakudo, M. *Proc. Jpn. Acad.* **1978**, *54*, 469-473.
(3) Fushiya, S.; Sato, Y.; Nozoe, S.; Nomoto, K.; Takemoto, T. *Tetrahedron Lett.* **1980**, 3071-3072.
(4) Nomoto, K.; Yoshioka, H.; Arima, M.; Fushiya, S.; Takagi, S.; Takemoto, T. *Chimia* **1981**, *35*, 249-250.
(5) Hider, R. C. *Struct. Bonding (Berlin)* **1984**, *58*, 25-87.
(6) Mino, Y.; Ishida, T.; Ota, N.; Inoue, M.; Nomoto, K.; Yoshioka, H.; Takemoto, T.; Sugiura, Y.; Tanaka, H. *Inorg. Chem.* **1981**, *20*, 3440-3444.
(7) Sugiura, Y.; Tanaka, H.; Mino, Y.; Ishida, T.; ota, N.; Inoue, M.; Nomoto, K.; Yoshioka, H.; Takemoto, T. *J. Am. Chem. Soc.* **1981**, *103*, 6979-6982.
(8) Mino, Y.; Ishida, T.; ota, N.; Inoue, M.; Nomoto, K.; Takemoto, T.; Tanaka, H.; Sugiura, Y. *J. Am. Chem. Soc.* **1983**, *105*, 4671-4676.
(9) Iwashita, T.; Mino, Y.; Naoki, H.; Sugiura, Y.; Nomoto, K. *Biochemistry* **1983**, *22*, 4842-4845.
(10) L-DDA has not been isolated yet as a natural product, whereas distichonic acid (L-DA) is a phytosiderophore excreted from the roots of beer barley (*Hordeum vulgare* L. distichum). L-DDA is thought to have an ability similar to that of the natural phytosiderophores, however, because the artificial phytosiderophore contains the six functional groups that participate in the complexation with metal ions such as Fe(III) and Co(III).
(11) Collum, D. B.; McDonald, J. H., III; Still, W. C. *J. Am. Chem. Soc.* **1980**, *102*, 2117-2118.
(12) Borch, R. F.; Bernstein, M. D.; Dust, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897-2904.
(13) Ohfune, Y., et al., manuscript in preparation.
(14) Ohfune, Y.; Nomoto, K. *Chem. Lett.* **1981**, 827-828.

0002-7863/89/1511-3436$01.50/0

Scheme I



meaning that no racemization took place during the course of the syntheses.

D- and L-AA complexes with Fe(III) gave identical ESR spectra (g = 7.66, 4.26; $S = {}^5/_2$), indicating that the structural configurations are mirror images of each other. The two isomers of DDA also exhibited identical ESR characteristics (g = 7.69, 4.24; $S = {}^5/_2$) for the Fe(III) complex in aqueous solution (pH 7). These g values are very close to those (g = 7.66, 4.26; $S = {}^5/_2$) for the MA-Fe(III) complex.[7,8] In addition, a chromatographic study using ^{59}Fe showed that essentially all of the iron present was the chelated form with both D and L ligands. These results clearly suggest that in both AA and DDA the D form as well as the L form makes an Fe(III) coordination complex similar to that of MA, namely, a nearly octahedral configuration in which two amine nitrogens and both terminal carboxylate oxygens bind to the metal ion as basal planar atoms and hydroxyl oxygen and intermediate oxygen coordinate as axial donors.[8]

Figure 1 shows the iron-uptake activities of the L and D forms of the phytosiderophore for the rice plant (*Oriza sativa* L. var. Koshihikari), which is susceptible to iron chlorosis because of the very limited ability to excrete its own phytosiderophore. The natural phytosiderophore L-AA remarkably stimulated ^{59}Fe uptake in the leaves of the water-cultured rice plant at pH 7. In contrast, the biological effect of its optical isomer, D-AA, was <30% of the L form. In the case of DDA, the L form also demonstrated high iron-uptake activity. On the other hand, the activity of the D form dramatically decreased and was comparable to that of the control. Thus the D form clearly differs from the L form in iron-uptake activity, although their metal coordination properties are very similar. It is most likely that there is a strict stereospecific recognition system (function) for the Fe(III) complex molecule on the membrane. Probably, a certain receptor protein that is able to bind selectively only for natural phytosiderophores exists on the root's membrane. In the plant kingdom, especially gramineous plants, the iron uptake may be regulated by both the excretion of phytosiderophores such as MA and the mobilization of the receptor protein for the iron complex molecule. A similar mechanism of iron regulation mediated by microbial siderophores such as enterobactin (catecholate type) and aerobactin (hydroxamate type) has been proposed for some microorganisms.[15,16]

In conclusion, the optical isomers of phytosiderophores reveal a significant distinction in iron-uptake ability. The present result indicates stereospecific iron-uptake mediated by phytosiderophore in gramineous plants.

Acknowledgment. We are grateful to Dr. Y. Ohfune for helpful suggestions in the synthesis of the phytosiderophores. This study was supported in part by a grant from the Ministry of Education, Science, and Culture, Japan.

(15) Neilands, J. B. *Struct. Bonding (Berlin)* **1984**, *58*, 1-24.

(16) Winkelmann, G.; Huschka, H.-G. In *Iron Transport in Microbes, Plants and Animals*; Winkelmann, G., van der Helm, D., Neilands, J. B., Eds.; Verlagesellschaft: Weinheim, 1987; pp 317-336.

New Phosphite Method: The Synthesis of Oligodeoxyribonucleotides by Use of Deoxyribonucleoside 3′-[Bis(1,1,1,3,3,3-hexafluoro-2-propyl) phosphites] as New Key Intermediates

Tetsuo Watanabe, Hirotaka Sato, and Hiroshi Takaku*

Laboratory of Bioorganic Chemistry
Department of Industrial Chemistry
Tsudanuma, Narashino, Chiba 275, Japan

Received December 19, 1988

Recently, the developments of phosphite, phosphoramidite, and H-phosphonate approaches by Letsinger[1], Caruthers[2], and Matteucci[3] have enabled the rapid chemical synthesis of oligo-

(1) Letsinger, R. L.; Finnan, J. L.; Heavner, G. A.; Lunsford, W. B. *J. Am. Chem. Soc.* **1975**, *97*, 3278. Letsinger, R. L.; Lunsford, W. B. *J. Am Chem. Soc.* **1976**, *98*, 3655.

(2) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859. McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245. Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucleic Acids Res.* **1984**, *12*, 4051.

0002-7863/89/1511-3437$01.50/0