Structure, Properties, and Transport Mechanism of Iron(III) Complex of Mugineic Acid, a Possible Phytosiderophore

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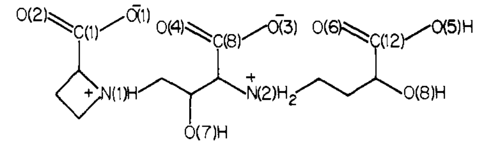
Plants require a continuing supply of iron to maintain proper growth. Since iron stress occurs in many alkaline soils, iron-inefficient plants often become chlorotic and die. Four chemical factors appear to be involved in iron absorption and transport in plants: (1) release of hydrogen ions from the roots, (2) release of reducing compounds from the roots, (3) reduction of ferric ion at the roots, and (4) increase of organic acid (particularly citrate) in the roots.[1] For the purpose of the availability of iron, on the other hand, aerobic microorganisms produce powerful low-molecular-weight chelating agents known as siderophores such as ferrichrome, ferrioxamine, and enterobactin.[2,3] The two most common siderophore classes contain hydroxamate and catecholate functional groups.

Mugineic acid, which is a unique and low-molecular-weight iron-chelating agent excreted from the roots of barley,[4] appears to play an important role for iron absorption under the condition of its deficiency. Indeed, recent experiment demonstrated that the addition of either mugineic acid or 2′-deoxymugineic acid to the medium of water-cultured rice at pH 7.0 increases the iron uptake and chlorophyll content.[5] Other graminaceous plants such as wheat and oats have also produced novel amino acids similar to mugineic acid.[6] Nevertheless, the chemical and biological properties of their iron compounds are little known at the present stage. In order to consider thoroughly a role of mugineic acid for iron uptake and/or transport by graminaceous plants, we have examined structural, spectroscopic, and electrochemical properties of the mugineic acid–iron(III) complex and obtained valuable information for the iron transport mechanism.

Mugineic acid (I) and 2′-deoxymugineic acid (II) were isolated according to the previous procedures.[4] Nicotianamine(III)[7] synthesized by the method of Kristensen et al.[8] was checked by elemental analysis and ^1H NMR spectrum.



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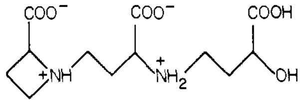

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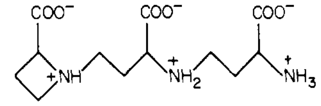

III

Ligand protonation constants of mugineic acid·2HCl were determined by nonlinear least-squares refinement of the potentiometric titration data under the condition of $\mu = 0.1(KNO_3)$ and 20 °C: $pK_1(COOH) = 2.39$, $pK_2(COOH) = 2.76$, $pK_3(COOH) = 3.40$, $pK_4(>N^+H) = 7.78$, and $pK_5(-N^+H_2-) = 9.55$. In the equilibrium curve for an equimolar solution (4.0 mM) of mugineic acid·2HCl and ferric ion, one important feature is that six protons from the ligand were finally released by the complexation. The result indicates that in addition to the five readily titratable ligand groups, there is one other group, probably a terminal OH group, which is dissociating to produce a proton only in the presence of ferric ion.[9] A classical Bjerrum $\bar{n}$ vs. pL plot[10] gave approximate values of the ligand–metal formation constants. Least-squares refinement determined the value ($\log K_{ML}{}^{M} = 18.1$) for the formation constant of the 1:1 mugineic acid–Fe(III) complex. The estimated formation constants for 1:1 divalent metal complexes of mugineic acid were as follows: the Cu(II) complex, $\log K = 18.3$; Zn(II) complex, 10.7; and Fe(II) complex, 8.1.

The Mössbauer spectra of the mugineic acid–^{57}Fe(III) complex in 0 and 600-G magnetic fields are shown in Figure 1. The present Mössbauer parameters ($\Delta E_Q = 0.24$ and $\delta_{Fe} = +0.39$ mm/s) obtained at 194 K are characteristic of those for high-spin ferric complexes ($S = {}^5/_2$). The high-spin ferric assignment was also supported by the large magnetic hyperfine interaction induced at low temperature by the applied magnetic field. The hyperfine field, H_{hf} = ca. −500 kOe, is the usual value for high-spin ferric ion.[11] Also, the X-band ESR spectrum of the mugineic acid–Fe(III) complex (g = 9.4, 4.51, 4.44, and 4.31) in an aqueous solution (pH 7.2) is typical of high-spin Fe(III). The g-tensor anisotropy for the Fe(III) complexes of mugineic acid and 2′-deoxymugineic acid (g = 9.4, 4.51, 4.44, and 4.31) is smaller than that for the nicotianamine–Fe(III) complex (g = 9.5, 4.56, 4.45, and 4.02). These ESR spectra were obtained at 77 K with a JES-FE-3X spectrometer. On the other hand, the mugineic acid–^{57}Fe(II) complex had the Mössbauer spectrum characterized by a single quadrupole doublet at 110 K. The quadrupole splitting ($\Delta E_Q = 2.88$ mm/s) and isomer shift ($\delta_{Fe} = +1.16$ mm/s) are typical of high-spin ferrous ion ($S = 2$), and these values fall in the range of values observed for ionic ferrous complexes. The pale yellow-colored mugineic acid–Fe(II) complex formed by reduction of the corresponding Fe(III) complex with sodium dithionite was ESR negative at 77 K and was reoxidized by air to give the original ESR spectrum of the mugineic acid–Fe(III) complex. In general, an $S = 2$ spin state has a relative short spin–lattice time, and such an ESR spectrum of high-spin Fe(II) complex is difficult to obtain.

At neutral pH, the golden yellow-colored mugineic acid–Fe(III) complex absorbed strongly in the visible and ultraviolet regions, with three peaks at λ_{max} = 350 (ϵ 1520), 250 (8350), and 204 nm (35 000). In the CD spectrum, three CD peaks were observed at 380 ($\Delta\epsilon$ −2.39), 270 (+2.22), and 235 nm (+9.11). Since high-spin ferric ion has no spin-allowed d–d transitions, the visible absorption band must be due to a charge-transfer transition. The structurally analogous mugineic acid–Co(III) complex exhibits

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

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(4) 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.

(5) In contrast, the addition of EDTA or NTA was without effect: Takagi, S., to be published.

(6) (a) Takagi, S. *Soil Sci. Plant Nutr.* (*Tokyo*) **1976**, *22*, 423–433. (b) Fushiya, S.; Sato, Y.; Nozoe, S.; Nomoto, K.; Takemoto, T.; Takagi, S. *Tetrahedron Lett.* **1980**, 3071–3072.

(7) It has been postulated that nicotianamine 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.; Ripperger, H.; Romer, A.; Scholz, G.; Schreiber, K. *Phytochemistry* **1980**, *19*, 2295–2297.

(8) Kristensen, I.; Larsen, P. O. *Phytochemistry* **1974**, *13*, 2791–2802.

(9) On the other hand, the 1:1 mugineic acid–divalent metal ion (Cu^{2+}, Zn^{2+}, and Fe^{2+}) complex system exhibited the release of five protons from the ligand. Indeed, our X-ray crystallographic result showed the axial coordination of the protonated OH group to Cu(II) ion: Mino, Y.; Ishida, T.; Ota, N.; Inoue, M.; Nomoto, K.; Yoshioka, H.; Takemoto, T.; Sugiura, Y.; Tanaka, H. *Inorg. Chem.* **1981**, in press.

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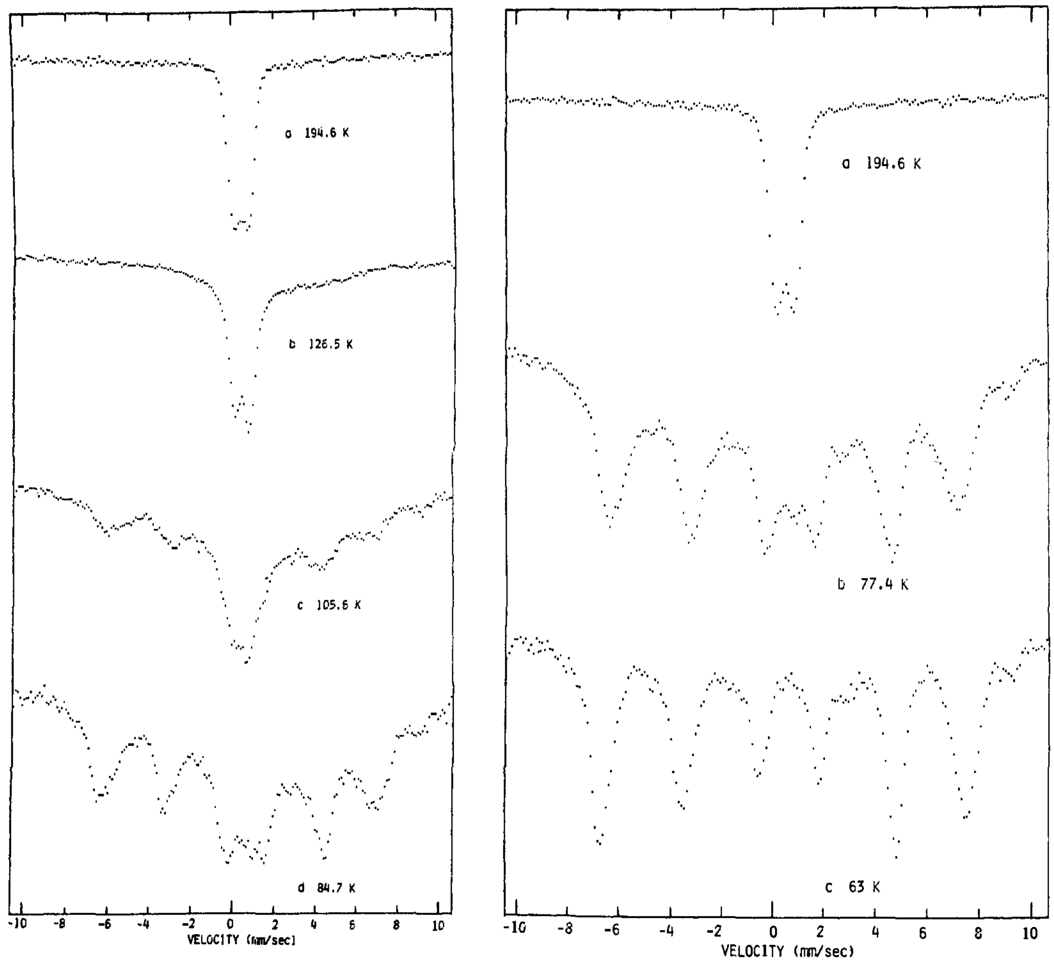

Figure 1. Mössbauer spectra of the mugineic acid–^{57}Fe(III) complex in 0 (left) and 600-G (right) magnetic fields.

absorption maxima [557 (ϵ 80), 393 (85), and 220 nm (7290)] and CD extrema [610 ($\Delta\epsilon$ −0.80), 485 (+0.03), 430 (−0.62), 270 (+6.72), and 225 nm (+8.22)]. On the basis of analogy with electronic spectra of well-known low-spin Co(III) complexes,[12] the band at 554 nm for the mugineic acid–Co(III) complex is assigned as the $^1A_1 \rightarrow {}^1E$ transition and that at 445 nm as the $^1A_1 \rightarrow {}^1A_2$ transition. A calculation of the in-plane field strength yielded the value of 2920 cm^{-1} for Dq_{xy}. The obtained out-of-plane crystal field $Dq_z(D_{4h})$, 1990 cm^{-1}, indicated that weaker ligands occupy the axial sites of Co(III). Indeed, the Fe(III) and Co(III) complexes of mugineic acid showed similar CD patterns in the ultraviolet region, suggesting structural resemblance of both and metal chelates. The X-ray crystallographic result of the isolated 1:1 mugineic acid–Co(III) complex clarified a nearly octahedral configuration in which the azetidine nitrogen, N(1), secondary amine nitrogen, N(2), and both terminal carboxylate oxygens, O(1) and O(5), coordinate to Co(III) ion as basal planar donor atoms, and the hydroxyl oxygen, O(8), and intermediate carboxylate oxygen, O(3), coordinate as axial donors.[13] The stereoscopic drawing of the mugineic acid–Co(III) complex and the coordination about the Co(III) ion are illustrated in Figure 2. The axial bond lengths, Co–O(8) and CoO(3), are considerably shorter than those (2.477 and 2.557 Å) in the corresponding Cu(II) complex.[14] The valency angles N(2)–Co–O(1) (174°) and N(1)–Co–O(5) (174°) suggest that the coordination geometry around the cobalt atom slightly distorts from square planar toward tetrahedral. The axial bonds, O(3)–Co–O(5) (166°), also slightly deviate from the normal of the basal plane. Probably, these distortions can be accounted for by the steric constrains of both a six-membered chelate ring, Co–N(1)–C(5)–C(6)–C(7)–N(2), and a seven-membered ring, Co–N(2)–C(9)–C(10)–C(11)–C(12)–O(5). However, these bond angles are larger than those (168.8, 168.8, and 144.3°) in the mugineic acid–Cu(II) complex which has a distorted tetragonal configuration. Full details will be published later. The similarity in the coordination chemistries between Fe(III) and Co(III) makes the present Co(III) complex a useful vehicle for probing the structure of the biological important mugineic acid–Fe(III) complex.[15]

Figure 3 shows a cyclic voltammogram for the mugineic acid–Fe(III) complex at pH 7.0. No additional well-defined reductions were observed at −1.5 V. The present ferric complex exhibited quasi-reversible one electron oxidation–reduction wave with $E_{1/2}$ value of −102 mV vs. the normal hydrogen electrode (NHE). The value $i_{p,c}/i_{p,a} \sim 1$ for the redox event also suggests effective chemical reversibility, whereas the nicotianamine–Fe(III) complex exhibited irreversible one electron redox wave at $E_{1/2}$ = −181 mV vs. NHE. The result is consistent with the order of O > N donor atoms for the positive shift of reduction potentials

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[12] Wentworth, R. A. D.; Piper, T. S. *Inorg. Chem.* **1965**, *4*, 709–714.

[13] A single crystal with dimensions of ca. 0.1 × 0.1 × 0.1 mm was used for 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 (λ = 1.5405 Å) using the ω–2θ scan technique. Crystal data were as follows: $C_{24}H_{33}N_4O_{16}Co_2Na\cdot 3H_2O$; M_r 828.44; monoclinic; space group $P2_1$; a = 9.425 (2), b = 17.350 (4), c = 9.428 (2) Å; β = 91.08 (2)°; V = 1541.4 (5) Å^3; D_m = 1.770, D_x = 1.785 g cm^{-3}; μ(Cu Kα) = 91.5 cm^{-1}. The structure was solved by the heavy-atom method and refined by least squares to R = 0.066 using 2718 independent reflections.

[14] Nomoto, K.; Mino, Y.; Ishida, T.; Yoshioka, H.; Ota, N.; Inoue, M.; Takagi, S.; Takemoto, T. *J. Chem. Soc., Chem. Commun.* **1981**, 338–339.

[15] Although the crystal structure of the mugineic acid–Fe(III) complex has not as yet been established by diffraction techniques, the coordination structure of the ferric complex should be sufficient alike to those of the demonstrated cobaltic complex. The crystal field stabilization for d^6 cobalt complexe (24Dq) is considerably greater than that for high-spin ferric complex (0Dq). Thus, the Co(III)-substituted complex of mugineic acid should be kinetically inert.

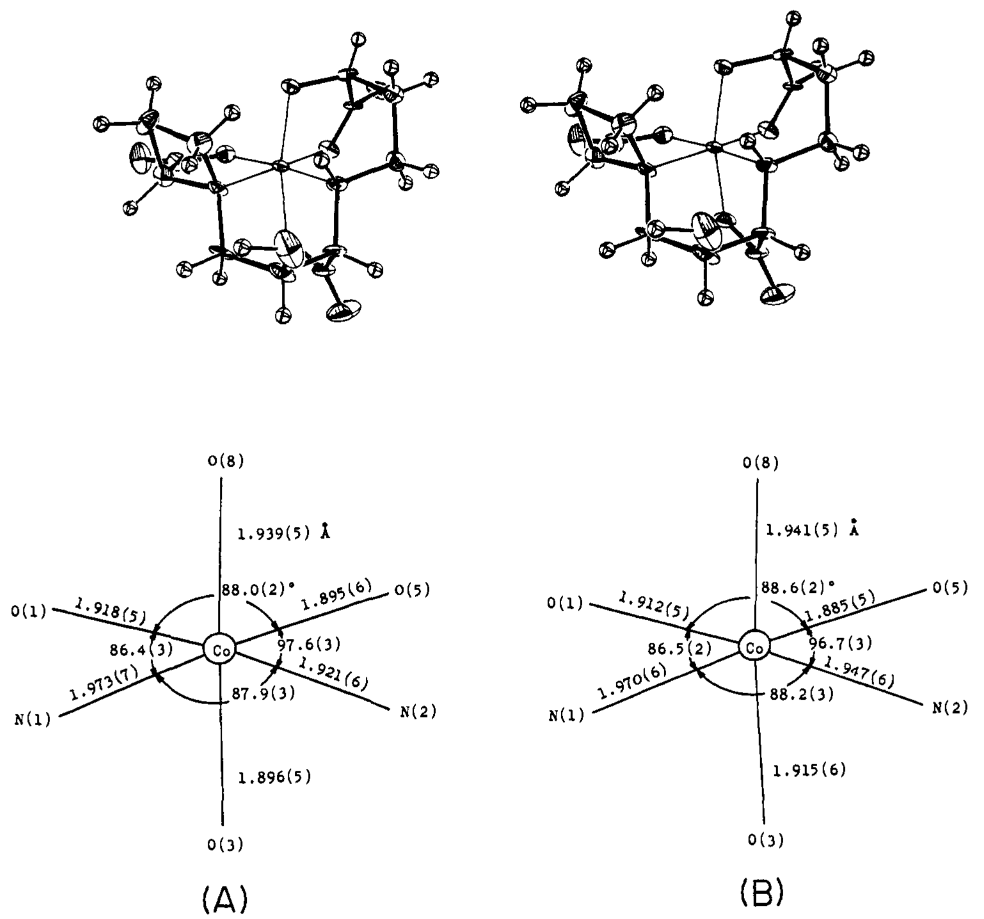

Figure 2. A perspective drawing of the molecule A of the mugineic acid-Co(III) complex and coordination about the cobalt ion in molecules A and B. Bond lengths and angles are shown in Å and deg, respectively. The bond angles O(3)-Co-O(8), N(2)-Co-O(1), and N(1)-Co-O(5) are as follows: 165.9(2), 174.1 (3), and 173.8 (3) (A molecule) and 166.0 (2), 174.5 (3), and 173.8 (3) (B molecule), respectively.

in Cu(II) complexes,[16] because the terminal hydroxyl group in mugineic acid is replaced by an amino group in nicotianamine. For a completely reversible one electron reduction, a peak potential separation $|E_{p,c} - E_{p,a}|$ of 59 mV is expected. The peak-to-peak separations for the Fe(III) complexes of mugineic acid and nicotianamine were 68 and 130 mV, respectively. The salient feature of the electrochemical results is an apparently higher reduction potential of the mugineic acid-Fe(III) complex than those of the microbial hydroxamates (from -350 to -450 mV vs. NHE) and ferric enterobactin chelates (~-750 mV vs. NHE).[17] The mugineic acid-Fe(III) complex has E (pH 7.0) = -102 mV vs. NHE as the redox potential and is readily reducible by physiologically available reductants such as NAD(P)H (ca. -0.32 V vs. NHE). Thus, the present results strongly indicate that the mechanism of iron transport in graminaceous plants probably includes iron reduction from the thermodynamically stable ferric mugineic acid complex ($\log K_{ML}{}^{M} = 18.1$) to the weakly bound ferrous complex (8.1). In fact, it has been suggested that intercellular iron release of microbial siderophores occurs by iron reduction in hydroxamates and by hydrolytic depolymerization in ferric enterobactin.[18] The formation constant of the mugineic acid-Fe(III) complex is closer to that of *N*-(2-hydroxyethyl)ethylenedinitrilo-*N*,*N'*,*N'*-triacetic acid (HEDTA)-Fe(III) complex ($\log K_{ML}{}^{M} = 19.8$)[19] rather than those of ferrichrome (29.1),[20] ferrioxamine B (30.6),[20] and ferric enterobactin (~52).[17,21] However, direct comparison of the formation constants of the siderophores with the present compound

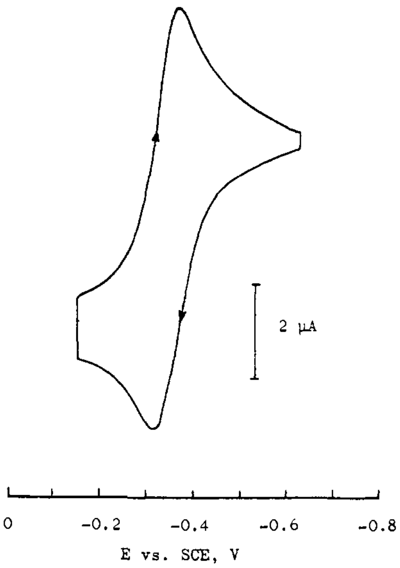

Figure 3. Cyclic voltammogram of the mugineic acid-Fe(III) complex at pH 7.0. Experimental condition was as follows: 0.1 M KCl, 0.05 M sodium borate/0.05 M sodium phosphate buffer, and 1.0 mM mugineic acid-Fe(III) complex. The electrochemistry was performed on a hanging mercury drop electrode with 100-mV/s scan rate.

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(16) Patterson, G. S.; Holm, R. H. *Bioinorg. Chem.* **1975**, *4*, 257-275.

(17) Harris, W. R.; Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2722-2727.

(18) Cooper, S. R.; McArdle, J. V.; Raymond, K. N. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3551-3554.

(19) (a) Skochdopole, R.; Chaberek, S. *J. Inorg. Nucl. Chem.* **1959**, *11*, 222-233. (b) Gustafson, R. L.; Martell, A. E. *J. Phys. Chem.* **1963**, *67*, 576-582: The ligand shows the same kind of hydrolytic behavior for Fe(III) and the absence of that behavior (for the hydroxyl coordination) for Cu(II). Therefore, this compound would be an adequate model for mugineic acid.

(20) (a) Schwarzenbach, G.; Schwarzenbach, K. *Helv. Chim. Acta* **1963**, *46*, 1390-1400. (b) Anderegg, G.; L'Eplattenier, F.; Schwarzenbach, G. *Ibid.* **1963**, *46*, 1409-1422.

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is not very informative because of the very different acidities of these ligands. The ligand, mugineic acid, is a much better complexing agent for ferrous iron than liquids which contain hydroxamate or catecholate. Thus, the great difference in redox potential would be explained by the relative stability of the ferrous complex of mugineic acid. The iron-solubilizing action of mugineic acid is strongly inhibited by the presence of cupric ion.[6] This can be explained by blockage of the Fe(III) binding to mugineic acid by Cu(II) ion, consistent with the order of the formation constant for the mugineic acid–metal complexes, Cu(II) ≥ Fe(III).

Most microbial siderophores have hydroxamate or phenolate groups as Fe(III)–ligand donors. The present phytosiderophore, mugineic acid, consists of carboxyl, amine, and hydroxyl groups as ligand functional groups. The mechanism for absorption and transport of iron in graminaceous plants involves the excretion of mugineic acid from the roots, which aids Fe(III) solubilization and reduction of Fe(III) to Fe(II).

Acknowledgment. We are grateful to Dr. Y. Maeda for Mössbauer measurements and to referees for useful comments. This study was supported in part by a grant from the Ministry of Education, Science, and Culture, Japan.

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Picosecond Laser Study of the Adiabatic Photodissociation of an Endoperoxide

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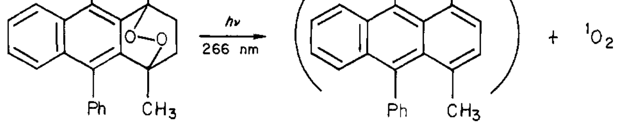
We wish to report the first direct evidence for the adiabatic photodissociation of an endoperoxide, the 1,4-endoperoxide of 1,4-dimethyl-9,10-diphenylanthracene (DMDPA-O_2). To establish that a photocleavage is adiabatic it is necessary to demonstrate that an excited electronic state photoproduct is produced.[1] The evidence that the photolysis of an endoperoxide is adiabatic has rested heretofore on the chemical evidence[2,3] that the oxygen fragment is produced in an excited state, $^1O_2$. In the present work we discovered a dissociation route in which the other fragment in the reaction, namely, the 1,4-dimethyl-9,10-diphenylanthracene (DMDPA), is produced in an excited singlet state rather than in the ground state. That the hydrocarbon fragment DMDPA is generated in an excited singlet state is established by observation of its fluorescence which in turn directly demonstrates that the photodissociation is adiabatic. The reaction can be written as



DMDPA-O_2 ^{1}DMDPA*

The precursor molecule, DMDPA-O_2 (1.5×10^{-4} M), in acetonitrile at room temperature, was excited with a picosecond laser pulse at 266 nm, the fourth harmonic of a Nd laser. The emission spectrum was recorded with a monochromator–optical multichannel analyzer, and the kinetics were measured with a streak camera.

We find that the fluorescence lifetime (0.5 ns) and spectrum (Figure 1) obtained on photoexcitation of the parent endoperoxide were indistinguishable from those of DMDPA, thus establishing the identity of the fragment. From the risetime of the fluorescence we found that the excited fragment DMDPA* appears in less than 5 ps. However, to demonstrate that the process is adiabatic, it is necessary to show that the observed fluorescence does not arise from a sequential two-photon process. Such a process would involve one photon exciting the endoperoxide and generating the ground-state fragment DMDPA. The DMDPA could then absorb a second photon from the same excitation pulse to produce DMDPA*, whose fluorescence we observe. Our experimental results show that the fluorescence varies linearly with the laser intensity, thus proving that this is indeed a one-photon process. We have also eliminated the possibility that DMDPA, present as an impurity in the endoperoxide sample or resulting from a buildup of the DMDPA photoproduct, is responsible for the observed fluorescence. This was done by determining that there was negligible emission following irradiation of the endoperoxide sample at 355 nm, where DMDPA adsorbs strongly but the endoperoxide does not.

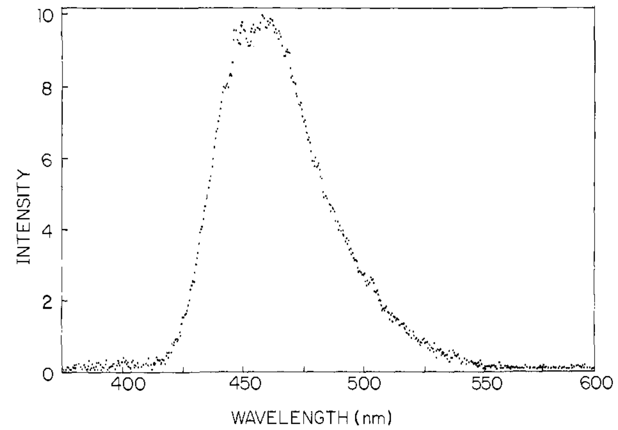


Figure 1. Fluorescence spectrum of the DMDPA* fragment following photodissociation of the precursor endoperoxide (1.5×10^{-4} M) in acetonitrile.

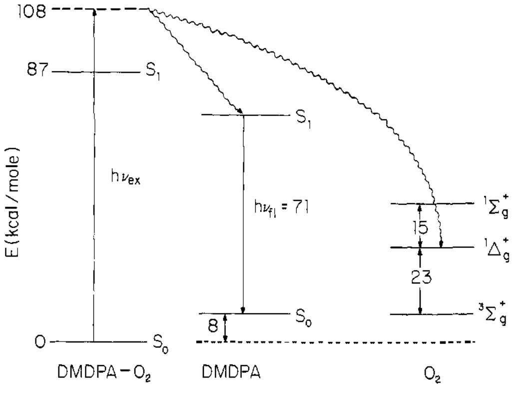


Figure 2. Energetics of endoperoxide photodissociation, $\lambda_{ex} = 266$ nm.

An interesting aspect of this photodissociative route is the particular state in which the oxygen is formed. It is reasonable to assume that the oxygen is released in an excited singlet state based on the time scale of the photolysis. We have shown that the photodissociation occurs in less than 5 ps as seen by the risetime of the ^{1}DMDPA* fluorescence. If oxygen were coming off in its triplet state the photodissociation process would be spin forbidden and highly unlikely to occur in such a short time. We can now consider the energetics of this route to decide on the particular excited state in which the $^1O_2$ is released. The two possible excited states for $^1O_2$ are the $^1\Sigma_g{}^+$ and the $^1\Delta_g$ separated by 15 kcal/mol. From the energy of dissociation[4-6] of DMDPA-O_2 and the energies

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