BIOSYNTHESIS OF AVENIC ACID A, A FERRIC CHELATING SUBSTANCE SECRETED FROM *AVENA SATIVA* L.

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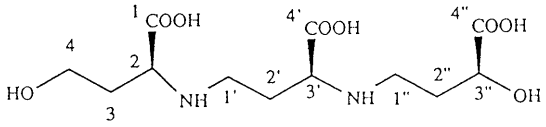
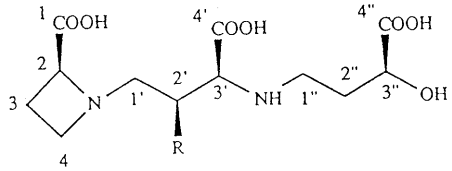
The biosynthesis of avenic acid A was studied by feeding L-methionine-1-^{13}C or D,L-methionine-3, 3, 4, 4-d_4 to iron-deficient oat roots (*Avena sativa* L. cv Amuri II). Both avenic acid A and 2'-deoxymugineic acid labeled with ^{13}C or ^{2}H were isolated from root-washings of the same plants. The ^{13}C-NMR elucidated that three molecules of L-methionine were incorporated into avenic acid A in the same way as 2'-deoxymugineic acid. The ^{2}H-NMR spectra indicated that all deuteriums from the methionine were incorporated into 2'-deoxymugineic acid, but one of the deuteriums at C-4 was lost in avenic acid A. A possible biosynthetic pathway is L-methionine→ →2'-deoxymugineic acid→avenic acid A.

KEYWORDS 2'-deoxymugineic acid; avenic acid A; biosynthesis; *Avena sativa*; L-methionine; ^{2}H-NMR; ^{13}C-NMR

It has been known that gramineous plants respond to iron stress by secreting iron chelating substances which have been generically termed "mugineic acids (MAs)".[1] These substances play a role in solubilization and transport of Fe(III). One of them, avenic acid A (**1**), was secreted from iron-deficient oat roots (*Avena sativa* L.).[2]



1; avenic acid A



2; R=H 2'-deoxymugineic acid
3; R=OH mugineic acid

Since mugineic acid (**3**) was discovered in barley plants and its structure was first determined,[3] the biosynthesis of MAs has been explored. Kawai *et al.* fed four ^{14}C-labeled amino acids and L-methionine-1-^{13}C to iron-deficient barley roots, and found that L-methionine is the most efficient precursor for mugineic acid (**3**) and 2'-deoxymugineic acid (**2**).[4] Shojima *et al.* also got the same results *in vitro*.[5,6] However, no experimental data on the biosynthesis of avenic acid A (**1**), or on its relation with other MAs, have been published. In the present study, we found that the precursor for avenic acid A (**1**) is the same as that for mugineic acid (**3**) and 2'-deoxymugineic acid (**2**). A possible biosynthetic pathway for avenic acid A (**1**) is suggested by the ^{2}H-NMR study.

Eighty 8-day-old oat plants (*Avena sativa* L. cv Amuri II) were cultured in 3-liter pots containing continuously aerated 1/5 strength Hoagland solution in an environmental chamber. The solution was adjusted to pH 6.0 and renewed once every two days. After 2 weeks, the plants were transferred to a solution free of iron. When the chlorosis developed moderately, 100 μM L-methionine-1-^{13}C or 200 μM D,L-methionine-3, 3, 4, 4-

d_4 were fed to the roots with 40 ppm surface-active agent "Decaglyn 1-L" at the end of the secretion. Root-washings were collected the following day by soaking the roots in distilled water from 3 to 5 hour after onset of the light period. The isolation was performed by various chromatographies.[3] As a result, ^{13}C-enriched avenic acid A (**1**) (10.8 mg) and 2'-deoxymugineic acid (**2**) (1.25 mg) and ^{2}H-enriched corresponding compounds (**1**, 1.8 mg; **2**, 0.5 mg) were obtained. The ^{1}H-, ^{2}H-, and ^{13}C-NMR spectra were measured at 500, 76.8, and 75.5 MHz spectrometers, respectively. The assignments were accomplished by ^{1}H-^{1}H and ^{13}C-^{1}H COSY, HMBC, and HMQC experiments.

The ^{13}C-NMR spectrum showed that labeled avenic acid A (**1**) (Fig. 1A) was ^{13}C-enriched by 11.2, 11.7, and 12.7-fold in their peak-heights for C-1, 4', and 4", respectively, compared to those of unlabeled avenic acid A (**1**) (Fig. 1B). This result revealed that three molecules of L-methionine were incorporated into avenic acid A (**1**) in the same way as mugineic acid (**3**) and 2'-deoxymugineic acid (**2**).[4] The precursor for these different MAs is thought to be the same.

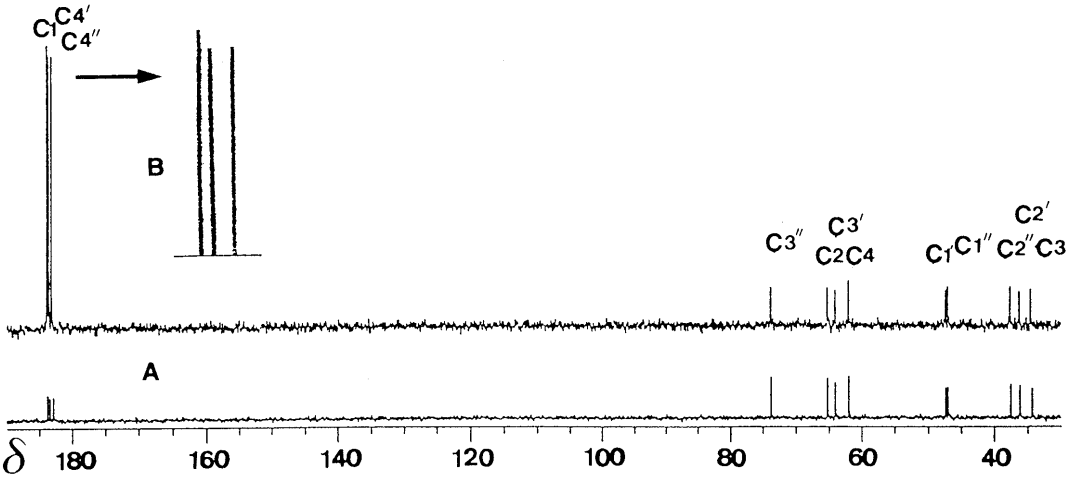


Fig. 1. ^{13}C-NMR Spectra of Avenic Acid A (**1**) Biosynthesized from L-Methionine-1-^{13}C (B) and Unlabeled Avenic Acid A (**1**) (A)

Avenic acid A (**1**) and 2'-deoxymugineic acid (**2**) were simultaneously isolated from the same plants; however, their relationship in the biosynthetic pathway is not clear. It is helpful, therefore, to know how protons in methionine change during the biosynthesis to avenic acid A (**1**) and 2'-deoxymugineic acid (**2**). To investigate this, both ^{2}H-enriched avenic acid A (**1**) and 2'-deoxymugineic acid (**2**) were isolated, and their ^{2}H-NMR was performed. As ^{2}H chemical shifts correspond to ^{1}H shifts aside from a small isotope effect and broadening, we can speculate the assignment of ^{2}H-NMR peaks based on that of ^{1}H-NMR. As shown in Fig. 2B, relative peak intensity at δ 4.12, 4.01, 3.51, 3.39, 3.22, 2.77, 2.57, 2.18, 2.06 in labeled 2'-deoxymugineic acid (**2**) was 1:1:1:1:2:1:1:3:1, corresponding deuteriums at C-4, C-1', C-1", C-3, C-2', and C-2", respectively. This result suggests that 12 deuteriums from three molecules of methionine-3,3,4,4-d_4 were wholly incorporated. In comparison, 11 deuteriums whose relative peak intensity at δ 3.60, 2.58, 1.76 was 1:4:6 were observed in the labeled avenic acid A (**1**) (Fig. 3B). One of the deuteriums at C-4 was lost in the labeled avenic acid A (**1**), but all other deuteriums were incorporated in the same way as the labeled 2'-deoxymugineic acid (**2**). These results suggest that avenic acid A (**1**) can not be the precursor of 2'-deoxymugineic acid (**2**). However, the reverse may be possible--that is, avenic acid A (**1**) is biosynthesized from 2'-deoxymugineic acid (**2**) by cleavage of azetidine ring. A possible biosynthetic pathway for avenic acid

A (**1**) may be suggested as L-methionine→ → 2'-deoxymugineic acid (**2**) →avenic acid A (**1**).

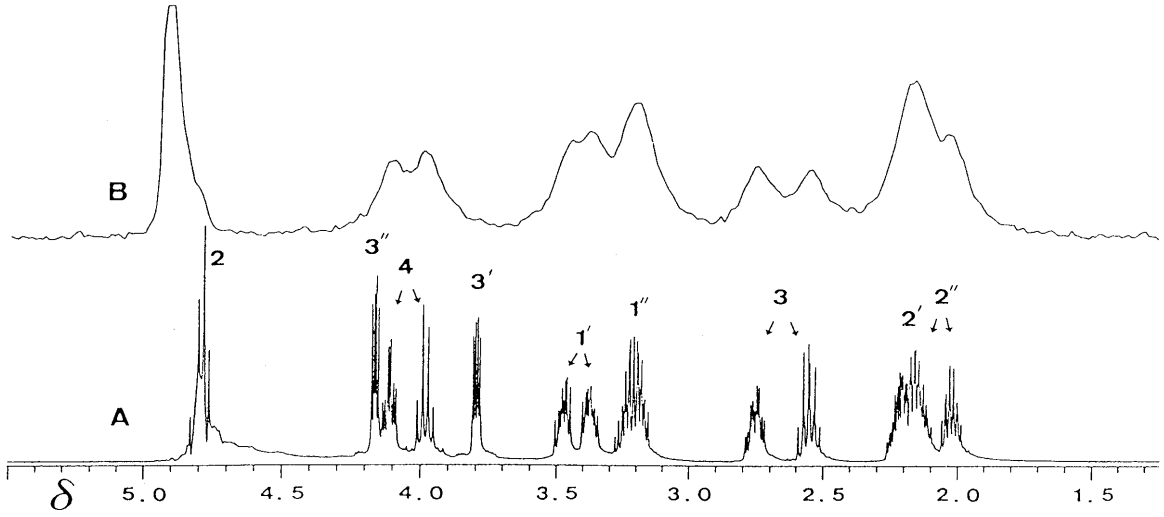


Fig. 2. ^{1}H-NMR Spectrum of 2'-Deoxymugineic Acid (**2**) (A) and ^{2}H-NMR Spectrum of 2'-Deoxymugineic Acid (**2**) Biosynthesized from D,L-Methionine-3,3,4,4-d_4 (B)
The peak at δ 4.93 represents the signal of water.

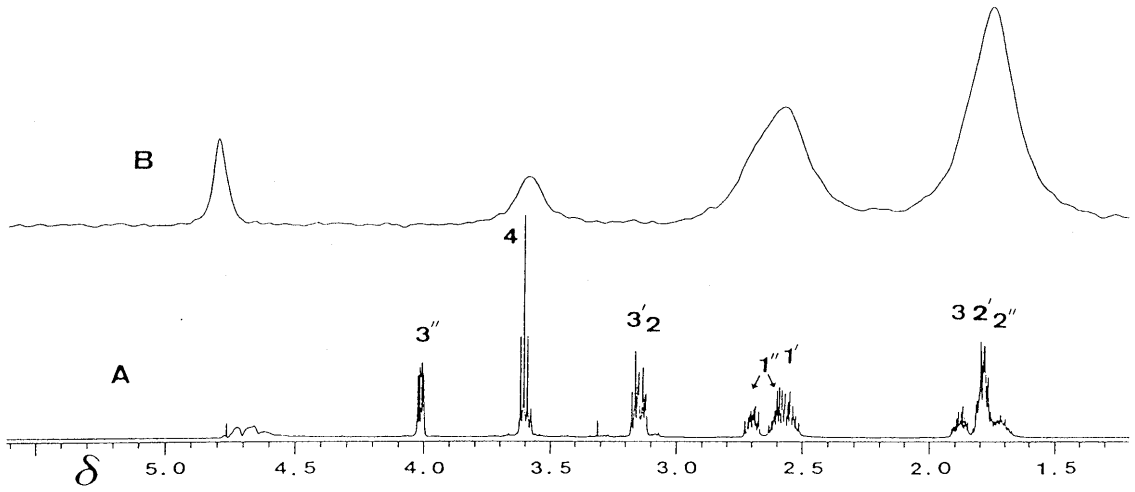


Fig.3. ^{1}H-NMR Spectrum of Avenic Acid A (**1**) (A) and ^{2}H-NMR Spectrum of Avenic Acid A (**1**) Biosynthesized from D,L-Methionine-3,3,4,4-d_4 (B)
The peak at δ 4.80 represents the signal of water.

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REFERENCES

1) Y. Sugiura and K. Nomoto, Structure and Bonding, **58**, 107 (1984).
2) S. Fushiya, Y. Sato, S. Nozoe, K. Nomoto, and T. Takemoto, Tetrahedron Lett., **21**, 3071 (1980).
3) T. Takemoto, K. Nomoto, S. Fushiya, R. Ouchi, G. Kusano, H. Hikino, S. Takagi, Y. Matsuura, and M. Kakudo, Proc. J. Acad., **54**, 469 (1978).
4) S. Kawai, K. Itoh, S. Takagi, T. Iwashita, and K. Nomoto, Tetrahedron Lett., **29**, 1053 (1988).
5) S. Shojima, N. K. Nishizawa, and S. Mori, Plant Cell Physiol., **30**, 673 (1989).
6) S. Shojima, N. K. Nishizawa, S. Fushiya, S. Nozoe, T. Irifune, and S. Mori, Plant Physiol., **93**, 1497 (1990).

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