Tetrahedron Letters Vol. 21, pp 3071 - 3072
© Pergamon Press Ltd. 1980. Printed in Great Britain

0040-4039/80/0801-3071$02.00/0

AVENIC ACID A, A NEW AMINO ACID POSSESSING AN IRON CHELATING ACTIVITY

Shinji Fushiya, Yoshikazu Sato, and Shigeo Nozoe*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980.

Kyosuke Nomoto and Tsunematsu Takemoto

Suntory Institute for Bioorganic Research, Shimamotocho,
Mishimagun, Osaka 618.

Sei-ichi Takagi

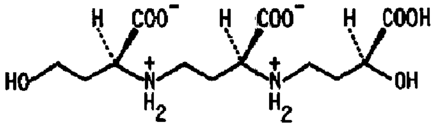
Department of Agricultural Chemistry, Iwate University, Ueda, Morioka 020.

Abstract: A new amino acid derivative possessing an iron chelating activity was isolated from root washings of water cultured *Avena sativa* under iron deficient conditions. The structure of this compound, avenic acid A has been determined as 2(S),3'(S),3"(S)-N-[3-(3-hydroxy-3-carboxypropylamino)-3-carboxypropyl]-homoserine (1) on the basis of chemical and spectroscopic evidence.

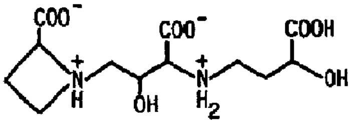
It has been suggested that some higher plants excrete iron chelators from their roots to absorb iron in the chelated form under conditions of its deficiency. Many studies[1] have been performed to isolate these chelating substances which play a role in iron uptake and transport in higher plants. Recently, Takagi[2] reported the detection of amphoteric iron chelating agents in the root washings of water cultured rice and oats under iron deficient conditions.
In a previous paper[3], we reported the isolation and structure elucidation of mugineic acid (2), one of these chelating substances from root washings of *Hordeum vulgare* L. Further studies of such substances excreted from the roots of oats has resulted in the isolation of a new amino acid, for which we propose the name avenic acid A. In the present paper, we describe the structure of this compound.

After cultivation of *Avena sativa* (ca. 800 plants) in iron containing nutrient solution up to full expansion of second leaf, culture was continued in an iron-less medium for another week when iron chlorosis developed gradually on the leaves. Root washings were collected by soaking the roots in distilled water for 2 hrs every morning for a period of ten days. The isolation and purification of the active substances was achieved by passing the combined solution through Amberlite IR-120B followed by separation on Dowex 50W monitoring the chelating activity by the *o*-phenanthroline method[2]. From the fractions eluted with NH_3-HCOOH buffer (pH 2.70), avenic acid A (120 mg) was obtained in the form of colorless fine crystals together with a minor congener (20 mg). That avenic acids are acidic amino acids is shown by their action on ion exchange resin and yellow coloration with BCG reagent.

Avenic acid A, mp.>300°C, $[\alpha]_D$+16.4° (c, 0.11, 2*N* HCl) has a molecular formula $C_{12}H_{22}N_2O_8$[4], ms (FD); m/e 345 $(M + Na)^+$. The ir spectrum (KBr disk) showed absorption at 3450 (NH and OH), 1710 (COOH), 1580, 1395 (COO^-), and 2850 cm^{-1} ($>NH_2^+$) and showed no amide absorption. The pmr spectrum[5] of 1 revealed the presence of two methylene groups (2.24-2.68, m, 4H) and two methine groups (2.95, t, J=7.6, 1H and 3.02, t, J=7.6, 1H), all of which are adjacent to a secondary ammonium group, a hydroxymethyl (3.52, t, J=7.6, 2H), a hydroxy bearing methine (3.92, dd, J=5.4 and 8.1, 1H) and three methylene groups (1.53-1.81, m, 6H). The cmr spectrum5) of 1 showed signals due to three carboxyl carbons at 182.3, 183.1 and 183.1 along with nine signals at 33.9, 35.0, 36.3 ($-CH_2-$); 60.2 ($-CH_2-O-$); 45.1, 45.4 ($-CH_2-\overset{+}{N}H_2-$), 72.1, 62.2, 63.3 ($CH-NH^+-$ or $CH-O-$). Upon irradiation at a broad methylene signal (1.53-1.81), three methine signals (2.95, 3.02 and 3.92) and a hydroxymethyl signal (3.52) changed to singlet and methylene signals at 2.24-2.68 simplified, indicating that all methylene and methine groups linked to N or O atoms are adjacent to the methylenes with no heteroatom functional groups. These findings revealed that avenic acid A has three parts of $X-CH_2-CH_2-CH\begin{smallmatrix}\diagup Y\\ \diagdown Z\end{smallmatrix}$ systems (X, Y=N or O functions; Z=COOH) in the molecule as well as a homoserine terminus. Since this compound contains no unsaturation, the formula 1 can be assumed to be the most probable structure for avenic acid A.



1



2

The structure deduced from spectral analyses was also supported by chemical degradation. $KMnO_4$ oxidation of 1 (65 μmole) yielded aspartic acid (2.6 μmole), homoserine (5.2 μmole) and malic acid (5.4 μmole). The characterization and identification of the amino acids and hydroxyacid were performed by comparison of their PC and HPLC behavior patterns with those of authentic specimens. The cd curves of aspartic acid, homoserine and malic acid obtained by $KMnO_4$ oxidation exhibit a positive Cotton effect ($[\theta]_{201}+13770$, $[\theta]_{197}+8380$, and $[\theta]_{206}+10340$ respectively) indicating the all chiral centers in this acid are S configuration.6)

From the data shown above, it is concluded avenic acid A is 2(S),3'(S),3"(S)-N-[3-(3-hydroxy-3-carboxypropylamino)-3-carboxypropyl]-homoserine (1). The structure of the minor congener and related substances as well as the biosynthetic relationship between these compounds are under investigation.

References and Notes

1. a) G.A.Snow, Bacteriol. Rev., 3, 99 (1970); b) J.C.Brown, Ann. Rev. Plant Physiol., 7, 171 (1956); J.C.Brown, L.O.Triffin, A.W.Spect and J.W.Resnicky, Agron. J., 53, 81 (1961); d) J.C.Brown, Adv. Agron., 13, 329 (1961); e) P.C.Dekock, Ann. Botany., 20, 133 (1956)
2. S.Takagi, Soil Sci. Plant Nutr., 22, 423 (1976)
3. T.Takemoto, K.Nomoto, S.Fushiya, R.Ouchi, G.Kusano, H.Hikino, S.Takagi, Y.Matuura and M.Kakudo, Proc. Japan Acad., 54 Ser B 469 (1978)
4. Satisfactory analytical data was obtained for this compound.
5. Avenic acid A is sparingly soluble in water, the pmr and cmr spectra were measured in 1 N NaOD.
6. a) J.C.Craig and S.K.Roy, Tetrahedron, 21, 1847 (1965); b) Idem. ibid., 21, 391 (1965)

(Received in Japan 10 May 1980)