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STRUCTURE AND SYNTHESIS OF AVENIC ACID B, A NEW AMINO ACID POSSESSING AN IRON CHELATING ACTIVITY

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A new amino acid derivative possessing an iron chelating activity was isolated from an ethanol extract from the roots of Avena sativa. The structure of this compound, avenic acid B, has been determined as 2(S),3'(S)-N-(3-hydroxy-3-carboxypropyl)-homoserine (**1**) on the basis of nmr analysis and by chemical synthesis.

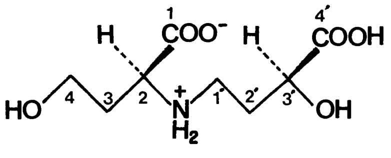
Recently, some amphoteric iron chelating agents, which play a role in iron uptake and transport have been detected in the root washings of rice and oats cultured under iron deficient conditions.1) Among these chelating substances, mugineic acid was first isolated from root washings of Hordeum vulgare L.2) Further studies have been undertaken to isolate such chelating compounds excreted from the root of oats and, in a previous paper,3) we reported the isolation and structural elucidation of avenic acid A (**2**) from root washings of Avena sativa. During the course of studies to isolate such amino acid derivatives, the presence of several other minor components in the root extracts of Avena sativa was observed beside avenic acid A (**2**). In the present communication, we describe isolation, structure and synthesis of a new substance closely related to avenic acid A (2). This new compound, avenic acid B also possesses iron chelating activity.1)

After cultivation of Avena sativa (ca. 2,000 plants) in iron deficient media, the roots were extracted with 70 % ethanol. The isolation and purification of the substance was achieved in the same manner used for the isolation of avenic acid A (**2**).3) On elution with a NH_3-HCOOH buffer (pH 2.60) on a Dowex 50W column, avenic acid B was obtained as a colorless non-crystalline material.

Avenic acid B has a molecular formula $C_8H_{15}NO_6$, ms (FD); 222 $(M + H)^+$. The pmr spectrum of **1** revealed the presence of a methylene group (2.34-2.76, m, 2H) and a methine group (3.12, t, J=7.0, 1H), both of which are adjacent to a secondary ammonium group, a hydroxymethyl (3.62, t, J=7.6, 2H), a hydroxy bearing methine (4.05, dd, J=5.4 and 8.1, 1H) and two methylene groups (1.65-1.97, m, 4H).

The cmr spectrum of 1 showed signals due to two carboxyl carbons at 181.1 and 181.9 along with six signals at 33.8, 35.3 ($-CH_2-$); 58.9 ($-CH_2-O-$); 43.9 ($-CH_2-\overset{+}{N}H_2-$); 61.1, 70.9 ($CH-\overset{+}{N}H$ or $CH-O$). The above-mentioned pmr and cmr signals of 1 show good correspondance with those based on $C_{(1)}-C_{(4)}$ and $C_{(1'')}-C_{(4'')}$ moieties of avenic acid A (2) (Table).

From the accumulated data together with circumstantial evidence indicating that avenic acid B coexists with avenic acid A (2), formula 1 can be proposed as the most probable structure for avenic acid B. And the cd curve of 1 exhibits a positive Cotton effect ($[\theta]_{198}$ +14590), indicating that all the chiral centers in this acid are S configuration.$^{5)}$ The structure deduced from spectral analysis was confirmed by the following synthesis.



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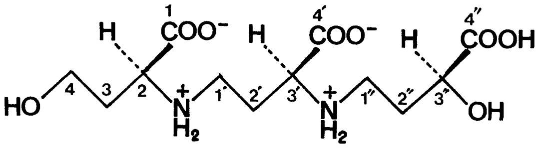

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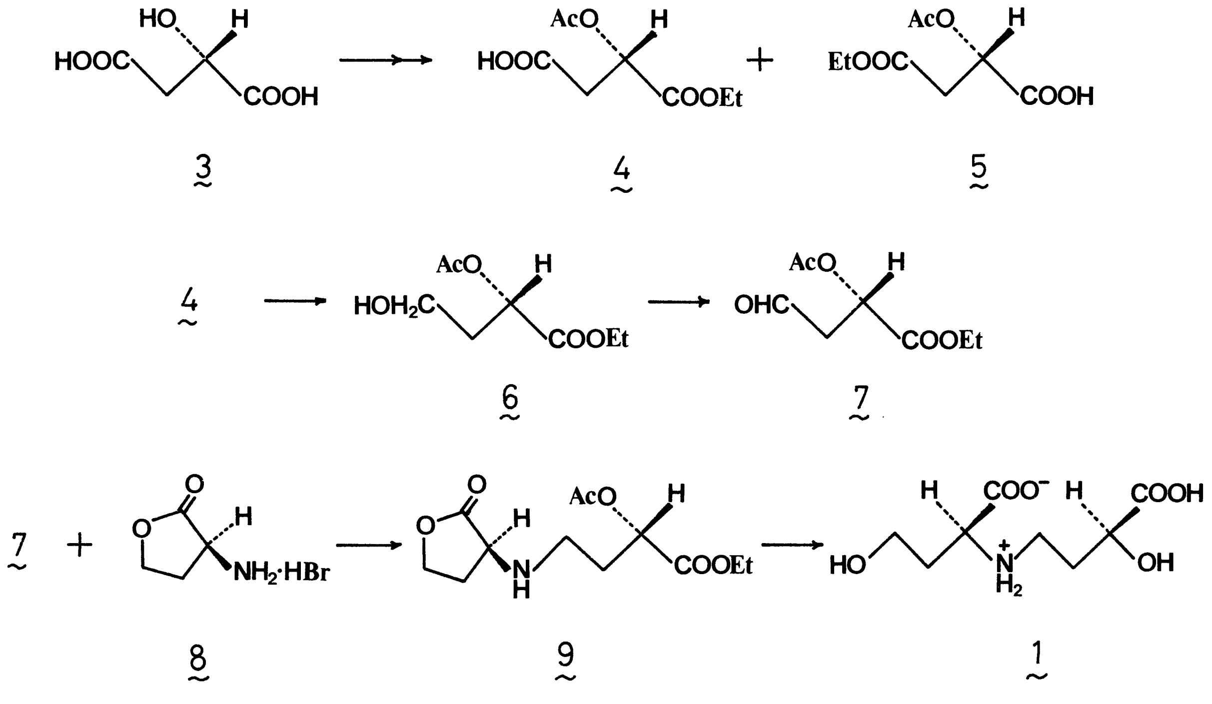
Table. Pmr and cmr spectra of avenic acid A and B$^{4)}$

avenic acid A (2)			avenic acid B (1)		
	pmr	cmr		pmr	cmr
$C_{(1)}$		183.1(s)	$C_{(1)}$		181.9(s)^{*e}
$C_{(2)}H$	2.95(t,J=7.6)^{*a}	63.3(d)^{*b}	$C_{(2)}H$	3.12(t,J=7.0)	61.1(d)
$C_{(3)}H_2$	1.53-1.81(m)	33.9(t)^{*d}	$C_{(3)}H_2$	1.65-1.97(m)	33.8(t)^{*f}
$C_{(4)}H_2$	3.52(t,J=7.6)	60.2(t)	$C_{(4)}H_2$	3.62(t,J=7.6)	58.9(t)
$C_{(1')}H_2$	2.24-2.68(m)	45.4(t)^{*c}			
$C_{(2')}H_2$	1.53-1.81(m)	35.0(t)^{*d}			
$C_{(3')}H$	3.02(t,J=7.6)^{*a}	62.2(d)^{*b}			
$C_{(4')}$		183.1(s)			
$C_{(1'')}H_2$	2.24-2.68(m)	45.1(t)^{*c}	$C_{(1')}H_2$	2.34-2.76(m)	43.9(t)
$C_{(2'')}H_2$	1.53-1.81(m)	36.3(t)^{*d}	$C_{(2')}H_2$	1.65-1.97(m)	35.3(t)^{*f}
$C_{(3'')}H$	3.92(dd,J=5.4,8.1)	72.1(d)	$C_{(3')}H$	4.05(dd,J=5.4,8.1)	70.9(d)
$C_{(4'')}$		182.3(s)	$C_{(4')}$		181.1(s)^{*e}

*a-f) These assignments may be interchanged.

Synthesis of **1** was achieved through reductive coupling of L-malic half-aldehyde derivative **7** to L-α-amino-γ-butyrolactone hydrobromide **8** by using of $NaBH_3CN$, a selective reducing agent, followed by hydrolysis.[6] The requisite aldehyde **7** was synthesized from L-malic acid **3** via alcohol **6** which was obtained by diborane reduction of the half-ester **4**.[7] Oxidation of the alcohol **6** with pyridinium chlorochromate gave an aldehyde **7** in 35 % yield ($[\alpha]_D$ -31.7° (c=0.5, $CHCl_3$); pmr, 9.78 (1H, t, J=1, $O\underline{H}C-CH_2-$)) along with a dimeric ester and an α,β-unsaturated aldehyde. Reaction of the aldehyde **7** and L-homoserine lactone hydrobromide **8** with $NaBH_3CN$ under controlled pH (6-7) afforded lactone ester **9** in 60 % yield: $[\alpha]_D$ -30.5° (c=0.4, $CHCl_3$); ms, m/e 273.1249 (calc'd for $C_{12}H_{19}NO_6$, 273.1285); ir ($CHCl_3$) 1773, 1737, 1225 cm^{-1}; pmr ($CDCl_3$) δ 1.27 (3H, t, J=7, $-CH_2C\underline{H}_3$), 2.14 (3H, s, $-COC\underline{H}_3$), 1.9-2.7 (4H, m, $C_{(3)}\underline{H}_2$, $C_{(2')}\underline{H}_2$), 2.84 (2H, m, $C_{(1')}\underline{H}_2$), 3.53 (1H, dd, J=8, 10, $C_{(2)}\underline{H}$), 4.18 (2H, q, J=7, $-OC\underline{H}_2CH_3$), 4.35 (2H, m, $C_{(4)}\underline{H}_2$), 5.10 (1H, t, J=6, $C_{(3')}\underline{H}$). Treatment of **9** with 1 % methanolic potassium hydroxide at room temperature followed by chromatographic separation on Dowex 50W afforded the compound **1** as a non-crystalline material in quantitative yield. The synthetic specimen of **1** was shown to be identical with natural avenic acid B in all respects including the Rf value of paper chromatography, Rt on HPLC and the pmr spectrum. Further, the cd curve of the synthetic **1** ($[\theta]_{198}$ +13880) is compatible with that of natural avenic acid B.



From the spectral data shown above as well as the chemical synthesis of the optically active compound, it is concluded that avenic acid B is 2(S),3'(S)-N-(3-hydroxy-3-carboxypropyl)-homoserine (**1**). Isolation and characterization of the other congeners of the amino acid derivative and the biosynthetic relationship between these substances as well as the biological function of these substances to iron chlorosis are under investigation.

References and Notes

1. S.Takagi, Soil Sci. Plant Nutr., **22**, 423 (1976).
2. T.Takemoto, K.Nomoto, S.Fushiya, R.Ouchi, G.Kusano, H.Hikino, S.Takagi, Y.Matsuura, and M.Kakudo, Proc. Japan Acad., **54** Ser B 468 (1978).
3. S.Fushiya, Y.Sato, S.Nozoe, K.Nomoto, T.Takemoto, and S.Takagi, Tetrahedron Lett., **21**, 3071 (1980).
4. These spectra were measured in 1N NaOD because of the sparing solubility in water.
5. The calculated cd curve obtained by the addition of the cd curve of L-homoserine to that of L-malic acid shows a positive Cotton effect ($[\theta]_{202}$ +17500).
6. Satisfactory spectral data (pmr, ms and ir) were obtained for all intermediates.
7. D.H.Horn and Y.Y.Pretorius, J. Chem. Soc., 1462 (1954).

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