NICOTIANAMINE AND ANALOGOUS AMINO ACIDS, ENDOGENOUS IRON CARRIERS IN HIGHER PLANTS

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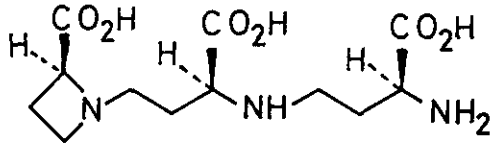
Abstract - The occurrence, structural elucidation and synthesis of nicotianamine (1) and of the analogous amino acids mugineic acid (4), 2'-deoxymugineic acid (5), 3-hydroxymugineic acid (6) as well as of avenic acid A (7) and B (8) have been reviewed. These compounds possess chelating properties for iron and other metal ions and are considered to be specific phytosiderophores of general importance for the iron (metal) transport and/or metabolism in higher plants.

1. INTRODUCTION

A series of papers, starting in 1960, deal with the "normalizing factor" for the tomato mutant 'chloronerva', which was recently shown to be identical[1] with the unusual, non-protein amino acid nicotianamine (1)[2,3] isolated some years ago from tobacco leaves[2] and beechnuts[3] and detected in other plants as well.[4] The semi-lethal mutant 'chloronerva' exhibits a severe growth and developmental inhibition, shows a chlorophyll defect in the intercostal areas of young leaves and possesses a disturbed iron metabolism, leading to an excessive iron absorption by the roots on the one hand and an irregular iron distribution within the young leaves on the other.[5,6] About 1 µg of nicotianamine (1) per mutant plant yielded a positive response after application to the leaves, while (2S:3'S)-N-(3-amino-3-carboxypropyl)-azetidine-2-carboxylic acid (2)[3] or S-azetidine-2-carboxylic acid (3) proved to be inactive. According to our present knowledge, nicotianamine (1) is an essential constituent of higher plants and is considered to be a possible specific phytosiderophore of general importance for the cellular iron transport and/or metabolism.[1]

+Dedicated with personal regards to Prof. Kyosuke Tsuda on the occasion of his 75th birthday.



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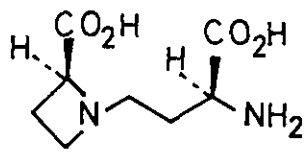

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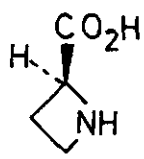

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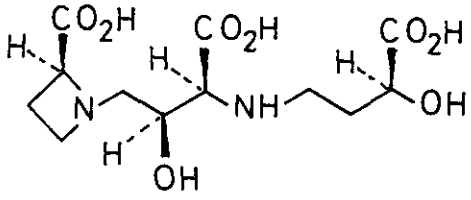

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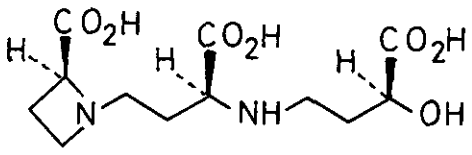

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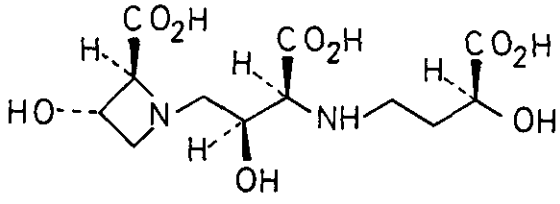

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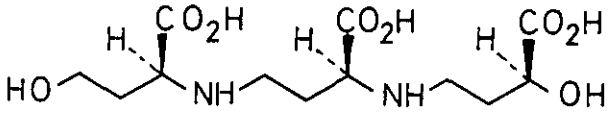

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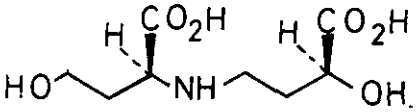

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Independently of these investigations, Japanese scientists were able to detect[7] and to isolate several amino acids possessing chelating properties for iron and other metals from root washings of gramineous plants grown under iron-deficient conditions.[8-13] According to the structural elucidation and synthetic work, these amino acids named mugineic acid (4)[8,9], 2'-deoxymugineic acid (5)[9,13],

3-hydroxymugineic acid (6)[9] as well as avenic acid A (7)[10,11] and B (8)[10,12] are closely related to nicotianamine (1) and the analogous amino acid 2. Most probably, the acids 4 - 8, at least in the investigated plants, are responsible for the iron uptake by the roots and seem to be metabolites of 1 (or one of its precursors) considered to be essential for the cellular iron transport in higher plants.

2. OCCURRENCE IN PLANTS

According to the results obtained by isolation in a preparative scale[1-3,14,15] or by screening experiments using an automatic amino acid analyzer[4,16] nicotianamine (1) has been detected in plants compiled in Table 1. As shown there, the occurrence of nicotianamine is restricted to cormophytes, that means to vascular plants. Up to now, the tomato mutant '*chloronerva*' is the only higher plant known where nicotianamine is absent, most likely due to a metabolic block in the biosynthesis of this amino acid.[17] The distribution of nicotianamine in different organs shows some differences. Generally, its highest content is in young growing leave tissue, its minimal concentration within seeds.[4,16,17] According to recent investigations, also cell cultures of *Lycopersicon esculentum* are able to synthesize nicotianamine.[18]

The amino acid 2 has been isolated from seeds of *Fagus silvatica* (beechnuts)[3], while S-azetidinecarboxylic acid (3) was shown to occur in species of Liliaceae and Agavaceae[19] as well as in *Delonix regia*[20], *Beta vulgaris*[21] and *Nicotiana tabacum*.[22]

The other metal chelating amino acids analogous to nicotianamine (1) have been isolated from the root washings of gramineous plants grown under iron-deficient conditions[7], thus mugineic acid (4) from barley (*Hordeum vulgare*)[8,9], 2-deoxymugineic acid from oat (*Avena sativa*)[9] and wheat (*Triticum aestivum*)[13], 3-hydroxymugineic acid (6) from rye (*Secale cereale*)[9] and avenic acid A (7) and B (8) from oat (*Avena sativa*).[10,11,12]

Table 1. Occurrence of nicotianamine (1) in plants

Family	Species	Organ	/umol/g dry wt[a]	References
CORMOPHYTA				
Solanaceae	Datura metel	leaves	1,2	4
	Lycium chinense	leaves	6,9	4
	Lycopersicon esculentum	leaves	0,63	16
	L. esculentum	leaves	0,5	4
	L. esculentum	fruits	0,14	16
	L. esculentum	seeds	traces	16
	L. esculentum mut. chloronerva	leaves	0,0	16
	L. esculentum mut. xantha$_5$	chlorotic leaves	(+)	16
	L. peruvianum	leaves	(+)	16
	L. hirsutum	leaves	(+)	16
	Nicotiana tabacum	upper leaves	2,1	4
	N. tabacum	middle leaves	1,6	4
	N. tabacum	lower leaves	traces	4
	N. tabacum	roots	2,6	4
	N. tabacum Xanthi	leaves	1,9	4
	N. glutinosa	leaves	0,7	4
	N. rustica	leaves	1,9	4
	N. arentsii	leaves	1,2	4
	N. alata	leaves	0,3	4
	N. debneyi	leaves	1,3	4
	Solanum melongena	leaves	0,5	4
	S. tuberosum	peeled tubers	0,10	16
Fabaceae	Medicago sativa	shoots	0,40	16
	M. sativa	seeds	0,04	16
	Melilotus officinalis	shoots	0,24	16
	Pisum sativum	shoots	0,12	16
	Trifolium incarnatum	shoots	0,16	16
	Trigonella coerulea	shoots	0,22	16

Table 1, contin.

Family	Species	Organ	/umol/g dry wt[a]	References
Brassicaceae	Arabidopsis thaliana	shoots	0,43	16
	Cardaria draba	leaves	0,01	14
Malvaceae	Malva verticillata	leaves	(+)	16
Chenopodiaceae	Beta vulgaris	leaves	0,08	1
Ulmaceae	Ulmus spec.	seeds	0,15	15
Fagaceae	Fagus silvatica	seeds	5,00	3
Convolvulaceae	Cuscuta europaea	shoots[b]	(+)	16
Liliaceae	Rohdea japonica	leaves	traces	4
Poaceae	Hordeum vulgare	etiolated leaves	(+)	16
	Zea mays	leaves	0,2	4
Aspidiaceae	Dryopteris filix-mas	leaves	0,05	16
THALLOPHYTA				
Polytrichaceae	Polytrichum commune	gametophyte	0,0	16
Sphagnaceae	Sphagnum spec.	gametophyte	0,0	16
Scenedesmaceae	Scenedesmus quadricauda		0,0	16
Tricholomataceae	Calocybe georgii		0,0	16
Saccharomycetaceae	Saccharomyces cerevisiae		0,0	16

[a] In the case of ref. 4 calculated from /umol per g fresh weight under the assumption of a water content of 90 %. (+) indicates a positive response in the bioassay using the tomato mutant 'chloronerva'.

[b] Sponging upon Euphorbia cyparissias.

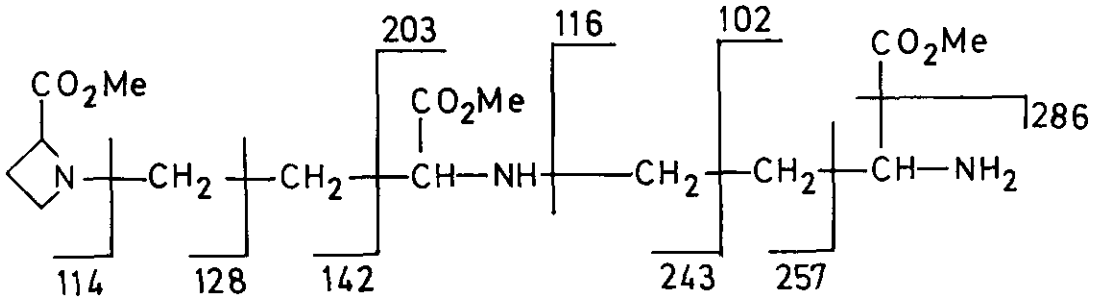
3. STRUCTURE ELUCIDATION AND SYNTHESIS

3.1. NICOTIANAMINE (1)

This amino acid with $[\alpha]_D$ -49.7° (water) decomposes above 250 °C.[1,23] A structure proposal was published by Noma et al.[2] in 1971 and was later slightly corrected to 1 by Kristensen and Larsen.[3] Structure 1 is in agreement with the results of Budesinsky et al.[1,23] and was derived from the following observations.

Elemental analysis gave the composition $C_{12}H_{21}N_3O_6 \cdot H_2O$.[2] When mass spectroscopy was used for the assessment of the elemental formula, a quasi-molecular ion $[M + H]^+$ could be obtained by the field desorption technique. With electron impact only $[M - H_2O]^+$ could be registered.[23] In the mass spectra of the methyl and the ethyl ester, parent peaks were observed at m/z 345 ($C_{15}H_{27}N_3O_6$ by high resolution) and 387, indicating three carboxyl groups and corroborating the molecular formula for nicotianamine.[2] This formula was later confirmed by Budešinský et al.[23] by mass spectroscopic investigation of the tetra-(trimethylsilyl)derivative. The proton noise decoupled ^{13}C nmr spectrum proved the presence of 12 C-atoms. The chemical shifts and the off-resonance proton decoupled spectrum specified the nature of the carbons as 6 CH_2, 3 aliphatic CH and 3 C=O groups. Since no further sp^2-hybridized carbon could be detected, the compound contains one cycle.[3,23] ^1H nmr investigations including proton homonuclear selective decoupling experiments led to the conclusion that the molecule contains three sequences of the type $N\text{-}CH_2\text{-}CH_2\text{-}CH(CO_2H)\text{-}N$ and that one of them forms an azetidine ring.[2,3,23] $KMnO_4$ oxidation of 1 yielded, in addition to aspartic acid and ß-alanine, azetidine-2-carboxylic acid, proving the existence of an azetidine ring.[2] The ms fragmentation of the methyl ester[2,3], the tetra-(trimethylsilyl)[23] and the bis-(4-bromobenzoyl)derivative[23] as indicated in the structures 9, 10 and 11 were very useful in the structure elucidation. The mass spectroscopic behaviour of nicotianamine itself has also been discussed.[23]

Heating of an aqueous solution of S-azetidine-2-carboxylic acid (3) with half an equivalent of NaOH to 100 °C for 24 h gave natural (-)-nicotianamine in 4 % yield. This synthesis established the configurations at all chiral C-atoms as S. Thus (-)-nicotianamine has the structure (2S:3'S:3"S)-N-[3-(3-amino-3-carboxypropylamino)-3-carboxypropyl]-azetidine-2-carboxylic acid (1).[3] Starting from R-azetidine-2-carboxylic acid (+)-nicotianamine ($[\alpha]_D$ +43,8° in water) has been obtained by Ripperger[24] in an analogous way in 2 % yield for biochemical experiments. An efficient synthesis of (-)-nicotianamine was published by Japanese authors.[10] S-2-Methoxycarbonylazetidine (12) was coupled with protected S-aspartic-ß-semialdehyde (13) to 14 by means of sodium cyanoborohydride. After elimination of the benzyloxycarbonyl residue, 15 reacted with the aldehyde 16



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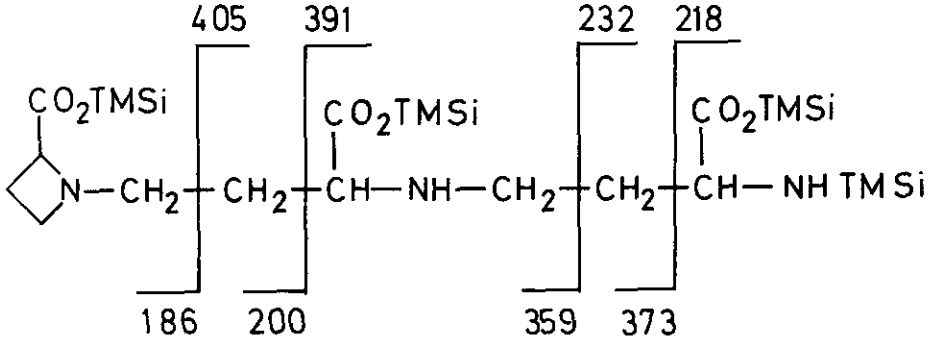

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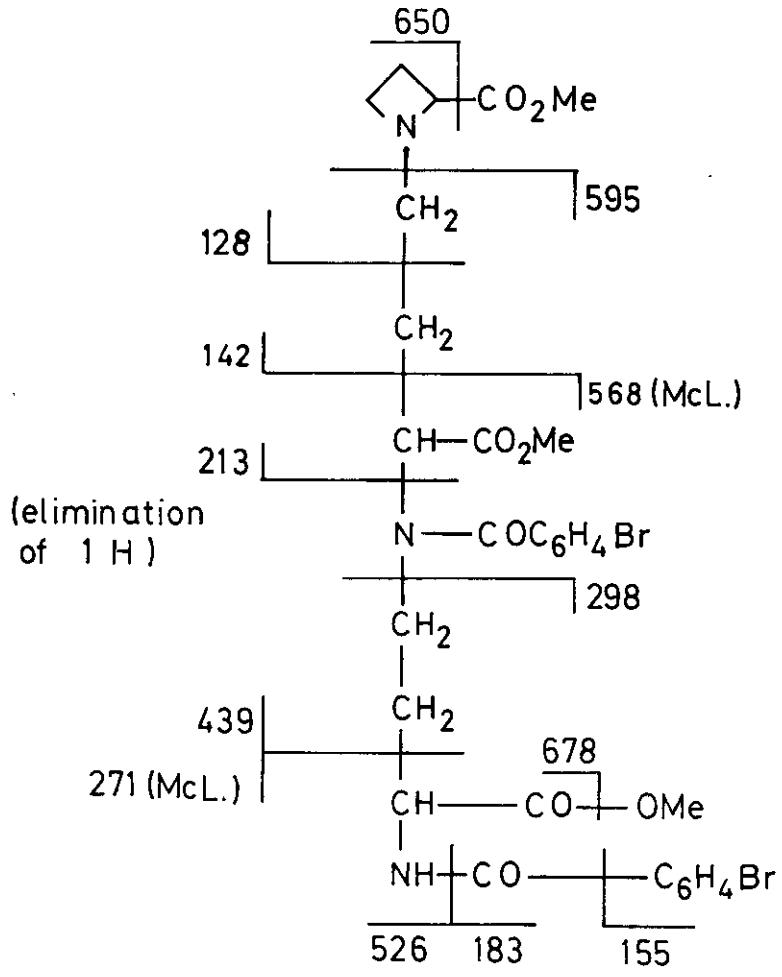
to give the nicotianamine derivative 17, which yielded nicotianamine (1) after catalytic hydrogenation, followed by treatment with CF_3CO_2H and finally with KOH.

(2S:3'S)-N-(3-Amino-3-carboxypropyl)-azetidine-2-carboxylic acid (2) with $[\alpha]_D$ -83° (water), structurally related to nicotianamine, has been isolated[3] and synthesized, either by dimerization of S-azetidine-2-carboxylic acid (3)[3] or by condensation of 12 with a protected S-aspartic-β-semialdehyde[10]; the antipode of 2 ($[\alpha]_D$ + 76.4° in water) was obtained by dimerization of R-azetidine-2-carboxylic acid.[24]



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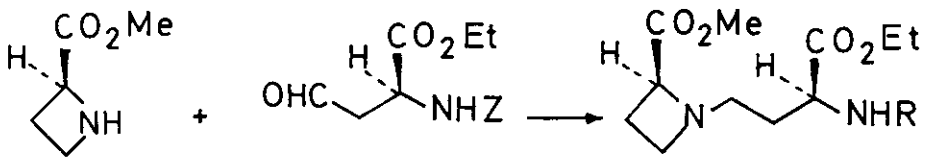
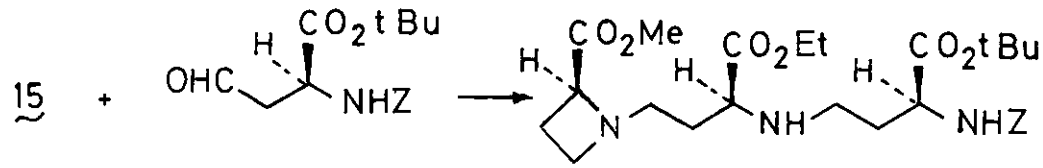
(McL. indicates McLafferty rearrangement)



12 13 14 : R = Z

15 : R = H·HCl



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As is evident from a Dreiding model, nicotianamine (1) has an optimal molecular structure for complex formation with iron (Fig. 1). Not only are six functional groups present, necessary for octahedral coordination, but the distances between the groups are optimal for the formation of three 5-membered and two 6-membered chelate rings.[1] The complex formation between 1 and Fe^{3+} ions has been proved by a positive Cotton effect at ca 250 nm (Fig. 2, see ref.[25]).

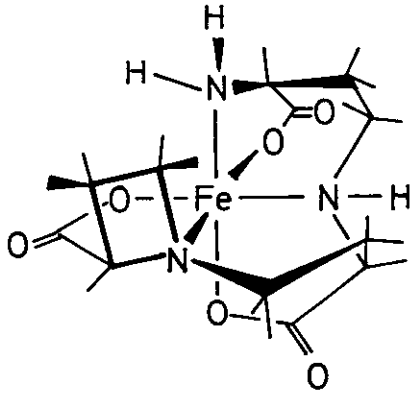


Fig. 1. Dreiding model of the iron nicotianamine complex

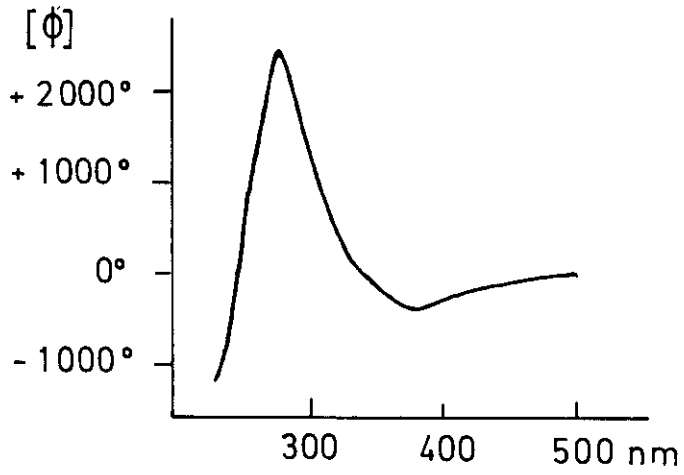


Fig. 2. Ord of the iron(III) nicotianamine complex (water, pH 2)

3.2. MUGINEIC ACID (4)

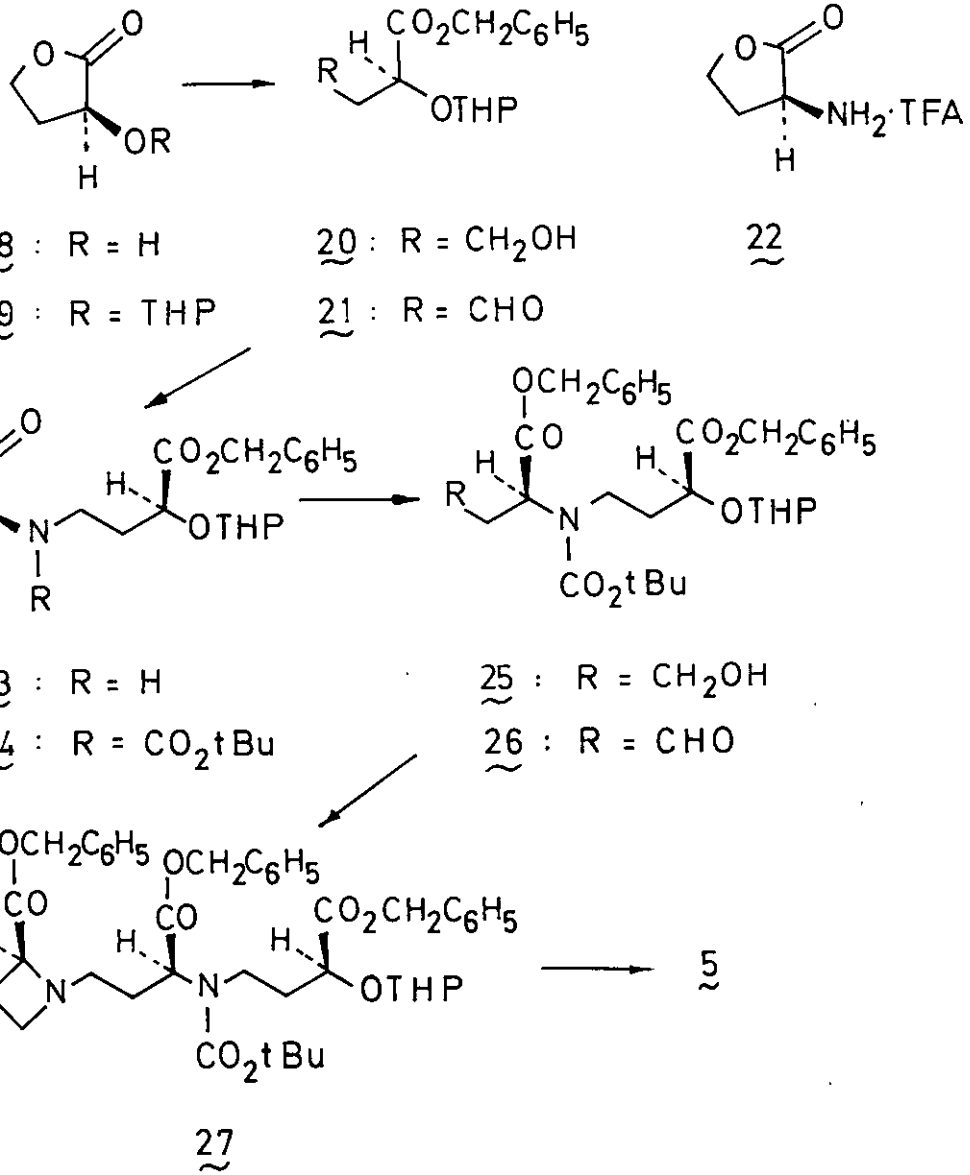
This compound is characterized by mp 210 - 212 °C (decomp.) and $[\alpha]_D$ -70.7° (water) and has the composition $C_{12}H_{20}N_2O_8$ according to elemental analysis. The field desorption ms revealed an $[M + H]^+$ ion. The ^1H nmr data indicated the presence of two CH_2CH_2CH and one CH_2CHCH moieties. The ^{13}C nmr spectrum demonstrated 5 CH_2, 4 aliphatic CH and 3 C=O groups. X-ray analysis established the structure to be 4.[8] The absolute configuration was determined by $KMnO_4$ oxidation, which afforded S-azetidine-2-carboxylic acid. Thus mugineic acid has the structure (2S:2'S:3'S:3"S)-N-[3-carboxy-3-(3-carboxy-3-hydroxypropyl-amino)-2-hydroxypropyl]-azetidine-2-carboxylic acid.[9]

The iron-solubilizing action of mugineic acid (4) is strongly inhibited by the presence of divalent metals (order of inhibition: Cu > Co ≫ Zn > Mn).[7,26] Thus, the amino acid 4 may preferentially chelate with divalent metal ions, forming a more stable complex than with Fe^{3+}.[26] According to an X-ray structural analysis, the copper(II) complex of 4 shows a quite analogous structure[26] as assumed for the iron(III) complex of nicotianamine (1) (Fig. 1).[1]

The isolation of an isomer of 4 (isomugineic acid) was reported, but no structure given.[9]

3.3. 2'-DEOXYMUGINEIC ACID (5)

This amino acid possesses mp 196 - 199 °C and $[\alpha]_D$ -66.6° (water).[13] The structure was elucidated by comparison of the spectroscopic properties (^1H and ^{13}C nmr) with those of mugineic acid (4) and nicotianamine (1)[8] and was finally established to be (2S:3'S:3"S)-N-(3-carboxy-3-(3-carboxy-3-hydroxypropylamino)-propyl)-azetidine-2-carboxylic acid (5) by total synthesis.[13]



S-α-Hydroxy-γ-butyrolactone (18) was converted into a diastereomeric mixture of tetrahydropyranyl derivatives 19, which was separated. Both isomers could be used for the following reaction sequence. Treatment with KOH followed by benzylation afforded the ester 20. Oxidation with pyridinium chlorochromate yielded the protected S-malic-β-semialdehyde 21. Coupling with S-homoserine lactone (22) by reaction with sodium cyanoborohydride afforded the lactone 23. Protection of 23 using di-tert-butyl dicarbonate gave the tert-butoxycarbonyl derivative 24. Analogous to 19, 24 was transformed into the aldehyde 26 via 25. 26 was reductively aminated with S-2-benzyloxycarbonylazetidine to give the 2'-deoxymugineic acid derivative 27. Catalytic hydrogenation followed by treatment with CF_3CO_2H gave 2'-deoxymugineic acid (5).

3.4. 3-HYDROXYMUGINEIC ACID (6)

6 is characterized by mp 205 - 213 °C (decomp.) and $[\alpha]_D$ -52.9° (water). The elemental composition was established to be $C_{12}H_{20}N_2O_9$. In the field desorption ms the $[M + H]^+$ ion could be detected. Especially on the basis of ^1H and ^{13}C nmr results the structure (2S:3S:2'S:3'S:3"S)-N-[3-carboxy-3-(3-carboxy-3-hydroxypropylamino)-2-hydroxypropyl]-3-hydroxyazetidine-2-carboxylic acid (6) was proposed.[9]

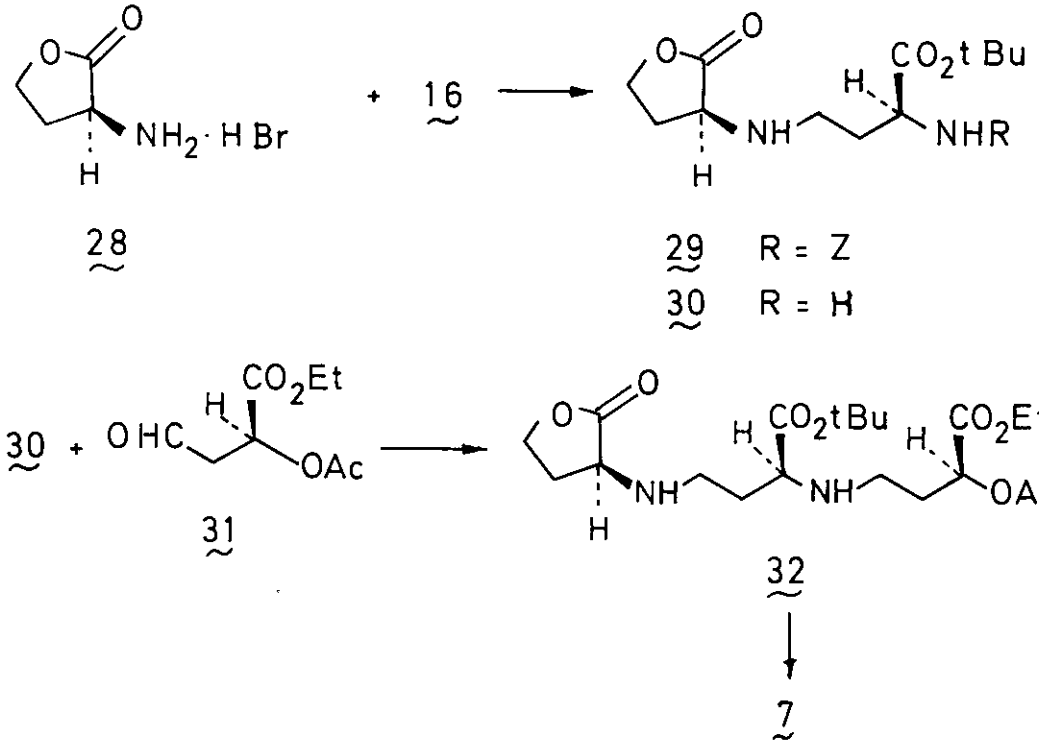
3.5. AVENIC ACID A (7)

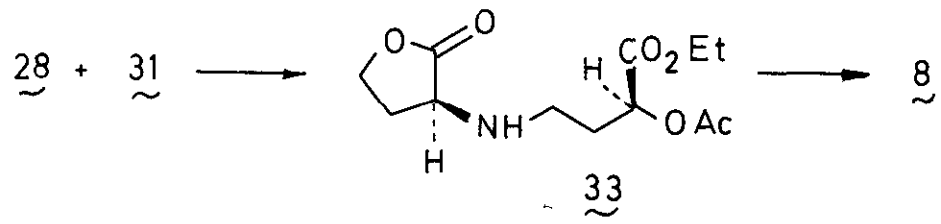
Avenic acid A possesses mp> 300 °C, $[\alpha]_D$ +16.4° (2N HCl) and, according to elemental analysis, a molecular formula $C_{12}H_{22}N_2O_8$. Its field desorption ms showed an $[M + Na]^+$ ion. The ^{13}C nmr spectrum displayed signals due to 6 CH_2, 3 aliphatic CH and 3 CO_2H groups. The ^1H nmr spectrum revealed the presence of 2 CH_2N and 2 CHN groups, a CH_2OH, a CHOH and 3 C-CH_2-C groups. Double resonance experiments evidenced 3 X-CH_2CH_2CH (CO_2H)Y systems (X, Y = N or O). The compound contains no unsaturation. $KMnO_4$ oxidation yielded aspartic acid, homoserine and malic acid. Thus avenic acid A has most probably the structure (2S:3'S:3"S)-N-[3-carboxy-3-(3-carboxy-3-hydroxypropylamino)-propyl]-homoserine (7), which was confirmed by synthesis (see below). The cd curves of aspartic acid, homoserine and malic acid obtained by oxidation exhibit positive Cotton effects indicating S-configurations of the chiral atoms.[11]

An efficient synthesis started from S-homoserine lactone hydrobromide (28), which was coupled with the protected S-aspartic-β-semialdehyde 16 by means of sodium cyanoborohydride to give 29. After elimination of the benzyloxycarbonyl residue, 30 reacted with the S-malic-β-semialdehyde derivative 31. The reaction product 32 was treated with TFA, followed by KOH, to yield avenic acid A (7).[10]

3.6. AVENIC ACID B (8)

This amorphous compound shows an $[M + H]^+$ ion in the field desorption ms and has the molecular formula $C_8H_{15}NO_6$. By comparison of the ^{13}C and ^1H nmr signals with those of avenic acid A the structure (2S:3'S)-N-(3-carboxy-3-hydroxypropyl)-homoserine (8) was proposed, which was confirmed by synthesis. The cd at 198 nm ($\Delta\varepsilon$ +4.42) corresponds to the calculated value ($\Delta\varepsilon$ +5,30) obtained by the addition of the Cotton effect of S-homoserine to that of S-malic acid, indicating that both chiral atoms possess S-configuration. The synthesis was achieved by reductive coupling of S-homoserine lactone (28) with the S-malic-β-semialdehyde derivative 31, followed by hydrolysis of the product 33 to yield avenic acid B (8).[12]





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