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STRUCTURE–FUNCTION RELATIONSHIPS OF NICOTIANAMINE ANALOGUES*

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Key Word Index—Nicotianamine; nicotianamine analogues; *Lycopersicon esculentum*; Solanaceae; phenotypical normalization of the tomato mutant *chloronerva*; structure–function relationships.

Abstract—The ability of nicotianamine analogues to bring about the phenotypical normalization of the nicotianamine-auxotroph tomato mutant *chloronerva* is correlated with the co-ordination behaviour of the analogues towards transition metal ions. This is in agreement with the proposed metal ion carrier function of nicotianamine.

INTRODUCTION

Nicotianamine (**1**) has been identified as the 'normalizing factor' which restores growth, development and chlorophyll synthesis of the nicotianamine-less mutant *chloronerva* of *Lycopersicon esculentum* Mill. cv. 'Bonner Beste' [2]. It is found in almost all multicellular plants [3, 4]. Biochemical experiments revealed a disturbed heavy metal ion metabolism in the mutant, the effects of which include excessive iron uptake by the plant and irregular iron distribution within young leaves [5–7]. Nicotianamine (**1**) forms stable 1:1 complexes with iron(II) and other divalent transition metal ions [8]. These complexes have high stability constants in comparison with those of bidentate amino acids. The stability constants of the 1:1 iron(II) complexes of nicotianamine and glycine are $10^{12.1}$ [8] and $10^{4.3}$ [9], respectively. As is evident from a Dreiding model, nicotianamine has an optimal three-dimensional molecular structure for complex formation with metal ions. Not only are six functional groups present, necessary for octahedral co-ordination, but the distances between the groups are also optimal for the formation of chelate rings. Three five-membered rings are formed by the α-amino acid residues and two six-membered rings by the 1,3-diaminopropane moieties [2, 4]. Starting from the hypothesis that strong co-ordination with heavy metal ions is a prerequisite to biological activity, a series of nicotianamine analogues were synthesized and tested.

RESULTS AND DISCUSSION

Root elongation of young mutant seedlings was taken as the quantitative parameter of biological acitivity. In addition, the greening of chlorotic intercostal areas of young mutant leaves was recorded, the results are summarized in Table 1. None of the analogues exhibited a higher activity than nicotianamine (**1**). Only hexadentate ligands of type **2** (**4–8**) with the exception of the pipecolic acid analogue **9** led to a significant increase of root growth and to greening of chlorotic leaf areas. The inactivity of **9** may be due to its lack of uptake through the leaf surface, since a white precipitate was discerned on the leaves at the end of the experiment, at least at the higher concentration tested. The related glycine analogue **3** was extremely insoluble and had to be tested at the unphysiologically high pHs of 8.4–9.3, dependent on its concentration. A similar precipitation was observed with **3**. Surprisingly, the stereochemistry of the ligands did not seem to be of crucial importance (cf. **6** and **7**), thus excluding a possible nicotianamine function via stereospecific binding to macromolecular surfaces such as membranes or to a receptor protein. It is more likely that nicotianamine performs its function(s) within the plant tissue as a chelating agent, interacting with transition metal ions. An effect of the hexadentate amino acid **10** on root growth was only observed at the highest concentration tested, and greening of chlorotic leaf areas occurred only occasionally. Although the iron(III) complex of **15** had a weak positive effect on chlorophyll pattern it cannot be regarded as active [5]. **15** alone proved to be inactive. Other nicotianamine analogues such as hexadentate homologues with 1,4-diaminobutane moieties (**11, 12**), quinquedentate (**13, 14**), quadridentate (**16–23**) and tridentate ligands (**24**) as well as peptides (**25–30**) and the phytosiderophore avenic acid A (**31** [10]) with a terminal hydroxy group instead of a primary amino group were biologically inactive in terms of the greening of chlorotic leaf areas. However, some of these compounds influenced root growth, but mostly only to a small extent. The inactivity of the hexadentate homologues **11** and **12** is in accordance with the observation that the chelating properties decrease from 1,3-diaminopropane to 1,4-diaminobutane [11]. The results of Table 1 together with some information on the stability constants of metal complexes of nicotianamine analogues [12] revealed that the biological activity is correlated with the co-ordination behaviour. The stability constants of the investigated 1:1 iron(II) complexes of active compounds (Table 1) are $10^{10.2}$ to $10^{12.8}$. The constants

*Part 28 in the series "The 'Normalizing Factor' for the Tomato Mutant *chloronerva*". For part 27 see ref. [1]

Table 1. Biological activity of nicotianamine analogues

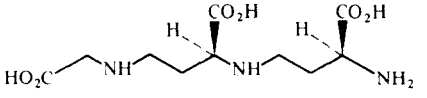
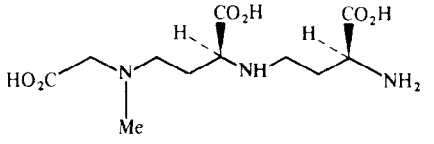
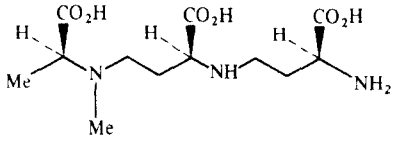
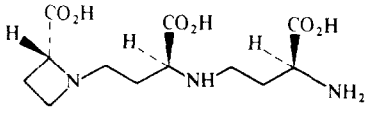
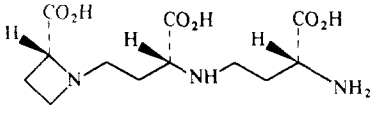
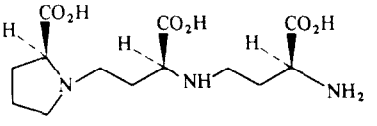
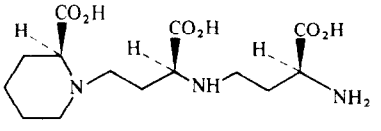
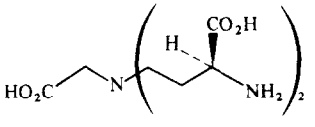
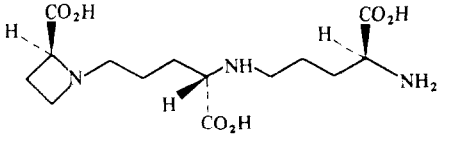
Compound		Root growth activity*† at concn of 0.1%	0.01%	0.001%	Greening of chlorotic leaves† at concn of 0.1%	0.01%	0.001%
3		—	—	—	—	—	—
4		0.79‡ ±0.31	0.66‡** ±0.29	NT	+	+	NT
5		0.54‡** ±0.29	0.14‖¶ ±0.20	NT	+	+	NT
6		1.00‡ ±0.31	1.04‡ ±0.29	—	+	+	+
7		0.70‡†† ±0.12	1.05§ ±0.42	0.78‡ ±0.42	+	+	+
8		0.73‡†† ±0.16	0.81‡ ±0.16	0.50‖** ±0.19	+	+	+
9		—	—	—	—	—	—
10		0.48‖†† ±0.25	—	—	+‡‡	+‡‡	+‡‡
11		—	—	—	—	—	—

Table 1. (*Continued*)

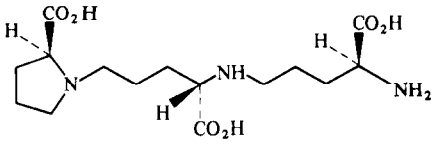
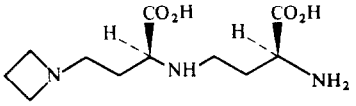
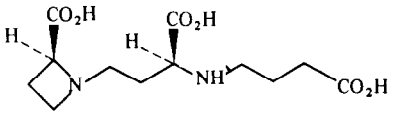
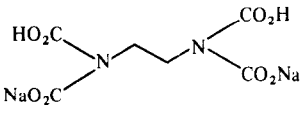
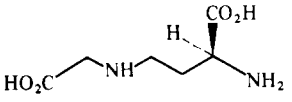
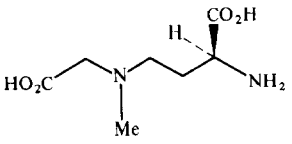
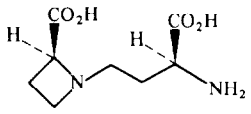
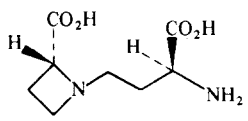
Compound	Root growth activity*† at concn of 0.1%	0.01%	0.001%	Greening of chlorotic leaves† at concn of 0.1%	0.01%	0.001%
12 	—	−0.14§¶ ±0.21	—	—	—	—
13 	—	0.60§ ±0.28	0.88‡ ±0.52	—	—	—
14 	—	—	—	—	—	—
15 	—	—	—	—	—	—
16 	—	—	—	—	—	—
17 	0.18‖¶ ±0.20	0.17‖¶ ±0.19	NT	—	—	NT
18 	NT	NT	NT	NT	—	NT
19 	0.48§†† ±0.18	—	—	—	—	—

Table 1. *(Continued)*

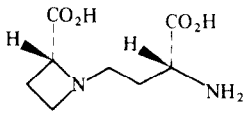
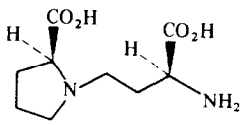
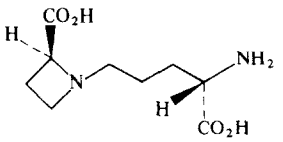
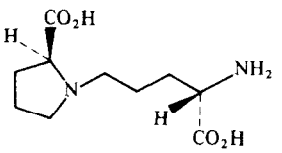
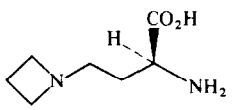
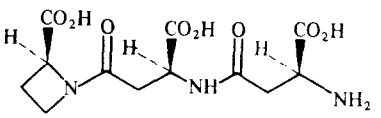
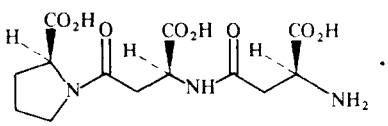
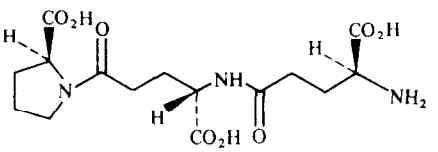
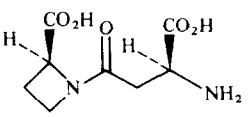
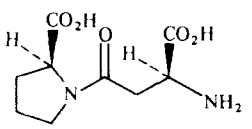
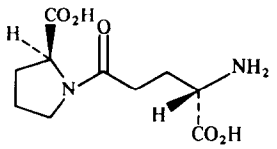
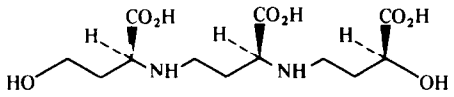
Compound	Root growth activity*† at concn of 0.1%	0.01%	0.001%	Greening of chlorotic leaves† at concn of 0.1%	0.01%	0.001%
20 	NT	NT	NT	NT	—	NT
21 	NT	—	NT	NT	—	NT
22 	—	—	—	—	—	—
23 	—	—	—	—	—	—
24 	NT	—	—	—	—	—
25 	—	—	—	—	—	—
26 	—	—	—	—	—	—
27 	—	—	0.13‖¶ ±0.11	—	—	—
28 	—	—	—	—	—	—
29 	—	—	—	—	—	—

Table 1. (*Continued*)

Compound	Root growth activity*† at concn of 0.1%	0.01%	0.001%	Greening of chlorotic leaves† at concn of 0.1%	0.01%	0.001%
30 	0.22§¶ ±0.11	0.22‖¶ ±0.15	0.20‡** ±0.08	–	–	–
31 	NT	NT	NT	NT	–	NT

$\frac{{}^{*}\bar{l}-\bar{l}_{H_2O}}{\bar{l}_{NA}-\bar{l}_{H_2O}}$ ± s.d. l root length after treatment with the analogue, l_{H_2O} after treatment with water, l_{NA} after treatment with nicotinamine at the same concn as the analogue.

†NT, not tested;—, not significantly different to water treatment.

‡Significantly different to water treatment at 0.1% level.

§At 1% level.

‖At 5% level.

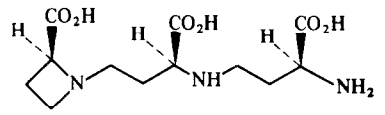
¶Significantly different to nicotianamine treatment (identical concn) at 0.1% level.

**At 1% level.

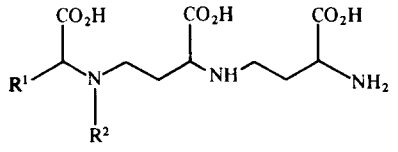
††At 5% level (*t*-test).

‡‡Poor reproducibility.

of inactive compounds are smaller. These observations are in agreement with our hypothesis that nicotianamine exerts its function(s) by complexing ferrous and other divalent transition element ions in plants [8].



1



2 R^1 = H or alkyl, R^2 = alkyl

EXPERIMENTAL

Nicotianamine (**1**) *and analogues* (**3–14, 16–30**). **1** was isolated according to ref. [2]. The following compounds were synthesized: **3**, **4**, **9**, **10**, **16** and **17** [Faust, J. and Schreiber, K., unpublished results], **5** [13], **6** and **19** [14], **7** and **20** [15], **8** and **21** [16], **11**, **12**, **22** and **23** [17], **13**, **14**, **18** and **24** [18], **25–30** [19].

Bioassay. Mutant seedlings were raised in a growth cabinet on Hoagland nutrient soln. After 12 days, test solns were supplied to the leaves with a smooth brush 5 times a day for 5 days. After 2 additional days main root length was measured and recovery from chlorosis was checked. A water and a nicotianamine control were run for comparison. The samples were dissolved at 0.1, 0.01 and 0.001% concn [7] in 0.05% Tween 20 (Atlas-Goldschmidt GmbH, Essen, FRG) as a wetting agent. Five seedlings were used per single concn, comprising 4.5 ml.

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