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OCCURRENCE OF NICOTIANAMINE IN HIGHER PLANTS

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Key Word Index—Solanaceae; Gramineae; Liliaceae; nicotianamine; distribution; azetidine-2-carboxylic acid.

Abstract—Nicotianamine is present in highest concentration in growing leaf tissue and has been found not only in the Solanaceae but also in the Liliaceae and Gramineae.

INTRODUCTION

In a previous paper [1], we reported the existence in tobacco leaves of a new amino acid, nicotianamine, which contains the azetidine-2-carboxylic acid (A2C) moiety. Recently, this acid was also found in the seeds of Fagus sylvatica [2].

Since the first finding of A2C in 1955 [3], distribution of the imino acid has been extensively examined. It has been detected in various species of Liliaceae and Agavaceae [4] and recently, 3 plants which belong to Leguminosae [5], Chenopodiaceae [6] and Solanaceae [7] were added to the list. Consequently, A2C is believed to be widespread in the plant kingdom.

This paper deals with the distribution of nicotianamine especially in species of Solanaceae and compares those of the Liliaceae.

RESULTS AND DISCUSSION

In tobacco plants, the content of free amino acids is generally higher in upper leaves than that in lower. However, some free amino acids, such as Gly, Ala and γ-amino butyric acid are known to be more highly concentrated in aged leaves [8]. To examine the variation in nicotianamine content of tobacco leaves according to their leaf age, the leaves from different stalk postitions were compared.

As shown in Table 1, the third leaf from the top of the plant contained 0.21 μmol/g fr. wt of nicotianamine which corresponds to about 1.2% of the total free amino acid except for amides ($AspNH_2$ and $GluNH_2$). Lower leaves, on the contrary, contained smaller amounts of the amino acid, the 12th leaf (over matured and yellow color) possessing negligible quantities of the amino acid. These results show that nicotianamine is present at a higher concentration in growing leaf tissue. The amino acid was also found in roots at a concentration similar to those of the third leaf. Accordingly, in the following experiments, younger or upper leaves of various plants were selected for the detection of nicotianamine.

As shown in Table 2, nicotianamine is found not only in the 5 species of Solanaceae examined but also in the more distantly related families of Gramineae and Liliaceae. The highest content so far found was in the leaves of *Lycium chinense*. They contained 0.69 μmol nicotianamine/g fr. wt which corresponds to 8.9% of the total free amino acid in the tissue except for the amides. This is the next major amino acid component following Glu and Pro, both the major amino acids in the leaf. Nicotianamine was also found in two other distantly related plants. In *Zea mays*, 0.02 μmol/g fr. wt was detected, the value is appreciable because the amino acid was identified not only by an amino acid analyzer but also by PC after partial purification of the amino acid. In *Rohdea japonica*, nicotianamine was detected by an amino acid analyzer, but the amount was too small for PC. From these results, together with the recent isolation of the amino acid from the seeds of *F. sylvatica*, it appears that nicotianamine is very widely distributed in the plant kingdom.

Table 1. Variation of nicotianamine content of *Nicotiana* leaves with stalk position

Stalk position*	Wt (g)	μmol/g fr. wt
3	0.72	0.21
6	2.0	0.16
9	4.5	0.04
12	4.3	trace
roots	8.7	0.26

* The stalk position was counted from top to bottom. The maximum leaf and bottom leaf were the 10th and the 15th stalk position, respectively.

Table 2. Distribution of nicotianamine in the leaves of various plants

Family		μmol/g fr. wt
Solanaceae	*Datura metel*	0.12
	Lycium chinense	0.69
	Solanum melongena	0.05
	Lycopersicon esculentum	0.05
	Nicotiana tabacum B.Y.	0.05
	N. tabacum Xanthi	0.19
	N. glutinosa	0.07
	N. rustica	0.19
	N. arentsii	0.12
	N. alata	0.03
	N. debneyi	0.13
Gramineae	*Zea mays*	0.02
Liliaceae	*Rohdea japonica*	tr

Table 3. Effect of pH and Na-concentration of the retention volumes of some amino acids eluted closely to nicotianamine

Na–citrate buffer Na-concentration (N)	pH	Retention volume (ml) Serine	Nicotianamine	Amides	Proline
0.10	3.32	100.8	135.0	106.7	133
0.12	3.36	87.1	103.3	91.7	114.2
0.15	3.27	84.6	94.2	90.0	110.8
0.20	3.20	70.0	70.8	74.2	90.8

A concept was suggested by Fowden that non protein amino acids found in relatively few species may well be synthesized in more types of plants, or even by all plants, but only in minute amounts [6]. Until very recently, A2C was only known in species of Liliaceae and Agavaceae [4], but it is now known to occur in distantly related plants such as *Delonix regia* [5], *Beta vulgaris* [6] and *N. tabacum* [7]. The wide distribution of nicotianamine, as well as of A2C, may also support Fowden's concept.

Nicotianamine can be prepared by polymerization of A2C under mild conditions [2], and we have also confirmed this by the same method. Therefore, there is a possibility that nicotianamine is synthesized from A2C by the same manner *in vivo*. If this is the case, there remains the problem of whether the reaction occurs enzymatically or nonenzymatically. If nicotianamine is synthesized from A2C nonenzymetically, plants which contain much A2C would be supposed to have much nicotianamine. In *N. tabacum*, nicotianamine accounts for *ca* 0.2 μmol/g fr. wt, whereas in *R. japonica* it occurs only in trace amounts. In the former, however, A2C is found in trace amounts detectable only by RI dilution [7], whereas in the latter it is found in appreciable amounts [5]. The co-existence of A2C and nicotianamine in such a manner suggests that nicotianamine may be formed from A2C enzymatically *in vivo*. However, experiments using isotopically labelled A2C will be needed to confirm this hypothesis.

A2C is known to be toxic to some organisms [9], while nicotianamine has shown no toxicity to any plants or microorganisms in our preliminary experiments. A2C is toxic to tobacco plants and a 100 ppm aqueous solution caused seeds to form radicles only after 4 days and killed the seedlings within 15 days. At 10 ppm, although the cotyledons grew like those of the control seedlings, the elongation of the radicles was completely inhibited. At 1 ppm, no effect was observed on the growth of the seedlings. The inhibitory activity of A2C on susceptible organisms is reported to be due to the lack of discriminating ability of prolyl-sRNA synthetase between A2C and Pro in contrast to the possession of this ability by the prolyl-sRNA synthetase of the plants which inherently contain A2C [10]. Therefore, the enzyme in tobacco plants probably cannot discriminate between Pro and A2C. These results suggest that nicotianamine may possibly be one of the detoxication (or activation) forms of A2C.

EXPERIMENTAL

Plants. Nicotiana tabacum (cv Bright Yellow) was grown by H_2O culture in a greenhouse. Leaves from different stalk positions and the roots were separately harvested at an early stage of flower breeding. *N. tabacum* cv. Xanthi, *N. glutinosa*, *N. arentsii*, *N. alata*, *N. debneyi*, *Solanum melongena* and *Lycopersicon esculentum* were grown in a greenhouse on soil. Autogenous plants *Datura metel*, *Lycium chinense* and *Rohdea japonica*, were used. Young leaves were harvested at 2:00 pm when their total free amino acid content is considered to be at maximum [11]. Harvested leaves or roots were homogenized with 20× their vol of 70% aq MeOH. After filtration, filtrates were dried *in vacuo*. Each dried residue was dissolved in an appropriate amount of 0.2 N Na–citrate buffer pH 2.20. The soln was centrifuged at 3000 rpm for 10 min and the clear supernatant was submitted to amino acid analysis.

Analysis of nicotianamine. Nicotianamine was estimated quantitatively using an amino acid analyzer. To obtain good resolution of nicotianamine from Ser, amides or Pro, the first buffer used for the usual physiological analysis was replaced by those shown in Table 3. By using 0.12 N Na-citrate buffer pH 3.36 instead of 0.2 N Na–citrate buffer pH 3.2 for the usual physiological analysis, homoserine and amides were completely separated from nicotianamine. The other analytical conditions were the same as the usual procedure for physiological analysis.

PC. To confirm the presence of nicotianamine in individual samples, 2D chromatography was also carried in (i) BuOH-HOAc–H_2O and (ii) PhOH-H_2O. Leaves (*ca* 5 g) were extracted with 400 ml of 70% aq MeOH and the extract evaporated to *ca* 30 ml *in vacuo*. Active carbon (1 g) was added to the soln and was stirred for 1 hr. After filtration, the clear soln was absorbed onto a column of 30 ml of Dowex 50WX4 ion exchange resin. The cationic fraction obtained by displacing with 2 N NH_4OH was submitted to amino acid analyzer analysis under the conditions described above without the ninhydrin reagent, and the eluates corresponding to nicotianamine were collected. The fraction was desalted and evaporated *in vacuo*. An aliquot of the concentrate was submitted to PC with authentic nicotianamine.

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