

## Tingitanine in etiolated plants of *Lathyrus tingitanus*

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### INTRODUCTION

Within the past several years 50 new free amino- and iminoacids were found in seed plants (Fowden 1958, Fowden 1960, Kaniuga 1959). The spread of these compounds is irregular. Some of them like e.g.  $\gamma$ -aminobutyric acid or  $\beta$ -alanine are observed in the majority of examined plants; others occur relatively seldom and are regarded as peculiar for the group or groups in question. These rare and peculiar amino acids are found sometimes in very distant taxonomic groups, so that no rules of their appearance based on the conventional classification can be established. Neither was any interrelation between the new amino acids and the plant organs found. Some of the compounds as e.g. canavanine in the legumes accumulate mostly in seeds (Bell 1958, Bell 1960, Tschiersch 1959, Tschiersch 1961). Others, like  $\gamma$ -methyleneglutamic acid and its amide in *Arachis hypogea*, do not occur at all in mature seeds and appear during the early developmental stages of the plant (Fowden 1954).

A considerable accumulation of certain rarely occurring free amino acids in plants belonging to definite systematic units initiated research after their role in the plant nitrogen metabolism. Detailed investigations of this kind were carried out by Reuter (1957) on citruline, by Tschiersch (1959) on canavanine and by Fowden (1954) on  $\gamma$ -methyleneglutamic compounds. All these authors arrive to conclusions that the amino acids they investigated play an important role in the nitrogen metabolism of plants; this in its turn reveals the existence of hitherto unknown and unforeseen metabolic pathways.

Last year a new free amino acid was isolated from seeds of *Lathyrus tingitanus* (Nowacki, Przybylska 1961); the term "tingitanine" was suggested for it. Probably the same compound — also from seeds of *Lathyrus tingitanus* — was isolated at the same time by Bell (1961), who described it as "lathyrine". The term tingitanine seems more appropriate, as within the genus *Lathyrus* there occur many new

compounds of an amino acid character (Przybylska, Nowacki 1961), so that the name of the newly isolated substance should rather derive from the species and not from the genus.

Tingitanine is probably a heterocyclic amino acid (Bell 1961, Nowacki, Przybylska 1961); an elementary analysis reveals its formula to be  $C_7H_{10}N_4O_2$ . After being reduced, tingitanine becomes converted to a compound revealing the presence of the guanidine group.

A chromatographic analysis of free amino acids in seeds of fifteen species of the genus *Lathyrus* revealed the presence of tingitanine also in *Lathyrus aphaca*, *Lathyrus maritimus* and *Lathyrus niger* (Przybylska, Nowacki 1961). The greatest, however, content of tingitanine — above 2 per cent of dry matter — was found on the basis of quantitative determinations in seeds of *Lathyrus tingitanus* (Nowacki, Przybylska 1961).

Primary analyses of green organs of *Lathyrus tingitanus* revealed that tingitanine occurs also in stems, leaves, pods and unripe seeds\*. The author took an interest in this problem because of the idea that the amino acid in question may play an outstanding role in the nitrogen metabolism of *Lathyrus tingitanus*. First of all, the changes in the content of tingitanine in etiolated plants were investigated.

#### MATERIAL AND METHODS

Seeds of *Lathyrus tingitanus* (originating from the Plant Breeding Station at Kosieczyn, 1960) were sterilized with 96 per cent ethanol, rinsed several times first with tap-water and after that with distilled water, and soaked for a few hours in distilled water. Swollen seeds were planted in boxes on quartz sand, rinsed with tap- and distilled water, and parched at  $140^{\circ}\text{C}$  for 24 hrs. Growth has counted from the time of germination which took place on the third day after planting. For the whole period of growth the plants were kept in a dark room, at a temp. of about  $21^{\circ}\text{C}$ , and were watered only with distilled water. Samples for analyses were taken during the following developmental stages:

- 0 — dry seeds,
- 1 — 3-day old plants, stem length 1.5—2.5 cm.,
- 2 — 7-day old plants, stem length 9—12 cm. 2—3 primordia of side leaves,
- 3 — 12-day old plants, stem length 23—27 cm. 3—4 primordia of side leaves, numerous side roots,

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\* Przybylska — unpublished data.

4 — 18-day old plants, stem length 34—36 cm. 5—6 primordia of side leaves, numerous side roots; growing points beginning to dry up.

From the first stage the cotyledons and stems with roots were analysed separately. The seed-coats were not tested in view of their insignificant role in the nitrogen metabolism of the plant. Offhand determinations of tingitanine in the seed-coats revealed only trace amounts of this amino acid.

In order to confirm the presumed synthesis of tingitanine in etiolated plants additional analyses of seeds from the following year of cultivation (1961) and of etiolated plants grown from these seeds were carried out. This time, however, the conditions of growth were different. In lieu of distilled water the plants were supplied with a nitrogen-free nutrient solution

2.11 g  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ; 0.25 g  $\text{KH}_2\text{PO}_4$ ; 0.51 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ;

and 2 drops of 1% solution of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 1 l of distilled water, in order to prolong their life. The temperature of the room was lower (16—17°C) so that the growth rate was lowered. Owing to technical difficulties the plants were only sampled in their 25-th day of development. At that time the stem length attained 42 cm, 6—7 side leaf primordia were to be seen, and the growing points were not found to dry up yet.

All the samples were analysed for the fresh and dry matter content, total nitrogen,  $\alpha$ -amino nitrogen, amido nitrogen and tingitanine. Determinations of the sums of aspartic acid and asparagine and of glutamic acid and glutamine as well as the chromatographic analysis of free amino acids were only performed in respect to unfed plants, tested at various stages of growth. The analyses of seeds of 1961 and of plants grown from them were only made to confirm the data concerning the possible synthesis of tingitanine; therefore only the results concerning the changing level of this amino acid shall be considered.

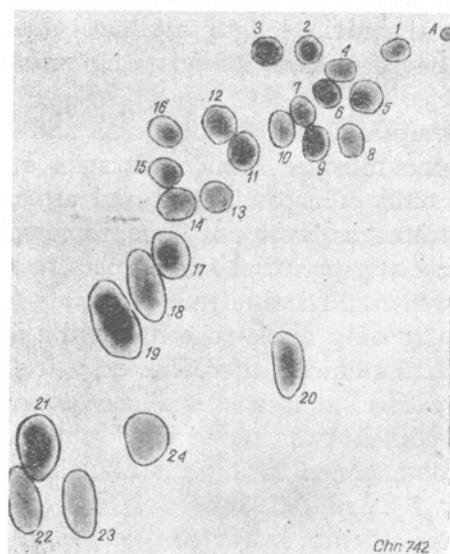
The dry matter content was determined with the use of a drier. Total nitrogen was determined in dry material according to Kjeldahl's method modified by Perrin (1953).

Fresh plant material was extracted with 75% ethanol until the reaction with ninhydrin disappeared. 20—80 stems with roots and 20—80 cotyledons were taken as samples for the extraction — the amount depending on the developmental stage. At least two separate extractions were performed. Chlorophyll was disposed of from the extracts by means of chlorophorm (Grzesiuk a. Kulka 1960).

The content of  $\alpha$ -amino nitrogen in the extract was determined according to Pope-Stephen's method modified by Albanese and

Irby (1944). Amido nitrogen was determined by the method of Varner and coworkers (1953). The tingitanine nitrogen was calculated from the amount of tingitanine on the basis of the chemical formula.

A two dimensional paper chromatography method of Wolfe (1957) modified to a certain degree by Przybylska (1960) was applied to separate the free amino acids. The chromatograms were developed with a 0.4% solution of ninhydrin in acetone with an addition of 0.2% cobaltic chloride (Haisa, Macek 1954). Before applying the extracts of free amino acids onto the chromatograms they were hydrolyzed with 1 n HCl at 100°C for 3 hrs in order to convert asparagine and glutamine to the respective acids, so as to make easier the chromatographic separation. In applying this technique a satisfactory separation of 23 amino acids (Phot. 1) is obtained — methionine and valine, however, remain unseparated. In order to find which of the two amino acids occurs in a considerable amount in stems and roots of etiolated plants, extracts of these organs from the last sampling were additionally separated on two dimensional chromatograms while a special set of solvents was applied (Przybylska 1960). The spot of the new amino acid-tingitanine overlaps on the chromatograms partly the glycine spot;



Phot. 1. Chromatogram of the standard solution of amino acids

A. Start-point. Individual spots correspond to the following amino acids: 1. cystine; 2. aspartic acid; 3. glutaminic acid; 4. canavanine; 5. lysine; 6. arginine; 7. glycine; 8. histidine; 9. serine; 10. homoserine; 11. alanine; 12.  $\beta$ -alanine; 13. proline; 14.  $\alpha$ -aminobutyric acid; 15.  $\beta$ -aminobutyric acid; 16.  $\gamma$ -aminobutyric acid; 17. tyrosine; 18. pipecolic acid; 19. methionine and or valine; 20. threonine; 21. isoleucine; 22. leucine; 23. phenylalanine; 24. tryptophan.

The same determinations are used in chromatograms of free amino acids of *Lathyrus tingitanus*

as, however, the coloured reaction of tingitanine with ninhydrin is very peculiar and the final colour different than in the case of the remaining amino acids, the identification is easy. Relative amounts of the various amino acids were determined on the basis of two dimensional chromatograms. Only the estimate of proline was performed with the use of circular technique, with a 0.4% solution of isatin in acetone as the developing agent (Przybylska, Kociałkowski, Wiewiórowski 1958).

Table 1  
Determinings of tingitanine in standard solutions

Amount of tingitanine in $\mu\text{g}$ .	Repetition	Extinction	Readings of tingitanine from the regression curve in $\mu\text{g}$ .	Mean	Mean per cent of error
10	I	0,072	10,2	10,37	+3,70
	II	0,075	10,7		
	III	0,072	10,2		
20	I	0,149	21,9	20,20	+1,00
	II	0,137	19,7		
	III	0,130	19,0		
30	I	0,207	30,2	30,00	—
	II	0,204	29,9		
	III	0,204	29,9		
40	I	0,267	39,2	38,40	—4,00
	II	0,253	36,8		
	III	0,267	39,2		
50	I	0,336	49,1	48,97	—2,06
	II	0,336	49,1		
	III	0,332	48,7		
60	I	0,420	61,5	61,00	+1,67
	II	0,420	61,5		
	III	0,408	60,0		

In order to illustrate not only the qualitative but also the quantitative changes in the composition of free amino acids extract amounts corresponding to 1/10 of the analysed organs were applied onto the chromatograms.

Quantitative determination of tingitanine was carried out in non-hydrolyzed extracts\*; it was separated from other amino acids by means of paper electrophoresis according to Wagner (1958). Known amounts of tingitanine were applied simultaneously onto the paper (10—60  $\mu\text{g}$ ). They served the purpose of plotting standard curves.

\* Tingitanine disintegrates partly when treated with acid (Nowacki, Przybylska 1961).

After the electropherograms had been run they were dried in a current of warm air and placed under a darkened globe over sulphuric acid. 24 hours later the electropherograms were developed with a 0.25% solution of ninhydrin in acetone, dried in dark at a room temperature and then placed again in a globe for a day; at the end of this procedure

Table 2

Content of tingitanine in extracts of non-defatted and defatted seeds in mg. per seed

Analysed sample	Extract	Individual determinings of tingitanine		Extract means	Sample means
Non-defatted seeds	I	2,27	2,20	2,24	2,26
		2,26	2,24		
	II	2,25	2,27	2,28	
		2,30	2,29		
Defatted seeds	I	2,19	2,20	2,21	2,25
		2,27	2,28		
	II	2,34	2,29	2,29	
		2,26	2,28		

the stains attained a maximal intensity. Coloured stains were eluted with 75% solution of ethanol with the addition of 0.005%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (Chorąży a. Chorąży 1959). An eluate of an unstained section of paper of the same surface served as a blank.

Extinctions of the eluates were measured in respect to the blank at a wave length  $470 \text{ m}\mu$  in a Unicam colorimeter SP 600. The Lambert-Beer law was followed within the examined range of concentrations. The mean error of this method did not amount to over 4 per cent (Table 1).

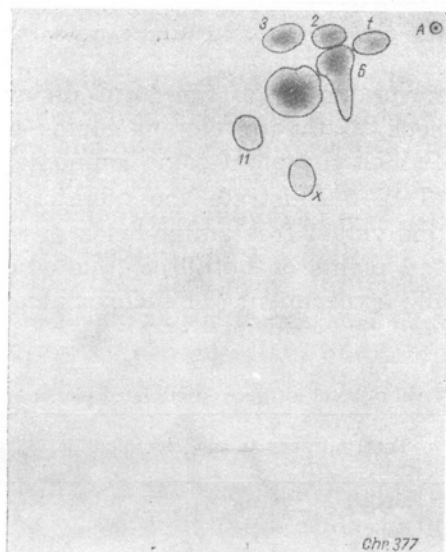
The level of tingitanine was also determined in extracts of seeds, the meal of which was defatted with ethyl ether. This was done in order to make sure whether the presence of fats in seeds does not affect the extraction of tingitanine. Should this happen, the results concerning the biosynthesis of this amino acid might be erroneously interpreted. Table 2 however shows that the process of defatting seeds does not influence the content of tingitanine in the extract.

Similarly to tingitanine, aspartic and glutamic acids were determined quantitatively. Nevertheless, in this case extracts hydrolyzed with  $\text{HCl}$  were used; within these extracts aspartic and glutamic acids represent the sum of these amino acids and of the respective amides. Extinctions of the eluates of aspartic and glutamic acids were determined at a wavelength  $520 \text{ m}\mu$ .

## DISCUSSION OF THE RESULTS

Changes in the level of fresh and dry matter during the growth of etiolated plants of *Lathyrus tingitanus* are shown in Tab. 3. Numerical values reveal that the content of dry matter per plant distinctly diminishes during the lasting of the experiment; this is a feature peculiar for growth in darkness.

Data concerning total nitrogen (Tab. 4) show there are no changes in its level until the 12-th day. In reckoning the nitrogen per plant a gradual fall in its level in the cotyledons, and a rise in the stems and roots can be seen. Towards the end of the growing period, between the 12-th and 18-th day, the level of total nitrogen in the whole plant slightly falls. A similar decrease in the amount of total nitrogen during the last developmental phase of etiolated *Lupinus albus* L. seedlings was observed by Meiss (1952). A partial outflow of soluble nitrogen



Phot. 2. Chromatogram of free amino acids of seeds of *Lathyrus tingitanus* Ting. — tingitanine (overlaps with glycine); *t*, *x* — unidentified amino acids. The amount of the extract applied onto the chromatogram corresponds to 1/10 of a seed

compounds into the substratum may be the underlying cause of this phenomenon. The percentage of total nitrogen on a dry matter basis remains on a more or less unchanging level.

While seeds germinate in the dark and etiolated plants develop, the level of free amino acids changes in a peculiar manner. A study of these changes, concerning both their quantitative and their qualitative

Table 3

Fresh and dry matter of etiolated plants of *Lathyrus tingitanus* at various periods of development

Period of growth (days)		Fresh matter			Dry matter				
		mg. per plant			mg. per plant			percentage	
		Cotyledons	Stem + root	Whole plant	Cotyledons	Stem + root	Whole plant	Cotyledons	Stem + root
Seeds 1960*		105.6	—	105.6	97.6	—	97.6	92.5	—
Unfed plants	3	219.3	105.7	325.0	81.5	7.3	88.8	37.2	7.4
	7	192.7	330.6	523.3	55.6	25.9	81.5	28.9	7.8
	12	193.3	617.6	810.9	35.7	43.2	78.9	18.6	7.5
	18	185.0	715.8	900.8	19.5	46.6	66.1	10.5	6.5
Seeds 1961*		123.4	—	123.4	112.2	—	112.2	90.9	—
25 Plants supplied with a nitrogen-free nutrient solution		137.5	862.5	1000.0	9.3	59.0	68.3	6.9	6.9

\* In view of the arrangement of the Table values concerning seeds are given in the column "cotyledons".

aspect, provides information as to the form under which nitrogen is carried from the seeds to the developing stems and roots; and also as to the form into which the plant toxic ammonia is fixed.

Phot. 2—4 and Tab. 5 illustrate the quantitative and qualitative changes concerning individual free amino acids in cotyledons and stems with roots of etiolated plants of *Lathyrus tingitanus*, and taking place throughout the whole experiment. The chromatogram of free amino

Table 4

The changes of the level of total nitrogen in etiolated plants of *Lathyrus tingitanus*

Period of growth (days)		Total nitrogen in mg. per plant			Total nitrogen as a dry matter percentage	
		Cotyledons	Stem + root	Whole plant	Cotyledons	Stem + root
Seeds 1960*		6.54	—	6.54	6.90	—
Unfed plants	3	5.87	0.68	6.55	7.20	9.30
	7	3.96	2.60	6.56	7.12	10.00
	12	2.61	4.06	6.66	7.31	9.40
	18	1.43	4.66	6.09	7.34	10.01
Seeds 1961*		6.53	—	6.53	5.82	—
25 Plants supplied with a nitrogen-free nutrient solution		0.47	5.59	6.06	5.04	9.47

\* In view of the arrangement of the Table values concerning seeds are given in the column "cotyledons".



acids of seeds (Phot. 2) reveals that tingitanine is the most important compound. Arginine, as well as aspartic and glutamic acids occur in much lesser amounts. Quantities of the remaining amino acids are also insignificant. In observing the changes taking place during the development of plants one can notice a continuous fall in the concentration of tingitanine in the cotyledons and, vice versa, a continuous increase in the stems and roots. These data show that tingitanine may be involved in the storing of nitrogen in seeds; and that nitrogen is carried to the developing organs in the form of this amino acid.

Beyond the fall in the level of tingitanine, a continuous rise in the content of arginine is a peculiar feature as regards the rate of changes in the amount of free amino acids in the cotyledons. It means that considerable amounts of arginine become liberated during the proteolysis of storage proteins in seeds. This assumption is confirmed by data from reference works which prove that arginine is an important compound of storage proteins in legumes (Block a. Weiss, 1956; Nehring a. Schwerdtfeger 1957).

Apart from a rise in the level of tingitanine in the stems and roots, also an increase in the concentration of such amino acids as lysine, serine and methionine and/or valine is observed. A special chromatogram of the extract of free amino acids from stems and roots during the last phase of development (see methods) revealed that the intensive stain in the position of methionine and valine (Phot. 4d) represents only valine. During the first few stages of growth of the etiolated plants the amount of aspartic and glutamic acid in the stems and roots increases; later however the concentration of these amino acids definitely falls. The level of the remaining amino acids does not change in a peculiar manner.

Quantitative determinations of tingitanine as well as of aspartic and glutamic acids confirmed the chromatographic data. Graphs representing changes in the quantity of these acids during the development of etiolated plants reveal a considerable fall in the level of tingitanine in the cotyledons (Fig. 1a) and a rise in the stems and roots (Fig. 1b). Data concerning the cotyledons together with those concerning the stems and roots furnish a pattern of changes in the whole plant. They are represented in Fig. 1c. It is evident that during the initial stages of development the content of tingitanine remains on a more or less unchanging level.

It means that the increase in the amount of tingitanine in stems and roots is solely due to the decrease in the cotyledons — no synthesis of this amino acid is observed. Later however, the increase in the concentration of tingitanine in the stems and roots exceeds its decrease in the cotyledons so that the total amount of tingitanine per plant

Table 5

Estimate of the contents of free amino acids in seeds and etiolated plants of *Lathyrus tingianus* on the basis of two-dimensional paper chromatography

Period of growth (days)	Tingitanine + glycine		Aspartic acid*		Glutamic acid*		Lysine		Arginine		Histidine		Serine		Alanine		$\gamma$ -amino butyric acid	Proline		Tyrosine		Methionine and or valine		Threonine		Isoleucine		Leucine		Phenyl- alanine		Cystine		Unidentified compound ****		
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B		
Seeds	5	—	2	—	2	—	—	—	2	—	—	—	—	—	—	1	—	—	$\frac{1}{2}$	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	
3	4 $\frac{1}{2}$	3 $\frac{1}{2}$	2 $\frac{1}{2}$	2	3	1	1	1	2	1	1 $\frac{1}{2}$	1	2	1	—	—	—	1 $\frac{1}{2}$	2	1	—	—	1 $\frac{1}{2}$	2	1	—	1	1	1	1 $\frac{1}{2}$	1	1	—	—	1	1
7	3 $\frac{1}{2}$	4	3	3	3	2	2	2	3	1	1	2	2	2 $\frac{1}{2}$	2	2	—	—	1 $\frac{1}{2}$	$\frac{1}{2}$	1	—	—	1 $\frac{1}{2}$	3	1	1	1	1	1	1	1	—	—	1	1
12	2 $\frac{1}{2}$	4 $\frac{1}{2}$	4 $\frac{1}{2}$	4	3	2	2	3	4	1	1	3	3	3	2	2 $\frac{1}{2}$	—	—	1 $\frac{1}{2}$	1	1 $\frac{1}{2}$	—	—	2	4	1	2	1	1	1	1	1	—	—	1	1
18	1 $\frac{1}{2}$	5	4 $\frac{1}{2}$	3	2 $\frac{1}{2}$	1	2	3	5	1	1	2	1 $\frac{1}{2}$	4	1	1 $\frac{1}{2}$	—	$\frac{1}{2}$	$\frac{1}{2}$	1	—	—	1 $\frac{1}{2}$	4 $\frac{1}{2}$	1	1	1	1	1	1	1	—	—	1	1	

A. Cotyledons. B. Stem + root

Conventional values\*\*\*:

5 — very large amount, 4 — large amount, 3 — average amount, 2 — small amount, 1 — very small amount, ½ — trace amount, — — unfound.

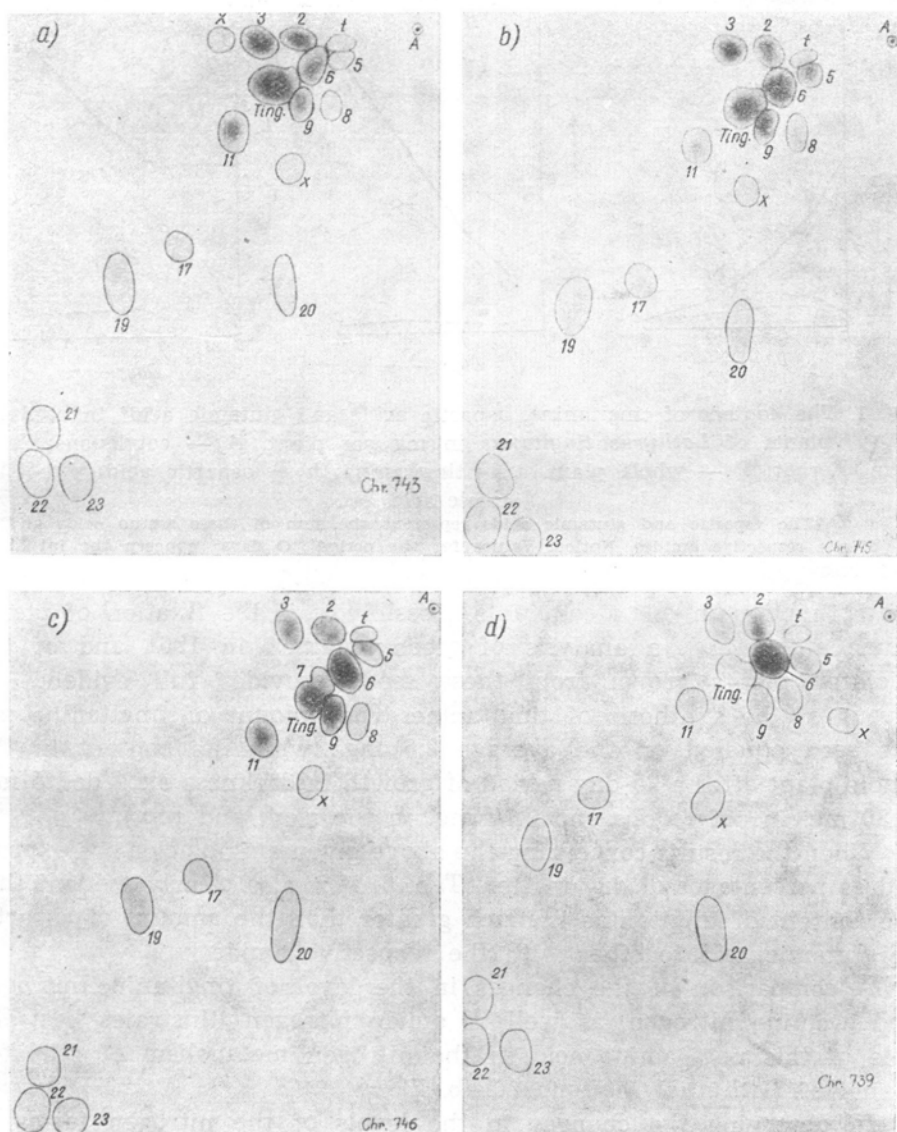
\* Aspartic and glutamic acids are given as the sums of these amino acids and corresponding amides.

\*\* The unidentified compound "t" gives a specific, coloured reaction with ninhydrin like tingitanine.

\*\*\* ½ placed after a whole number denotes an intermediate value.

Remark: Unidentified compounds which appear only at certain periods of growth are not given in the Table.

becomes larger. It seems rather unlikely that this amino acid is a component of the storage protein in seeds of *Lathyrus tingitanus*, and therefore one must assume that, during the later periods of growth of etiolated plants, there must take place a synthesis of tingitanine.



Phot. 3a—3d. Chromatograms of free amino acids of cotyledons of etiolated plants of *Lathyrus tingitanus* during successive stages of development (correspondingly: 3, 7, 12, 18 days). *Ting.* — tingitanine; *t*, *x* — unidentified amino acids. Amounts of extracts applied onto the chromatograms correspond to 1/10 of cotyledons

This synthesis would also occur at that period when the total amount of aspartic acid and its amide already falls and the level of glutamic acid and glutamine is very low. The above described data seem to provide evidence in favour of a supposition that tingitanine has a de-

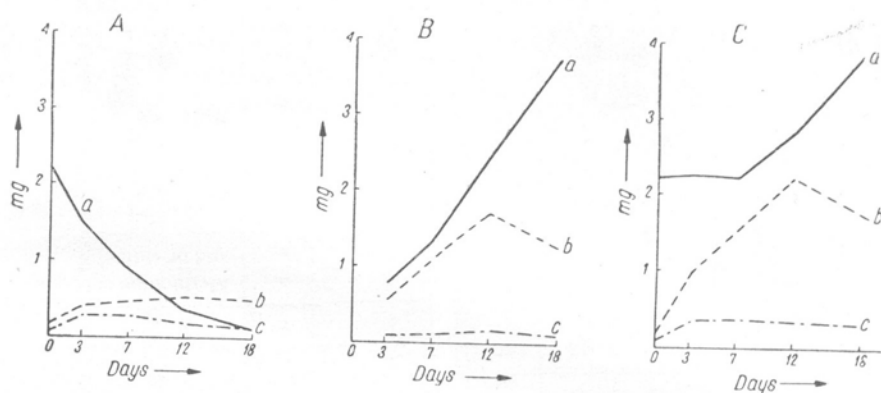


Fig. 1. The content of tingitanine, aspartic acid\* and glutamic acid\* in etiolated plants of *Lathyrus tingitanus* in mg per plant. A — cotyledons; B — stem + root; C — whole plant. a — tingitanine; b — aspartic acid; c — glutamic acid

\* The aspartic and glutamic acids represent the sum of these amino acids and of the respective amides. Notice: Values for the period "0 days" concern the initial seeds.

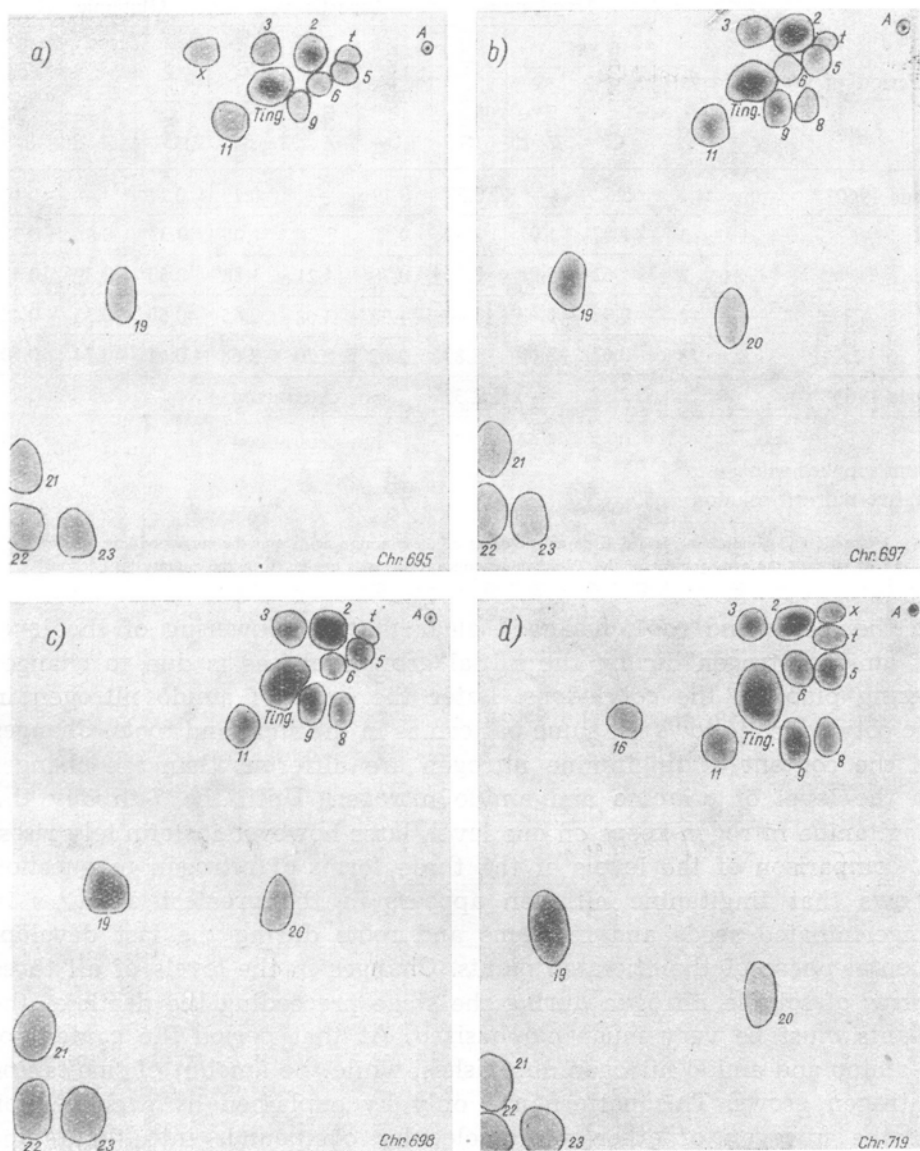
finite function in etiolated plants. Possibly it is the fixation of plant-toxic ammonia. An analysis of seeds obtained in 1961 and of the etiolated plants grown from these seeds provides full evidence in favour of the synthesis of tingitanine. The amount of tingitanine per one seed equaled on the average 2.6 mg., while its content in the whole plant after a 25-day period of growth in darkness exceeded 5 mg. (0.30 mg. in cotyledons and 5.10 mg. in the stem and root).

When the results concerning the above given amino acids are expressed as percentages of dry matter (Tab. 6) it is also clearly evident that the content of tingitanine is much greater than the amount of aspartic or glutamic acid together with the respective amide.

A comparison of the changes in the level of tingitanine nitrogen and  $\alpha$ -amino nitrogen, as well as amido nitrogen illustrates best the role of this new amino acid in the nitrogen metabolism of etiolated plants of *Lathyrus tingitanus* (Tab. 7).

In observing the changes in the levels of the nitrogen forms in question per 1 plant (Tab. 7A) it becomes evident that the content of  $\alpha$ -amino nitrogen increases until the 12-th day of growth (counting from the time of emergence of the seedlings) and then begins to decrease. These changes are due in the first place to the stem and root, as  $\alpha$ -amino nitrogen in the cotyledons remains on a more or less

unchanging level. The level of amido nitrogen follows a similar pattern, with the only difference that it falls slightly during the first few days of growth. A study of the changes taking place in the cotyledons and



Phot. 4a—4d. Chromatograms of free amino acids of stems and roots of etiolated plants of *Lathyrus tingitanus* during successive stages of development (correspondingly: 3, 7, 12, 18 days)

*Ting.* — tingitanine; *t*, *x* — unidentified amino acids.

Amounts of extracts applied onto the chromatograms correspond to 1/10 of the analysed organs

Table 6

Content of tingitanine, aspartic and glutamic acids\* in seeds and etiolated plants of *Lathyrus tingitanus* in percentages of dry matter

Period of growth (days)		Tingitanine			Aspartic acid			Glutamic acid		
		Cotyledons	Stem + root	Whole plant	Cotyledons	Stem + root	Whole plant	Cotyledons	Stem + root	Whole plant
Seeds 1960**		2.32	—	2.32	0.19	—	0.19	0.11	—	0.11
Unfed plants	3	1.87	1.07	2.59	0.50	7.26	1.06	0.33	0.96	0.38
	7	1.62	5.21	2.76	0.79	4.21	1.88	0.50	0.39	0.47
	12	0.98	5.43	3.43	1.38	3.68	2.65	0.50	0.35	0.42
	18	0.62	8.09	5.89	2.62	2.70	2.68	0.61	0.24	0.50
Seeds 1961**		2.32	—	2.32	not determined					
25		0.32	8.65	7.51	not determined					
Plants supplied with a nitrogen-free nutrient solution										

\*Aspartic and glutamic acids are given as the sums of these amino acids and the corresponding amides.

\*\* In view of the arrangement of the Table values concerning seeds are given in the column "cotyledons".

in the stems and roots makes it clear that the lowering of the level of amido nitrogen during the initial growth phases is due to changes taking place in the cotyledons. Later the level of amido nitrogen in the cotyledons follows the same pattern as in the stem and roots. Changes in the content of tingitanine nitrogen are different than the changes in the level of  $\alpha$ -amino and amido nitrogen. Until the 7-th day the tingitanine nitrogen keeps on one level, later however it definitely rises. A comparison of the levels of the three forms of nitrogen in question shows that tingitanine nitrogen appears in the greatest amounts in ungerminated seeds and in stems and roots during the last developmental phase of the etiolated plants. Changes in the levels of all three forms of soluble nitrogen during the stage preceeding the death of the plants must be very much emphasized. At that period the content of  $\alpha$ -amino and amido nitrogen diminishes, while the amount of tingitanine nitrogen grows. This pattern can only be explained by a conversion of the nitrogen of other micromolecular compounds into tingitanine nitrogen. After 18 days of growth in darkness the tingitanine nitrogen is equal to the sum of  $\alpha$ -amino and amido nitrogen. Similar relations occur in etiolated plants grown from seeds of 1961. In these seeds the predominance of tingitanine nitrogen is even more distinct. In the etiolated plants the amount of tingitanine nitrogen is also equal to the sum of  $\alpha$ -amino and amido nitrogen.

Table 7

Changes in the content of  $\alpha$ -amino nitrogen, amido nitrogen and tingitane nitrogen\* during the development of etiolated plants of *Lathyrus tingitanus*

Period of growth (days)	A						B				C											
	Cotyledons			Stem + root			Whole plant			Cotyledons		Stem + root		Cotyledons			Stem + root					
	$\alpha$ -amido N	amido N	tingitane N	$\alpha$ -amido N	amido N	tingitane N	$\alpha$ -amido N	amido N	tingitane N	$\alpha$ -amido N	amido N	tingitane N	$\alpha$ -amido N	amido N	tingitane N	$\alpha$ -amido N	amido N	tingitane N				
Seeds 1960**	0.21	0.22	0.70	—	—	—	0.21	0.22	0.70	0.22	0.23	0.72	—	—	—	3.21	3.36	10.70	—	—		
United plants	3	0.20	0.11	0.47	0.18	0.08	0.24	0.38	0.19	0.71	0.25	0.14	0.58	2.47	1.10	3.29	3.41	1.87	8.01	26.47	11.76	35.29
	7	0.25	0.14	0.28	0.48	0.26	0.42	0.73	0.40	0.70	0.45	0.25	0.50	1.85	1.00	1.62	6.31	3.54	7.07	18.46	10.00	16.15
	12	0.21	0.18	0.11	0.75	0.33	0.77	0.96	0.51	0.88	0.59	0.50	0.31	1.74	0.76	1.78	8.05	6.90	4.22	18.47	8.13	18.97
Seeds 1961**	18	0.20	0.12	0.04	0.62	0.28	1.16	0.82	0.40	1.20	1.03	0.62	0.21	1.33	0.60	2.49	13.99	8.39	2.80	13.31	6.01	24.89
		0.18	0.04	0.80	—	—	—	0.18	0.04	0.80	0.16	0.04	0.71	—	—	—	2.76	0.61	12.25	—	—	—
25																						
Plants supplied with a nitrogen-free nutrient solution		0.18	0.06	0.01	1.07	0.29	1.57	1.25	0.35	1.58	1.94	0.65	0.11	1.81	0.49	2.66	38.30	12.77	2.13	19.14	5.19	28.09

A — in mg. per plant

B — as percentages of dry matter

C — as percentages of total nitrogen

\* Tingitane nitrogen was calculated on the basis of its content and chemical formula (Bell 1961; Nowacki a. Przybylska 1961)

\*\* In view of the arrangement of the Table values concerning seeds are given in the column "cotyledons",

The predominance of tingitanine nitrogen over  $\alpha$ -amino and amido nitrogen is evident also when the results are counted over in respect to dry matter (Tab. 7B) and total nitrogen (Tab. 7C). It is most interesting to investigate the changes in the levels of the nitrogen forms in question on the basis of total nitrogen.

In the cotyledons the content of  $\alpha$ -amino nitrogen expressed as percentage of total nitrogen definitely rises. The same holds true for amido nitrogen, with the exception of the above mentioned lowering in its level during the initial growth phases. On the basis of the decomposition of storage proteins which takes place during the germination of seeds and growth of plants these observations are fully consistent. On the other hand, the lowering relative amount of tingitanine nitrogen in the cotyledons seems to support the above given assumption that tingitanine is one of the forms in which nitrogen is carried to the developing plants. This supposition seems also to be confirmed by the large percentage (ca 35%) of tingitanine nitrogen in the total nitrogen of stems and roots in 3-day old plants. The occurrence that also  $\alpha$ -amino nitrogen represents at that period a high percentage (ca 26%) of total nitrogen reveals the high rate of decomposition of seed storage protein during the initial developmental phases. Later probably the rate of the proteolysis of seed proteins and of the transport of soluble nitrogen compounds to the plants falls. At this period protein synthesis in vegetative tissues commences, and therefore a relative lowering of the levels of  $\alpha$ -amino nitrogen and tingitanine is observed. The proportion of amido nitrogen in total nitrogen of the stems and roots becomes gradually smaller as the plants develop. During the final stages of development the relative amount of  $\alpha$ -amino nitrogen also falls, while the proportion of tingitanine nitrogen — save the initial fall — definitely rises.

### CONCLUSIONS

The above given data show that tingitanine plays an important role in the nitrogen metabolism of *Lathyrus tingitanus*. The present knowledge, however, does not enable to determine more precisely its function. In order to investigate this problem thoroughly analyses of green plants growing under normal conditions are necessary, as well as experiments with the use of radioactive tracers and enzyme tests. Further research of the role of tingitanine in the nitrogen metabolism of *Lathyrus tingitanus* is being carried out.

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## STRESZCZENIE

Celem niniejszej pracy było zbadanie roli nowego wolnego aminokwasu — tingitaniny w metabolizmie azotowym etiolowanych roślin *Lathyrus tingitanus*.

Przeprowadzono jakościową analizę wolnych aminokwasów nasion oraz etiolowanych roślin w różnych stadiach rozwoju. Osobno analizowano liścienie, osobno łodygi z korzeniami. Ilościowo oznaczono nowy wolny aminokwas tingitaninę, sumę kwasu asparaginowego i asparaginy oraz sumę kwasu glutaminowego i glutaminy. Ponadto oznaczono kształtowanie się świeżej i suchej masy, azotu ogólnego, azotu  $\alpha$ -aminowego i azotu amidowego.

W wyniku przeprowadzonych analiz stwierdzono duży udział nowego wolnego aminokwasu — tingitaniny w gospodarce azotowej etiolowanych roślin *Lathyrus tingitanus*. Tingitanina jest dominującym wolnym aminokwasem nasion tej rośliny. W trakcie rozwoju roślin w ciemności zmniejsza się stopniowo ilość tingitaniny w liścieniach, wzrasta natomiast jej zawartość w łodydze i korzeniu. W końcowym okresie rozwoju etiolowanych roślin zawartość tingitaniny w łodydze i korzeniu jest wyższa niż w nasieniu, co świadczy o syntezie tego związku.

Uzyskane dane wskazują, że nowy aminokwas może spełniać funkcję magazynowania azotu w nasionach oraz wiązania toksycznego amoniaku w etiolowanych roślinach. Możliwe jest również, że odgrywa on pewną rolę w transporcie azotu w roślinie.

Bliższe sprecyzowanie roli nowego związku w *Lathyrus tingitanus* wymaga dalszych badań.

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