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VARIATION IN QUANTITATIVE AND QUALITATIVE ALKALOID COMPOSITION IN *PHALARIS ARUNDINACEA* (*POACEAE*)

Summary

Research of the identification and determination of alkaloid contents in the aboveground parts of Phalaris arundinacea (Poaceae) during various periods of the vegetation season was conducted. Analyses were performed using an apparatus system by Hewlett-Packard (USA), consisting of a gas chromatograph model 589011 and a mass detector model 5971 A. Analyses were conducted at the laboratory of the phytochemistry at the Institute of Bioorganic Chemistry, PAS in Poznań. A total of six alkaloids were identified in the analysed material, i.e. tryptophol and gramine (indole alkaloids) as well as lupanine and 13-OH lupanine and lupanine esters – 13α- isovalericlupanine and 13α-tigloyloxylupanine (quinolysidine alkaloids). Gramine and lupanine are the dominant alkaloids in the growth stage.
***Key words**: indole alkaloids, alkaloidy chinolizydynowe, Phalaris arundinacea*

ZRÓŻNICOWANIE SKŁADU ALKALOIDOWEGO POD WZGLĘDEM ILOŚCIOWYM I JAKOŚCIOWYM U *PHALARIS ARUNDINACEA* (*POACEAE*)

Streszczenie

Badania dotyczące identyfikacji i określenia zawartości alkaloidów w części nadziemnej rośliny pod względem ilościowym i jakościowym, przeprowadzono u Phalaris arundinacea w różnych okresach sezonu wegetacyjnego na aparacie firmy Hewlett - Packard (USA), w skład którego wchodzi chromatograf gazowy model 589011 oraz detektor masowy model 5971 A, w pracowni Fitochemii Instytutu Chemii Bioorganicznej PAN w Poznaniu. Zidentyfikowano występowanie 6 alkaloidów: tryptofol i graminę (alkaloidy indolowe) oraz lupaninę i 13 -OH lupaninę oraz estry lupaniny: 13α-izowaleroksylupaninę i 13α-tigloyloksylupaninę (alkaloidy chinolizydynowe). Dominującym alkaloidem w fazie wzrostu była gramina, a także lupanina.
***Słowa kluczowe**: alkaloidy indolowe, alkaloidy chinolizydynowe, gramina, Phalaris arundinacea*

1. Introduction

Many plant species, including those from the family *Poaceae*, contain in their tissues species-specific substances, such as e.g. alkaloids. Similarly to certain protein and non-protein amino acids (arginine, lysine, histidine, ornithine and its homologues) and biogenic amines (putrescine, cadaverine, spermine and spermidine), alkaloids are nitrogenous basic compounds found in plant organs. These compounds serve very important physiological functions in plants, e.g. allelopathic in plant-plant interactions, bacteriostatic in plant–bacteria interactions, and herbivolar in plant-consumer interactions [1, 2, 3]. Apart from defence functions against aggression of herbivorous animals, insects, fungi, bacteria and viruses, they also definitely serve an essential regulatory function in transcription, translation and replication processes [4]. Studies conducted to date have shown considerable variability in the composition of these compounds in plants, although the total content of these compounds remains constant. This means that an increase in the contents of e.g. some alkaloids is accompanied by a decrease in the level of the others (biogenic amines or basic amino acids) and vice versa [5].

From the nutritional point of view a high accumulation of alkaloids in the plant material is an adverse phenomenon. Alkaloids are bitter in taste and as such are the primary factor limiting plant material uptake by animals. *Phalaris arundinacea* is a species containing as many as 9 alkaloids. These compounds vary in the degree of their toxicity [6, 7, 8, 9]. Some researchers ascribe them with toxic properties [10]. For example, in the case of lupanine found in grasses acute toxicity expressed in LD50 (mg/kg body weight) is 177, whereas at intragastric administration (at intravenous injection in rats) it is as high as 1464 [11]. However, these values are considered disputable, since the authors did not supply information on the form of lupanine - free base or salt. As it is generally known, alkaloids in the plant physiological environment, i.e. at pH of approx. 7, are found in the salt form, thus they are bound with a specific anion of an organic acid. For this reason only toxicity tests conducted on the latter alkaloid form may provide reliable data on its toxicity to a given organism [12].

2. Aim of the study

The aim of this study was to identify and determine alkaloid contents in the aboveground parts of *Phalaris arundinacea* (Poaceae) during various periods of the vegetation season.

3. Methods

Analyses were conducted at the laboratory of the phytochemistry at the Institute of Bioorganic Chemistry, PAS in Poznań. Alkaloid contents in the aboveground parts of *Phalaris arundinacea* were determined at various stages of ontogenesis: the growth stage, the shooting stage, the onset of ear formation, the stages of earing and flowering. Additionally, analyses were performed on grass harvested in another area (Rogalinek) at the stage corresponding to the initial growth stage. Each time samples were harvested from the same single-species phytocenosis of reed canary grass located in Sobota near Poznań.

Analyses were conducted on green aboveground parts of specimens belonging to the species *Phalaris arundinacea*., i.e. stems, leaves and ears.

Plant material was ground for 2x15 seconds and dried for 2 h at 90°C. An analytical sample of 0.5 g material was supplemented with 5 ml 5% TCA (trichloroacetic acid). It was sonicated in an ultrasound bath 3 x 15 min. Each time it was centrifuged for 5 min. (3000 rpm) and the precipitate after the third extraction was discarded. Supernatants were combined (approx. 15 ml) and supplemented with 1 ml 10 M NaOH. Extraction was run 3 x 15 ml Ch_2Cl_2 (in separators).

In the case when the layers were not separated the samples were centrifuged for 5 min at 3000 rpm. Combined Ch_2Cl_2 layers (approx. 45 ml) were dried using anhydrous Na_2SO_4 and next the solvent was evaporated in a recirculating evaporator. Obtained alkaloids were transferred from the flask to a Pyrex test tube (the Ch_2Cl_2 solvent was evaporated in a stream of nitrogen). Next the internal standard 10 nanoliter coffein was added at 1 mg/nanoliter. The sample was dissolved in 50 nanoliter Ch_2Cl_2 and it was injected. The results are given in equivalents of lupanine.

Analyses were performed using an apparatus system by Hewlett-Packard (USA), consisting of a gas chromatograph model 589011 and a mass detector model 5971 A. Separations were run on a SPB tm 5 Capillary Column (30 m x 0.25 mm x 0.2 Supelco tm). The temperatures of the injection chamber 250°C, detector 300°C, the initial temperature of the oven 160°C were maintained for 3 min., followed by an increase in temperature at 7 C/min to 300°C final temperature maintained for 10 min.

Helium was used as the carrier gas at a flow rate of 1 ml/min. Analyses were conducted at the division of the carrier gas introduced to the column at the 1:20 rate. The volume of 1 nanoliter sample was injected into the injection chamber.

4. Results of study

Nitrogen content and calculated protein content in Phalalris arundinacea plants in different periods of ontogenesis are given in Table 1.

A total of six alkaloids were identified in the analysed material, i.e. tryptophol and gramine (indole alkaloids) as well as lupanine and 13-OH lupanine and lupanine esters – 13α- isovalericlupanine and 13α-tigloyloxylupanine (quinolysidine alkaloids).

Table 1. Dates, periods of ontogenesis and contents of the % protein in *Phalaris arundinacea* tested for alkaloid contents
Tab. 1. Terminy, okresy ontogenezy oraz zawartość % protein u Phalaris arundinacea badanej na zawartość alkaloidów

Sample no.	Date	Plant height (cm)	Growth and development stages	Chemical composition	
				N%	% protein
1.	09.05.2003	34	Growth	3.82	23.86
2.	21.05.2003	83	Shooting	2.51	15.70
3.	31.05.2003	99	Onset of ear formation	1.45	9.05
4.	15.06.2003	168	Earing	1.32	8.27
5.	26.06.2003	179	Flowering	1.07	6.71
6.	Rogalinek	48	growth	2.36	14.74

Contents of alkaloids in the vegetative parts of *Phalaris arundinacea* assayed at various stages of ontogenesis are presented in Table 2 and 3.

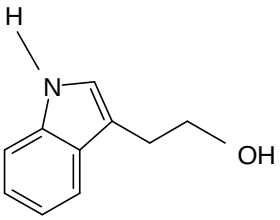
Source: own work / Źródło: opracowanie własne

Table 2. Percentage alkaloid contents in analysed grass samples of *Phalaris arundinacea*
Tab. 2. Zawartość alkaloidów w badanych próbkach Phalaris arundinacea w %

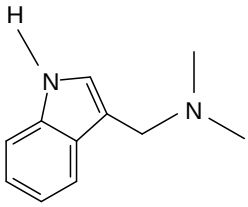
Sample no.	Total alkaloid content in 100 mg DM	Tryptophol	Gramine	Lupanine	13-OH Lupanine	1. esters 2.		Σ
						13α-isovalericoxy lupanine	13α-tigloyloxy lupanine	
1.	0.0005%	—	82.67	17.33	—	—	—	100%
2.	0.0004%	12.56	56.63	30.82	—	—	—	100%
3.	0.0226%	—	—	88.9	0.41	0.82	9.78	100%
4.	0.002%	16.19	20.09	56.26	—	—	7.45	100%
5.	0.000066%	—	—	100.00	—	—	—	100%
6.	0.0036%	—	34.01	65.99	—	—	—	100%

Source: own work / Źródło: opracowanie własne

The formulas of alkaloids identified in *Phalaris arundinacea* formulas:
a) indole:

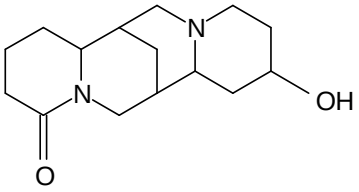


** tryptophol*

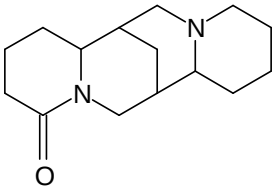


*** gramine*

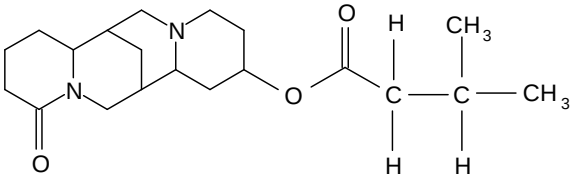
b) quinolysidine:



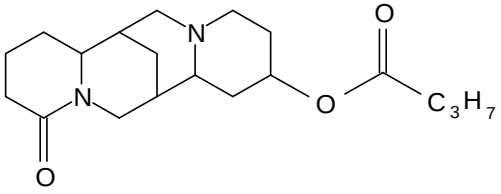
** lupanine*



*** 13 -OH lupanine*



**** 13α- izowaleroksylupaninę*



***** 13α-tigloyloksylupaninę*

Table 3. Percentage alkaloid contents in analysed grass samples from reed canary grass meadows collected at the shooting stage in various locations
Tab. 3. Zawartość alkaloidów w badanych próbkach traw z łąk mozgowych pobranych w fazie strzelania w źdźbło w różnych miejscowościach w %

Sample no.	Sample collection site	Plant height in cm	Total alkaloid content in 100 mg DM	Tryptophol	Gramine	Lupanine	Σ
1.	Meadow in Brody	25	0.0170%	16.73%	80.9%	2.37%	100%
2.	Meadow in Pniewy	35	0.0108%	22.44%	74.69%	2.87%	100%
3.	Meadow in Turowo	70	0.0052%	35.9%	62.88%	1.22%	100%
4.	Ditch slope	50	0.0032%	—	97.64%	2.36%	100

Source: own work/ Źródło: opracowanie własne

However, all of these alkaloids are not found at the same plant development stages. Gramine is the dominant alkaloid in the growth stage (at 82.67% share in the alkaloid pool). At that stage apart from gramine also lupanine is synthesised. With plant growth (the shooting stage) the content of gramine decreases and a new indole alkaloid, tryptophol, is synthesised. Lupanine content also increases in this case. At the onset of ear formation the synthesis of indole alkaloids declines, while the level of lupanine increases to reach 88.9% share in the alkaloid pool and its esters appear, i.e. 13α- isovaleric oxylupanine and 13α- tigloyloxylupanine. At the onset of ear formation we observe the greatest accumulation of alkaloids in the vegetative growth stage of grasses (0.02226%). In further stages (earing, flowering) the accumulation of alkaloids declines markedly to the level of 0.066 mg% in the flowering stage. Lupanine is a trace alkaloid detected at this stage. In grass harvested in Rogalinek at the initial growth stage we detected gramine and lupanine, i.e. the same alkaloids as in the samples from Sobota, although at different percentage shares in the total alkaloid pool. These differences may be explained by the differing habitat factors. Moreover, we may not exclude the effect of the ecological factor (i.e. climate and soil conditions).

In grasses harvested in September from meadows in the different geographical locations a marked diversification was observed in the total alkaloid content. It ranged from 0.0032% (a slope of the ditch in Brody) to 0.0170% (a meadow in Brody) (Table 3).

Qualitative differences are also observed, although in this case it is highly conserved in terms of their proportions. Gramine was the dominant alkaloid in all grasses, while lupanine was least abundant. Tryptophol is found at intermediate levels. The diversification of the quantitative and qualitative alkaloid composition in *Phalaris arundinacea* coming from various locations may be explained by soil conditions, primarily water availability, which seems to be a very important environmental factor affecting alkaloid contents. This is confirmed by the research results reported by many authors, e.g. [1, 13, 14, 15, 16].

5. Conclusions

1. In present study a total of six alkaloids were identified in the analysed material, i.e. tryptophol and gramine (indole alkaloids) as well as lupanine and 13-OH lupanine and lupanine esters – 13α- isovalericlupanine and 13α-tigloyloxylupanine (quinolysidine alkaloids).
2. Gramine is the dominant alkaloid in the growth stage (at 82.67% share in the alkaloid pool). At that stage apart from gramine also lupanine is synthesised.
3. The diversification of the quantitative and qualitative alkaloid composition in *Phalaris arundinacea* was observed. These differences may be explained by the differing habitat factors, primarily water availability, which seems to be a very important environmental factor affecting alkaloid contents.

6. References

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