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ISOXAZOLIN-5-ONE DERIVATIVES IN *LATHYRUS ODORATUS* DURING DEVELOPMENT AND GROWTH

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Key Word Index—*Lathyrus odoratus*; Leguminosae; sweet pea; non-protein amino acids; lathyrism; isoxazolin-5-ones.

Abstract—Variations in the concentrations of isoxazolin-5-ones and of some non-protein amino acids in the dry seeds, seedlings and various parts of mature *Lathyrus odoratus* plants were examined. The lathyrogenic compounds α-amino-γ-(isoxazolin-5-on-2-yl)-butyric acid, 2-cyanoethyl-isoxazolin-5-one and γ-glutamyl-β-aminopropionitrile were major products during development and growth.

INTRODUCTION

Some legume seedlings belonging to the genera *Pisum*, *Lathyrus* and *Lens*, contain high concentrations of isoxazolin-5-one derivatives [1]. In sweet pea (*Lathyrus odoratus*) α-amino-γ-(isoxazolin-5-on-2-yl)-butyric acid (**1**) can reach a concentration of 3.5% of the dry weight [1]. This product is metabolized to the neurolathyrogen, 2,4-diaminobutyric acid (DABA) in young chicks and can cause neurolathyrism [2]. In the same plant species 2-cyanoethyl-isoxazolin-5-one (**2**) can reach a concentration of 0.8% of the dry weight. Compound **2** causes osteolathyrism in rats [3]. It is also a major constituent of the sweet pea seedlings exudate [4], and can be degraded to β-aminopropionitrile (BAPN) [3].

In this paper we describe the variations in the concentrations of isoxazolin-5-ones, particularly the lathyrogenic compounds **1** and **2** in *Lathyrus odoratus* during development and growth.

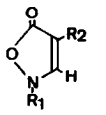
RESULTS AND DISCUSSION

The variations in the concentrations of isoxazolin-5-ones in seedlings of *Lathyrus odoratus* during the germination are given in Fig. 1 on a logarithmic scale. The concentrations of these compounds and of some other non-protein amino acids in different parts of the mature plant and in seedlings of different ages are given in Table 1.

Seedlings of *Lathyrus odoratus* contain β-(isoxazolin-5-on-2-yl)-alanine (**3**), β-(2-β-D-glucopyranosyl-isoxazolin-5-on-4-yl)-alanine (**4**), 2-aminoethyl-isoxazolin-5-one (**5**) and its γ-glutamyl derivative (**6**), **1**, **2** and 2-β-D-glucopyranosyl-isoxazolin-5-one (**7**). These different heterocyclic nitrogen compounds increased during 3 to 12 days growth and then gradually decreased (Fig. 1). Virtually no **3**, **4**, **5** and **7** were found in extracts of dry seeds and of 1-day-old seedlings. Dry seeds contain low concentrations of **1** (0.006% of the dry weight) and **2** and **6** (both 0.001% of the dry weight).

During imbibition of the seeds, **2** leaks out of the seeds and is absent from imbibed seeds. In the extracts from 12-day-old seedlings, **1** is the major free amino acid, making up 2.73% of the dry weight, while **3** (0.59%), **6** (0.64%) and **2** (0.09%) are present in lower concentrations.

Table 1 also gives the distribution of isoxazolin-5-ones and some other non-protein amino acids in different parts of *L. odoratus* at the flowering stage. The highest concentrations of the lathyrogens **1** and **2** are found in young seedlings, flower buds, petals and immature pods. In the roots, only **1** is a major constituent (together with smaller concentrations of **3**, **6**, **7** and γ-glutamyl-BAPN). While the highest concentrations of **1** are found in young seedlings, the highest concentrations of **2** are found in the



	R_1	R_2
1	$CH_2-CH_2-CH(NH_2)-COOH$	H
2	CH_2-CH_2-CN	H
3	$CH_2-CH(NH_2)-COOH$	H
4	β-D-glucopyranose	$CH_2-CH(NH_2)-COOH$
5	$CH_2-CH_2-NH_2$	H
6	$CH_2-CH_2-NHCO-CH_2-CH_2-CH(NH_2)-COOH$	H
7	β-D-glucopyranose	H

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Table 1. Variations in the concentrations of isoxazolin-5-one derivatives and some free amino acids in *Lathyrus odoratus* during development and growth

Plant organ	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6	Comp. 7	BAPN*	γ-glutamyl-BAPN	Har*	GABA*
Dry seeds	0.26†	0.06	—	—	—	0.06	—	0.39	2.92	6.68	0.07
3-day-old seedlings	7.35	1.79	5.92	0.17	+‡	1.05	2.18	—	12.86	0.98	0.33
7-day-old seedlings	12.83	0.95	5.26	0.18	+	2.45	2.64	—	36.98	—	0.96
12-day-old seedlings	12.31	0.55	2.97	0.15	+	2.14	1.39	—	21.26	—	0.66
16-day-old seedlings	8.15	0.41	2.21	0.13	+	1.78	1.02	—	20.70	—	0.48
21-day-old seedlings	6.65	0.27	1.70	0.11	+	1.52	0.77	—	15.87	—	0.30
Flower buds	1.23	4.66	—	—	+	0.46	—	—	3.93	—	1.20
Flower (no petals)	0.17	2.77	—	—	+	0.08	—	—	15.04	—	2.11
Petals	0.50	7.13	—	—	+	—	—	—	12.44	—	2.96
Immature pods	0.16	5.75	—	—	+	0.12	—	—	7.21	—	0.78
Immature seeds	0.95	0.99	—	—	+	0.20	—	0.47	15.60	—	7.70
Flower stems	0.11	0.13	—	—	+	0.08	—	—	4.56	—	0.60
Stems	0.19	0.14	—	—	+	0.09	—	—	9.24	—	(0.92)
Leaves	0.20	0.69	—	—	+	0.18	—	—	(5.88)	—	1.62
Roots	1.35	0.05	0.45	0.14	+	0.38	0.87	—	0.91	—	0.57

*BAPN = β-aminopropionitrile; Har = homoarginine; GABA = 4-aminobutyric acid.

†Concentrations of compounds were estimated by an automatic amino acid analyser as described in the Experimental and before [4] and are shown in μmol/g fresh weight.

‡+ stands for the presence of a compound when the peak was too small to be calculated.

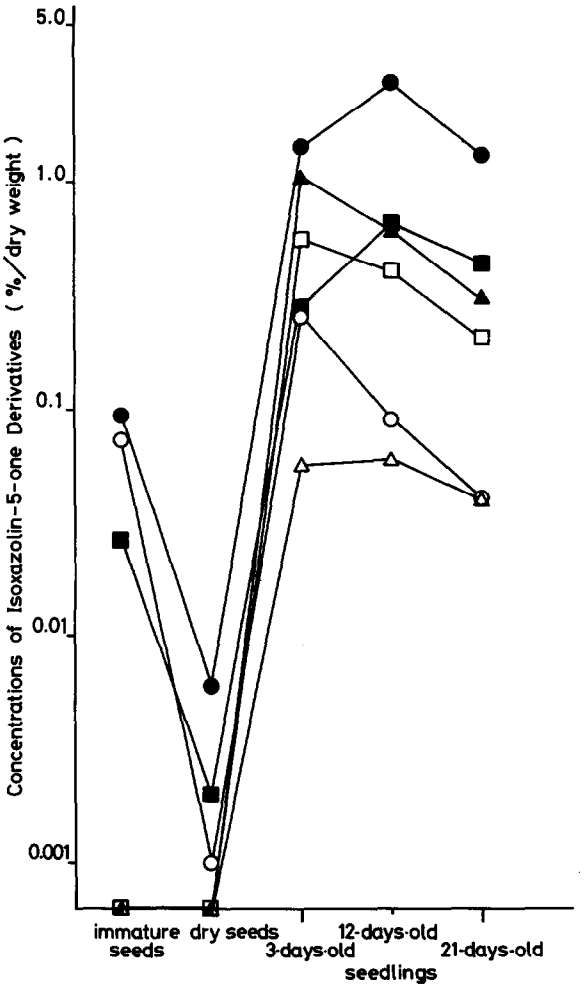

Fig. 1. Variations in the concentrations of isoxazolin-5-one derivatives in *Lathyrus odoratus* during development and growth. (●) Comp. **1**; (○) Comp. **2**; (▲) Comp.**3**; (△) Comp.**4**; (■) Comp. **6**; (□) Comp. **7**.

petals and in the immature pods; in these parts the concentration of **2** on a fresh weight basis is about 10 times greater than in 12-day-old seedlings and *ca* 100 times greater than in the dry seeds.

In the immature seeds three lathyrogens: **1**, **2** and γ-glutamyl-BAPN are major soluble compounds. During the ripening process 80% or more will disappear from the seeds. During germination the concentration of these three compounds again rises 10 to 20-fold.

In the flowering stage these compounds are present mainly in the reproductive parts of the plant. Other isoxazolin-5-one derivatives (**3**, **4**, **5** and **7**) are important soluble compounds in the seedling stage and disappear in older plants.

Both **2** and γ-glutamyl-BAPN are toxic in experimental animals [5, 6] and can be regarded as bound forms of BAPN. Free BAPN has been reported to inhibit seed germination [7]. Compound **2** is present in the seedlings of about one fifth of the species in the genus *Lathyrus* [8] and in the sweet pea seedling root exudate [4]. From our results it seems likely that these compounds may play a role in the chemical ecology of the genus *Lathyrus*. An unknown acidic isoxazolin-5-one derivative was also found in the immature seeds and pods.

EXPERIMENTAL

Plant materials. Sweet pea (*Lathyrus odoratus* L. cv Spencer) seeds were germinated in the dark at 25–26°. After 1, 3, 7, 12, 16 or 21 days, the seedlings were collected (cotelydons removed), ground with a chilled mortar and pestle and then extracted with 70% EtOH overnight at 2–4°. Sweet pea seeds were also grown in our botanical garden and collected at the flowering stage in October, 1981.

Determination of isoxazolin-5-ones and some free amino acids. The 70% EtOH extracts were concd *in vacuo* to 2 ml and then examined by 2-D TLC using *n*-BuOH–HOAc–H_2O (12:3:5) in one direction and PhOH–H_2O (80% by wt) in the other, and by using an automated amino acid analyser (JEOL, model JLC-5AH) coupled to a UV-monitoring flow system (JEOL) with detection at 260 nm according to the method of ref. [4].

Identification of homoarginine. The presence of homoarginine was confirmed also by HPLC of the *o*-phthalaldehyde derivatives with fluorescence detection after partial purification and separation from lysine by PC. After hydrolysis in 2.5 M LiOH at 105° for 18 hr the formation of lysine was shown by 2-D TLC, automated amino acid analysis and by HPLC.

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REFERENCES

1. Lambein, F., Kuo, Y. H. and Van Parijs, R. (1976) *Heterocycles* **4**, 567.
2. Lambein, F. and De Vos, B. (1981) *Arch. Int. Physiol. Biochim.* **89**, B66.
3. Van Rompuy, L., Lambein, F., Van Parijs, R. and Ressler, C. (1974) *Experientia* **30**, 1379.
4. Kuo, Y. H., Lambein, F., Ikegami, F. and Van Parijs, R. (1982) *Plant Physiol.* **70**, 1283.
5. Bell, E. A. (1980–81) *Food Chem.* **6**, 213.
6. Janzen, D. H., Juster, H. B. and Bell, E. A. (1977) *Phytochemistry* **16**, 223.
7. Wilson, M. F. and Bell, E. A. (1978) *Phytochemistry* **17**, 403.
8. Ikegami, F., De Blauwe, M. and Lambein, F. (1981) *Arch. Int. Physiol. Biochim.* **89**, B174.