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# SHORT REPORTS

## DISTRIBUTION OF STIZOLAMINE IN SOME LEGUMINOUS PLANTS

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**Key Word Index**—Lotoideae; Leguminosae; stizolamine; chemotaxonomy.

**Abstract**—The seeds of 38 species of 29 genera in 21 tribes of the Leguminosae were screened for stizolamine. It was detected in 19 species of the subfamily Lotoideae. Its occurrence in Lotoideae was wide (82% in tribes, 56% both in genera and species) but sporadic. The content was fairly varied (0·249–9540 nmol/g of seeds), the largest amount occurring in *Stizolobium hassjoo*.

### INTRODUCTION

A nitrogen-rich compound, stizolamine, was isolated from the seeds of *Stizolobium hassjoo* Piper and determined as 1-methyl-3-guanidino-6-hydroxymethylpyrazine-2-one [1]. This amine was also detected in etiolated plants.

Plants of the subfamily Lotoideae in Leguminosae are known to be rich in non-protein amino acids and their derivatives, e.g. canavanine [2], vicine [3] and lathyrine [4]. The distribution of stizolamine in Leguminosae was

Table 1. Distribution and content of stizolamine in leguminous seeds.

| Subfamily<br>Tribe<br>Species | Stizolamine content (nmol/g) |
|---|---|
| Caesalpinioideae | |
| Caesalpinieae | |
| *Delonix regia* Raf. | 0 |
| Cassieae | |
| *Cassia occidentalis* L. | 0 |
| Cerceae | |
| *Cercis chinensis* Bunge. | 0 |
| Lotoideae | |
| Sophoreae | |
| *Sophora japonica* L. | 9·08 |
| *S. fomentosa* L. | 1·17 |
| Cytiseae | |
| *Cytisus scoparius* Link. | 0 |
| Lupineae | |
| *Lupinus luteus* L. | 0 |
| Robinieae | |
| *Robinia pseudacacia* L. | 3·31 |
| Millettieae | |
| *Wistaria floribunda* DC. | 7·80 |
| Indigoferaeae | |
| *Indigofera decora* Lindl. | 0·249 |
| *I. pseudo-tinctoria* Matsum. | 16·8 |
| Astragaleae | |
| *Astragalus sinicus* L. | 0 |
| Cajaneae | |
| *Rhynchosia acuminatifolia* Makino | 0 |
| Lotoideae —*cont.* | |
| Diocleae | |
| *Canavalia lineata* DC. | 27·5 |
| *Pueraria thunbergiana* Benth. | 2·11 |
| Erythrineae | |
| *Erythrina* sp. | 0 |
| *Stizolobium hassjoo* Piper | 9540 |
| Phaseoleae | |
| *Dolichos lablab* L. | 0 |
| *Phaseolus vulgaris* L. var. *humilis* Alef. | 14·7 |
| *P. angularis* W. F. Wight | 0 |
| *P. aureus* Roxb. | 0 |
| Glycineae | |
| *Amphicarpa edgeworthii* Benth. var. *japonica* Oliver | 0 |
| *Dumasia truncata* Sieb. et Zucc. | 0·753 |
| *Glycine soja* Sieb. et Zucc. | 0 |
| *G. max* Merrill | 0 |
| Vicieae | |
| *Lathyrus odoratus* L. | 10·8 |
| *Pisum sativum* L. | 0 |
| *Vicia sativa* L. | 2·21 |
| *V. unijuga* Al. Br. | 0·781 |
| *V. faba* L. forma *anacarpa* Makino | 1·83 |
| Trifolieae | |
| *Trifolium repens* L. | 0 |
| *T. pratense* L. | 0 |
| Desmodieae | |
| *Desmodium racemosum* DC. | – |
| Stylosantheae | |
| *Arachis hypogaea* L. | 2·14 |
| Lespedezeae | |
| *Kummerowia striata* Schindl. | 150 |
| *Lespedeza pilosa* Sieb, et Zucc. | 0·499 |
| *L. cuneata* G. Don | 185 |
| Mimosoideae | |
| Eumimoseae | |
| *Leucaena glauca* Benth. | 0 |

therefore of interest in relation to these other nitrogenous substances.

## RESULTS AND DISCUSSION

The seeds of 38 species of 29 genera in 21 tribes were examined (Table 1). Stizolamine was detected in 19 species of the subfamily Lotoideae. It could not be detected in *Delonix regia* Raf., *Cassia occidentalis* L., and *Cercis chinensis* Bunge. belonging to Caesalpinioideae or in *Leucaena glauca* Benth. of the Mimosoideae, but its absence from these two subfamilies cannot be assumed until a proper sample has been examined. Its occurrence in Lotoideae was wide (82% in tribes, 56% both in genera and species tested) but very sporadic. The content was fairly varied (0.249–9540 nmol/g of seeds), and always less than that originally found in *Stizolobium hassjoo*. The maximum content was obtained in *Lespedeza cuneata* G. Don., and the minimum in *Indigofera decora* Lindl.

Turner and Harborne [5] have reported that canavanine is found only in the subfamily Lotoideae and stizolamine, also a guanidino compound, seems to have a similar distribution.

## EXPERIMENTAL

*Plant materials.* The seeds of *Cercis chinensis* Bunge., *Indigofera pseudotinctoria* Matsum., *Glycine soja* Sieb. et Zucc. and *Vicia unijuga* Al. Br. were collected at Saruhashi in Yamanashi Prefecture, *Rhynchosia acuminatifolia* Makino, *Pueraria thunbergiana* Benth., *Lespedeza pilosa* Sieb. et Zucc., *Kummerowia striata* Schindl., *Dumasia truncata* Sieb. et. Zucc. and *Desmodium racemosum* DC. at Kakio in Kanagawa Prefecture and *Erythrina* sp. at Izu-kogen in Shizuoka Prefecture. The seeds of *Sophora japonica* L., *Cytisus scoparius* Link., *Indigofera decora* Lindl., *Amphicarpa edgeworthii* Benth. var *japonica* Oliver and *Lespedeza cuneata* G. Don. were gathered at Fukazawa, Setagaya-ku in Tokyo, *Wistaria floribunda* DC. in Akasaka, Minato-ku in Tokyo and *Cassia occidentalis* L., *Sophora fomentosa* L., *Canavalia lineata* DC. and *Leucaena glauca* Benth. at Ogasawara in Tokyo. Further, the seeds of *Delonix regia* Raf., *Kobinia pseudo-Acacia* L., and *Vicia sativa* L. were supplied by Dr. S. Kobayashi, Makino Herbarium, Tokyo Metropolitan University. The other plant materials were all commercial sources.

*Isolation and determination of stizolamine.* Five g (or 3 g) of seeds were crushed in 30 ml hot $H_2O$ and extracted with 4 × 100 ml hot 50% MeOH containing 1 ml of HOAc for each 1 hr. The combined extracts were concentrated *in vacuo*, the remaining residue dissolved in 100 ml of hot $H_2O$ and insoluble materials filtered off. The filtrate was passed through the Amberlite IRA 410 ($OH^-$ form) column, the effluent was concentrated and dissolved in 100 ml 0·01 N NCl. The solution was concentrated and dissolved in 5 ml of $H_2O$ (or 3 ml of $H_2O$). 50 μl of this crude extract was spotted on 2 cm wide papers and developed with *n*-BuOH–HOAc–$H_2O$ (6:1:2) and 6% HOAc, separately. Stizolamine was detected on chromatograms by its blue fluorescence and colour reactions with alkaline nitroprusside–ferricyanide and Dragendorff's reagents at $R_f$ value 0.3 (the former solvent) and at 0·7 (the latter solvent) [the limitation value of detectable concentration by fluorescence; 3.33 pmol per 2 cm width]. An aliquot (1.5 ml) of the crude extract was spotted on paper (0.5 ml/20 ml of width) and developed with the former solvent. The blue fluorescent substance at $R_f$ 0.3 was extracted with 50% MeOH, concentrated and dissolved in 20 ml $H_2O$. The soln was applied on CM-cellulose column (1.6 × 20 cm) and column was washed with 50 ml $H_2O$. The amine adsorbed on the column was then eluted with 1 N HOAc. The eluate was concentrated *in vacuo*, dissolved in 10 ml of $H_2O$, passed through the Amberlite IRA 410 ($OH^-$ form) column. The effluent was concentrated, dissolved in 1.5 ml of $H_2O$, applied on Sephadex G-10 column (2 × 49 cm) saturated with 0.1 M $NH_4Ac$ and chromatographed with the same solvent (flow rate; 6 ml/hr, fraction volume; 2 ml). In this procedure, the most abundant amount of stizolamine was detected at 34th fraction. Stizolamine was measured fluorometrically (Excited at 350 nm, Analysed at 390 nm).

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# FUCOIDAN IN *PADINA SANCTAE-CRUCIS* SPORES

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**Key Words Index**—*Padina sanctae-crucis*; Dictyotaceae; fucoidan; sulphated polysaccharide, spore.

**Abstract**—The presence and distribution of fucoidan in the vegetative fronds and spores of the brown alga *Padina sanctae-crucis* Børg. was studied. Autoradiography using $^{35}S$ showed that fucoidan is localized in the walls of the

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