

***Linum mucronatum*: Organ to Organ Lignan Variations**

Abdolali Mohagheghzadeh^{a,*}, Azra Gholami^a,
Mohammad Soltani^a, Shiva Hemmati^{a,b},
and A. Wilhelm Alfermann^b

^a Department of Pharmacognosy, Faculty of Pharmacy,
Shiraz University of Medical Sciences and Health
Services, P.O. Box 71345-1583, Shiraz, I.R. Iran.
Fax: +98-711-2426070. E-mail: Mohaghegh@sums.ac.ir

^b Institut für Entwicklungs- und Molekularbiologie
der Pflanzen, Heinrich-Heine-Universität Düsseldorf,
Universitätsstr. 1, D-40225 Düsseldorf, Germany

* Author for correspondence and reprint requests

Z. Naturforsch. **60c**, 508–510 (2005);
received January 24, 2005

The percentage of podophyllotoxin (PTOX) and its congener lignans were measured by HPLC in *Linum mucronatum* ssp. *mucronatum* (Linaceae) fresh plant organs. The highest amounts of PTOX ($0.595 \pm 0.060\%$ g/g dry wt) and 6-methoxypodophyllotoxin (MPTOX) ($1.491 \pm 0.125\%$ g/g dry wt) were found in the plant sexual organs. Whereas, the highest levels of β -peltatin, 5'-demethoxy-MPTOX and yatein were found in not developed buds, petals and sepals, respectively.

Key words: Aryltetralin Lignans, Linaceae,
Linum mucronatum ssp. *mucronatum*

Introduction

Linaceae, one of the flowering plant families, consists of genera including *Anisadenia*, *Cliococca*, *Hesperolinon*, *Linum*, *Radiola*, *Reinwardtia*, *Sclerolinon* and *Tirpitzia* (Heywood, 1985). The genus *Linum* is the largest genus in this family consisting of a large group of herbs and sub-shrubs divided into the sections *Syllinum*, *Cathartolinum*, *Dasylinum*, *Linum* and *Linastrum* according to classical taxonomy based on morphological characters (Ockendon and Walters, 1968). The occurrence of lignans in the *Linum* genus is documented since 1975 (Weiss *et al.*, 1975; Berlin *et al.*, 1986; Broomhead and Dewick, 1990; Wichers *et al.*, 1990), aryltetralin lignans occur in the section *Syllinum* (Weiss *et al.*, 1975; Broomhead and Dewick, 1990; Mohagheghzadeh *et al.*, 2003; Konuklugil *et al.*, 1999, 2001), whereas aryl-naphthalene lignans (Mohagheghzadeh *et al.*, 2002) and simple acyclic, furanofuran- and dibenzylbutyrolactone-type lignans (Meagher *et al.*, 1999) have been found in the section *Linum*, and dibenzylbutyrolactone lignans has been recorded in species of

Linastrum (Mohagheghzadeh *et al.*, 2003). All studied species from *Linum* genus that produce podophyllotoxin (PTOX) show the production of 6-methoxypodophyllotoxin (MPTOX) besides PTOX in different ratio (Mohagheghzadeh *et al.*, 2003; Konuklugil *et al.*, 1999, 2001). Lignans are present at different levels of abundance in plant parts including roots, stems, leaves, petals and flowers (Mohagheghzadeh *et al.*, 2003).

The presence of PTOX and MPTOX was reported in *L. mucronatum* ssp. *armenum* cell cultures previously (Konuklugil *et al.*, 2001). According to the previous work (Mohagheghzadeh *et al.*, 2003), the section *Syllinum* was divided into three sub-groups. Our evaluation consists of the analysis and determination of lignans in different parts of *L. mucronatum* Bertol. ssp. *mucronatum*, one of species belonging to the sub-group b of section *Syllinum*.

Results and Discussion

The percentage contents of PTOX and related lignans in different parts of *L. mucronatum* ssp. *mucronatum* are summarized in Table I. The results show a variation in the distribution and relative abundance of lignans from plant organs. The highest amounts of PTOX (0.595% g/g dry wt) and MPTOX (1.491% g/g dry wt) were found in the sexual organs ($p < 0.001$). Furthermore, considerable amounts of MPTOX were found in petals and not developed buds (0.951 and 0.946% g/g dry wt, respectively). MPTOX has been reported from different plant parts of *Linum flavum* and agar-grown plants results showed that roots and stems are important accumulation sites for MPTOX (Wichers *et al.*, 1990). Like the other yellow flower *Linum* spp. belonging to the sub-group b of the section *Syllinum* (Mohagheghzadeh *et al.*, 2003), the *L. mucronatum* plant is rich in MPTOX. The highest levels of β -peltatin (β -PLT, 0.928% g/g dry wt), 5'-demethoxy-MPTOX (0.994% g/g dry wt) and yatein (YAT, 1.480% g/g dry wt) were found in not developed buds, petals and sepals, respectively. Lignan study in different organs will guide us to more elucidation of the biosynthetic pathway in the plant. On the whole, *L. mucronatum* ssp. *mucronatum* is rich in aryltetralin lignans and the plant cultivation would be an interesting method for the production of aryltetralin lignans in the large scale.

Table I. Lignan contents in different organs of *Linum mucronatum* ssp. *mucronatum*^{a,b}.

Plant part	PTOX	Significance differences ^c	β -PLT	Significance differences	5'-Demethoxy-MPTOX	Significance differences	MPTOX	Significance differences	YAT	Significance differences
Root	0.125 $\pm$ 0.033	Ψ, ξ	0.306 $\pm$ 0.268		0.053 $\pm$ 0.046	$\perp$	0.701 $\pm$ 0.052	Ψ	–	Ψ
Peripheral branches	0.081 $\pm$ 0.008	Ψ, ξ	0.350 $\pm$ 0.072		0.064 $\pm$ 0.001	Ψ	0.215 $\pm$ 0.015	Ψ	0.049 $\pm$ 0.043	Ψ
Leaves	0.061 $\pm$ 0.008	Ψ, ξ	$\uparrow^d$	Ψ	–		0.109 $\pm$ 0.065	Ψ	–	Ψ
Bud (not developed)	0.201 $\pm$ 0.166	Ψ	0.928 $\pm$ 1.066	Ψ (***)	0.656 $\pm$ 0.038		0.946 $\pm$ 0.154	ξ	0.077 $\pm$ 0.005	Ψ
Bud (developed)	0.139 $\pm$ 0.013	Ψ	$\uparrow$	Ψ	0.599 $\pm$ 0.052		0.672 $\pm$ 0.056	$\perp$	0.121 $\pm$ 0.028	Ψ
Receptacle	0.124 $\pm$ 0.036	Ψ	0.061 $\pm$ 0.054	Ψ	0.208 $\pm$ 0.006		0.342 $\pm$ 0.142	Ψ	$\uparrow$	Ψ
Sepals	0.096 $\pm$ 0.034	Ψ, ξ	0.854 $\pm$ 0.061		0.133 $\pm$ 0.027	Ψ	0.140 $\pm$ 0.016	Ψ	–	Ψ (*)
Petal	0.280 $\pm$ 0.025	$\perp, \xi$ (***)	– ^e	Ψ	0.994 $\pm$ 0.140	Ψ (***)	0.951 $\pm$ 0.151	$\perp$	–	Ψ
Sexual organs	0.595 $\pm$ 0.060	Ψ (*), $\perp$ (**)	–	Ψ	0.854 $\pm$ 0.057	Ψ (***)	1.491 $\pm$ 0.125	ξ (***)	0.212 $\pm$ 0.185	Ψ

^a Values calculated on dry plant materials (% g/g dry wt) are mean $\pm$ S.D. ($n = 3$), ANOVA test.
^b 5'-Demethoxy-MPTOX, MPTOX and YAT were quantified according to commercial PTOX.
^c Ψ , $\perp$, ξ : significant different from other indicated values in the same column; (*) $p < 0.001$, (**) $p < 0.01$, (***) $p < 0.05$.
^d Trace ($t < 0.001$).
^e No peak at the retention time of this compound was detected.

Experimental

Plant material

Linum mucronatum Bertol. ssp. *mucronatum* (Linaceae) plant materials were collected in April 2003 from Tang-e-Abol Hayat, Shiraz-Kazeroun road, Fars province, Iran, at an altitude of 1000 m. Voucher specimens (No. 237) were deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Shiraz University of Medical Sciences. The samples were identified by Iraj Mehregan (Institut für Spezielle Botanik und Botanischer Garten, Johannes Gutenberg-Universität Mainz, Germany).

Different parts of the perennial herbaceous plant were separated carefully based on the morphology for the lignan assay as follows: roots perennial thick, slightly branched and twisted; stems up to 40 cm high, with peripheral sterile branches at base bearing imbricate, spatulate leaves; fertile stems bearing alternate 1–3 veined leaves and ending to cymes of yellow flowers; stem leaves 5–30 mm long and 2–6 mm width, simple, linear-oblong, mucronate to acute, basal ones smaller than upper ones; not opened buds lanceolate, petals are hidden by lanceolate sepals; flowers actinomorphic; receptacle bearing 5 sepals, 5 petals, 5 stamens alternating with 5 staminoids, and a 5-loculate superior ovary becomes a $\pm$ spherical capsule; sepals lanceolate, acute, 7–9 mm long and 1–2 mm width, membranous at margins; petals yellow, 15–28 mm long, connected with claws at base.

Quantitative analysis

Fresh plant materials were frozen and then lyophilized by freeze-dryer. Extraction and determination of lignans were performed as described by Empt *et al.* (2000). PTOX, β -PLT, 5'-demethoxy-MPTOX, MPTOX and YAT were determined at $R_t = 9.15$ min, 10.12 min, 12.53 min, 13.03 min and 26.26 min, respectively. Calibration curves for PTOX and β -PLT standards were drowning (inter-day and intra-day injections) in the range of 0.9–400 $\mu\text{g/ml}$ and 0.7–200 $\mu\text{g/ml}$, respectively. 5'-Demethoxy-MPTOX, MPTOX and YAT were quantified according to commercial PTOX (Roth 3946.1). Accuracy of each quantified lignan was admitted by retention time, co-chromatography and measuring on-line UV spectra using a Thermo Quest HPLC system (Egelsbach, Germany) equipped with a spectra system KO 6000 LP photodiode array detector.

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