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ALKALOID ACCUMULATION DYNAMICS IN *Veratrum lobelianum* GROWING IN GEORGIA AND BIOLOGICAL ACTIVITY OF JERVINE

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The alkaloid accumulation dymanics in Veratrum lobelianum *were investigated. Jervine was found from 0.02 to 0.11% in the subterranean part at all vegetation stages. The jervine content was greatest in subterranean plant organs, reaching a maximum (0.3%) during natural dying off of aerial organs. Jervine, a natural analog of serotonin, can be used as a specific fibroblast growth factor.*

Key words: *Veratrum lobelianum*, alkaloid accumulation dynamics, jervine, biological activity.

Veratrum lobelianum growing in various regions of Georgia has yielded 12 pure alkaloids. One of the main alkaloids is jervine [1-4]. Jervine exhibited activity in screening experiments in a test system of isolated organs sensitive to serotonin.

We studied the qualitative and quantitative composition of the total alkaloids and the jervine content in various plant organs by vegetative phases in order to find the optimal collection times of the raw material and to isolate biologically active compounds from *V. lobelianum*. According to our results, total alkaloids in *V. lobelianum* varied as functions of development phase and plant habitat [1, 3].

The jervine accumulation dynamics in aerial organs of *V. lobelianum* (Table 1) showed that the alkaloid is present in practically all stages of active vegetation. The content was maximum at a plant height of 20-30 cm. The variability of the alkaloid content separately in stems and leaves showed that jervine accumulated more in leaves at the early stage of plant development whereas the content was higher in stems than in leaves at the start of flowering. The situation changed during fruiting. The amount of jervine decreased in stems and increased in leaves although it remained less than at the start of vegetation.

Pseudojervine appeared in the total alkaloids slightly later after the plant height reaches 20-30 cm. Veralosine dominated compounds containing the 22,26-iminocholestane skeleton among total alkaloids in subterranean organs of *V. lobelianum* in the early vegetative stages. The veralosidine and veralosinine contents remained practically constant from the appearance of young shoots until the plant reached a height of 20-30 cm.

A spot for rubijervine was observed by TLC in glucoalkaloid fractions from *V. lobelianum* that attained a height of 20-30 cm.

The variability of jervine, pseudojervine, and rubijervine contents in rhizomes with roots showed that the jervine concentration gradually increased from the moment of appearance of aerial organs and reached a maximum during natural dying off of aerial organs whereas the accumulation of pseudojervine was maximum at the start of vegetation and then gradually decreased and reached a minimum when aerial organs start to die off. The rubijervine content remained practically constant until flowering, decreased slightly during flowering, and decreased toward the end of vegetation.

The presence of verazine was observed chromatographically in roots at all vegetative stages and in the aerial part of plants from 3 to 30 cm high.

The content of total alkaloids in rhizomes with roots of *V. lobelianum* showed that the amount of alkamines increased from the very start of the appearance of aerial organs and continued until fruiting, then decreased slightly, and increased again during natural dying off of aerial organs, reaching a maximum (Table 2).

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TABLE 1. Accumulation Dynamics of Pure Alkaloids in Aerial and Subterranean Parts of *Veratrum lobelianum*, % of Absolutely Dry Raw Material

Height of aerial plant part, cm	Jervine			Pseudojevine		Rubijervine	Veralosine	Veralosinine	Veralosidine
	leaves	stems	subterranean part	aerial part	subterranean part	subterranean part	aerial part		
3-5*	-	-	0.095	+	0.190	0.100	0.057	0.020	0.030
10-15	0.110	0.017	0.100	+	0.087	-	0.045	0.023	0.032
20-30	0.100	0.030	0.130	0.059	0.058	0.110	0.010	0.020	0.340
50-60	0.048	0.075	0.170	0.040	0.052	-	+	+	+
70-80	0.080	0.020	0.200	0.008	0.055	0.140	-	-	-
90	0.078	0.050	0.180	+	0.052	0.073	-	-	-
100	+	+	0.300		0.040	0.035			

*0.110% jervine was observed in the aerial part.
(-), not observed chromatographically.
(+), observed chromatographically.

TABLE 2. Accumulation Dynamics of Alkamines and Glucoalkaloids in *Veratrum lobelianum* Rhizomes with Roots by Vegetative Phases

Vegetative phase	Total alkamine content, % of absolutely dry raw material		Total glucoalkaloid content, % of absolutely dry raw material	
	rhizomes	roots	rhizomes	roots
Start of vegetation	1.50	1.00	0.70	1.00
Flowering	1.60	1.10	1.00	1.20
Fruiting	1.38	1.50	0.80	0.61
Natural dying off of aerial organs	2.35	1.59	0.60	0.45

The opposite picture was seen for accumulation of total glucoalkaloids. As the plant grew and developed, the alkaloid concentration decreased to a minimum toward the end of vegetation. Thus, the content of alkamine compounds increased as the plant grew and developed whereas the glucoalkaloid concentration decreased.

The investigation showed that rhizomes with roots during natural dying off of aerial organs are recommended for preparing the biologically active alkaloid jervine.

As noted above, jervine exhibited stimulatory activity in a test system of isolated organs sensitive to serotonin (guinea pig ileum and rat stomach and uterus). The role of serotonin circulating in blood is not yet clear [5]. However, this bioamine in fibroblast cultures at concentrations close to the physological ones stimulates cell multiplication and adhesion as a result of interaction with the corresponding membrane receptors [5, 6].

The effect of jervine on the behavior of fibroblasts as the principal cell elements of connective tissue was studied in this model system. As it turned out, jervine had an effect on fibroblasts similar to that of serotonin. In particular, jervine at micromolar concentrations (10^{-7} M) stimulated proliferation. Furthermore, cells grew in the presence of jervine even with a minimal serum content (2.0-2.5% instead of the standard 10%) in the medium. The ability of jervine to stimulate fibroblast growth was also apparent upon its addition to a medium of fibroblasts "starving" (depleted of serum) for 48 h. In this instance a micromolar solution of jervine in a medium with 0.5% serum renewed the growth of cultures similar to nutrient medium with 10% serum. Jervine did not exhibit a stimulating effect in the presence of a serotonin pharmacological antagonist (ciproheptadine). This indicates that jervine must interact with serotonin receptors of fibroblasts to activate culture growth.

As a rule, jervine increased the fibroblast population by 40-80%. An increase in the number of cells less than 40% was disregarded (the difference is unreliable at this probability level, P = 0.05, about 15% of the instances). Adding antagonist beforehand blocked completely the stimulating action of jervine. Simultaneous administration of ciproheptadine and a double dose of jervine stimulated completely culture growth. Consequently, jervine activates proliferation by interacting with serotonin receptors in the fibroblast membrane. Therefore, it is obvious that jervine is a natural serotonin analog and can be used as a specific fibroblast growth factor. From a practical viewpoint, jervine is proposed for use in development of "serum-free" media for fibroblast cultures and preparations that stimulate wound healing by topical application.

EXPERIMENTAL

Jervine was determined quantitatively by a chromatography—spectroscopy method developed by us earlier [7].

The alkaloids pseudojervine, rubijervine, veralosine, veralosinine, and veralosidine were determined using a planimetric analytical method based on measuring areas of spots in chromatograms [8-12]; for TLC, silica gel LS 5/40 plates (Czech Rep.) and kieselgel (Merck); for PC, Leningrad "M" impregnated with formamide. Spot areas were determined using the formula $S = 3.14Rr\ mm^2$. A calibration curve was constructed using the mathematical expression for the logarithm of the spot area as a function of the logarithm of the amount of substance. The layer thickness was determined by calculating silica gel or kieselgel (2.0 g) + distilled water (4 mL) on plates of dimension 4 × 12. For TLC, we used benzene:ethanol (9:1.5 and 9:2.5), chloroform:methanol (6:1), chloroform:ethanol (9:1), and chloroform:methanol:ammonia (25%) (86:14:1 and 25:1:0.2). For PC, we used formamide-saturated chloroform and formamide-saturated chloroform:benzene (1:1 and 1:2). The accuracy of a single TLC determination approached the conditions of PC.

V. lobelianum was collected in Kazbeg region in 2003 during various vegetative phases. Total alkamines and glucoalkaloids from rhizomes with roots were obtained by the literature method [13]. The yield of total compounds and fraction s were determined gravimetrically.

Air-dried aerial plant parts (100 g) were extracted with ethanol (1:7) in a Soxhlet apparatus. The alcohol extracts were condensed and dissolved in acetic acid (10%). The aqueous-acid solution was extracted with $CHCl_3$. The $CHCl_3$ was distilled off. The jervine content in the solid was determined by a chromatography—spectrophotometry method. The remaining aqueous-acid solution was treated with $(NH_4)_2CO_3$ (3 M). The resulting nonalkaloid solid was filtered off. The filtrate was made basic with NH_4OH (25%) until the pH was 8, extracted with diethylether, made basic until the pH was 10, and extracted with $CHCl_3$. The contents of veralosine, veralosidine, and veralosinine were determined planimetrically in the ether extract; of pseudojervine, in the $CHCl_3$ extract.

Air-dried aerial plant parts (100 g) were extracted with ethanol (1:5) in a Soxhlet apparatus. The alcohol extract was condensed and dissolve in tartaric acid (5%). The aqueous-acid solution was washed with $CHCl_3$ (1:1). The remaining aqueous-acid solution was neutralized with NH_4OH (25%) until the pH was 6 and extracted with $CHCl_3$ to produce a fraction in which the contents of jervine and rubijervine were determined. The weakly acidic aqueous solution was then made basic until the pH was 10 and extracted with $CHCl_3$. The pseudojervine content in the extract was determined.

Primary cultures of mice embryo fibroblast-like (MEF) cells and passed lines of L-197 and L-929 mouse fibroblasts were used in the experiments. The cultivation method was described before [14]. The cell concentration on innoculation of primary cultures was 8×10 cell/mL; for passed cultures, 5×10 cell/mL. Jervine and the competing serotonin antagonist ciproheptadine were added to the culture medium at the time of cell innoculation and to 4-day cultures starved for 48 h. The final alkaloid and ciproheptadine concentrations were 10^{-7} M. The corresponding starting doses of these agents were 4.0 ng/mL (jervine) and 2.9 ng/mL (ciproheptadine). The density of a monolayer in experimental and control cultures was determined after 24 h.

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