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Note

Determination of vasicine and related alkaloids by gas chromatography–mass spectrometry

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Goat's rue, *Galega orientalis* Lam., is a promising perennial forage legume for Finnish climatic conditions[1]. However, its fodder value is thought to be impaired by the bitter-tasting quinazoline alkaloid vasicine[2]. Plants containing vasicine have long been used in folk medicine, and a number of pharmacological activities have been described[3]. In addition to effects on the respiratory system, vasicine has ben reported to have oxytocic, abortifacient and even insecticidal properties[4,5]. Its acute toxicity is 290 mg/kg (LD_{50}, oral, mouse)[6].

Thin-layer chromatography has traditionally been used for the determination of alkaloids in *Galega* species[2,7]. Recently, a high-performance liquid chromatographic method was developed to determine vasicine and vasicinone levels in *Adhatoda vasica* leaves[8].

As only traces of vasicine could be found in our preliminary tests on goat's rue samples, the need arose for a sensitive and rapid technique. This paper reports a gas chromatographic–mass spectrometric (GC–MS) method for the determination of vasicine in *Galega* species.

EXPERIMENTAL

Most of the *G. orientalis* used for experiments in Finland originates from goat's rue populations cultivated in the Estonian SSR[1,9]. The following amounts of material were used for the preparation of a single sample: 4 g of seeds of *G. orientalis* and dried herbs at different stages of development grown both in the field and in greenhouses; 1 g of seeds of wild *Galega officinalis* L. plants collected from Manawatu River Valley, New Zealand (by Dr. K. Lindström), and dried herbs at different stages of development grown in greenhouses; 1 g of dried herbs of *Peganum harmala* L. from the Botanical Garden of Turku University; and 1 g of dried herbs of *Linaria vulgaris* L. collected from Southern Finland.

Alkaloid samples were prepared by modifying the method described earlier[8]. Alkaloids were extracted with 20 ml of 40% ethanol and 1 ml of scopolamine

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hydrobromide solution (1 mg/ml, scopolamine · HBr · $2.5H_2O$; Sigma, St. Louis, MO, U.S.A.) was added as an internal standard. Of this extract, 2.5 ml was made alkaline with 10% ammonia solution and extracted with 1 ml of chloroform (E. Merck, Darmstadt, F.R.G.). The chloroform extract was dried with anhydrous sodium sulphate and evaporated to 100 μl.

Mass spectrometric identification was performed on a Hewlett-Packard (HP) 5890 gas chromatograph coupled to an HP 5970 quadrupole mass-selective detector. The latter was operated at 70 eV with a scan rate of 1100 a.m.u./s, an electron multiplier voltage of 1800 V and an ion source temperature of 250°C. The mass spectrometer was controlled by an HP 9825B desktop computer with an HP 9134 disc memory for data storage. The samples were analysed on a fused-silica capillary column coated with NB-54 (15 m × 0.20 mm I.D.; Nordion, Finland). The oven temperature was 240°C and helium was used as the carrier gas at a flow-rate of 0.5 ml/min.

GC–MS identification was based on computer matching against library spectra built up from samples of pure vasicine, and on published MS data for vasicine, vasicinone, desoxyvasicine and desoxyvasicinone[8,10–12]. In quantitative analyses by selected ion monitoring (SIM), the fragment ions were of *m/z* 187 (vasicine), 146 (vasicinone), 171 (desoxyvasicine), 185 (desoxyvasicinone) and 94 (scopolamine). When analysing vasicine levels in *G. orientalis*, the fragment ion of *m/z* 188 was also used (Fig. 2).

RESULTS AND DISCUSSION

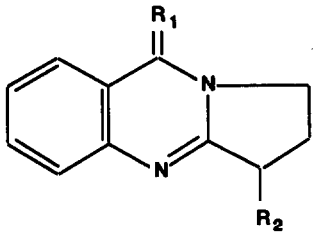
The ten most abundant fragments of the four quinazoline alkaloids are presented in Table I. The concentrations of vasicine found in *P. harmala*, *G. officinalis* and *L. vulgaris* were high enough to obtain a reliable spectral comparison with an

TABLE I

FRAGMENTATION AND NORMALIZED ABUNDANCES OF VASICINE AND ITS DERIVATIVES

Desoxyvasicine (1)[a]		*Vasicine (2)*[a]		*Desoxyvasicinone (3)*[a]		*Vasicinone (4)*[a]	
m/z	*Abundance (%)*	*m/z*	*Abundance (%)*	*m/z*	*Abundance (%)*	*m/z*	*Abundance (%)*
171	100	187	100	185	100	146	100
172[b]	42	188[b]	50	186[b]	79	202[b]	79
116	8	131	26	130	12	119	58
68	8	159	23	76	10	145	24
129	6	77	12	102	10	118	24
77	6	104	8	187	9	130	17
51	6	89	8	129	9	147	16
41	6	169	7	50	9	129	15
143	5	189	6	103	8	187	11
85	5	171	5	77	7	203	11

[a] Elution order on NB-54 column: *cf.*, Fig. 2.
[b] $[M]^+$, electron impact mode.



Vasicine	$R_1 = H_2$, $R_2 = OH$
Vasicinone	$R_1 = O$, $R_2 = OH$
Desoxyvasicine	$R_1 = H_2$, $R_2 = H$
Desoxyvasicinone	$R_1 = O$, $R_2 = H$

Fig. 1. Structures of vasicine and related alkaloids.

authentic sample. The spectra of desoxyvasicine, vasicinone and desoxyvasicinone, corresponding to the data presented in the literature[8,10,11], were obtained from analyses of *P. harmala* and were used as the basis for comparison in the remaining experiments.

Vasicine is the major alkaloid in *G. officinalis*[7,13] accompanied by its oxidation product vasicinone (Fig. 1). In order to avoid further oxidation of vasicine during extraction, ethanol (40%) was used as the solvent according to an earlier report[8]. Galegine, a guanidine alkaloid which is another poisonous compound in this plant[13,14], could not be confirmed by means of the present analyses, probably because of thermal decomposition in the GC–MS system. No significant amounts of galegine have been found in *G. orientalis*[15].

The reproducibility of the GC–MS–SIM method is shown in Table II. The mean relative standard deviations (R.S.D.) for vasicine and vasicinone contents were 3.5% and 3.4%, respectively, in the analyses of *G. officinalis* samples. With *G. orientalis*, the SIM analyses were unreliable when the fragment ion of *m/z* 187 was used for vasicine (R.S.D. = 13.1%). However, the reproducibility was considerably improved by selecting the ion of *m/z* 188 (R.S.D. = 6.9%) (Table II).

TABLE II

REPRODUCIBILITY OF QUANTITATIVE ANALYSES OF VASICINE AND VASICINONE BY GC–MS–SIM TECHNIQUE (n = 6)

Compound	*G. officinalis*		*G. orientalis*			
	Mean (μg/g)	*R.S.D. (%)*	*Mean (μg/g)*	*R.S.D. (%)*	*Mean (μg/g)*	*R.S.D. (%)*
Vasicine	3470[a]	3.5	1.4[a]	13.1	1.6[c]	6.9
Vasicinone	258[b]	3.4	–	–	–	–

[a] Fragment of *m/z* 187.
[b] Fragment of *m/z* 146.
[c] Fragment of *m/z* 188.

TABLE III

CONTENT OF QUINAZOLINE ALKALOIDS IN DRIED PLANT MATERIALS

Plant material	*Vasicine (%)*	*Vasicine (literature)*		*Vasicinone (%)*	*Desoxyvasicine (%)*	*Desoxyvasicinone (%)*
		%	*Ref.*			
G. orientalis herbs	$(1.4–1.6) \cdot 10^{-4}$	+	2			
G. officinalis herbs	0.1–0.35	0.0–0.3	13	0.03	$6.5 \cdot 10^{-4}$	$11.3 \cdot 10^{-4}$
G. officinalis seeds	0.03	+	2	$9.3 \cdot 10^{-4}$	$12.2 \cdot 10^{-4}$	$3.1 \cdot 10^{-4}$
P. harmala herbs	0.35	0.2–0.5	16	0.03	0.12	0.01
L. vulgaris herbs	0.03	0.5–0.8	17	$30.8 \cdot 10^{-4}$		

According to the GC–MS–SIM analyses, the content of vasicine varied between 0.1 and 0.35% in *G. officinalis* herbs of different development stages (Table III, Fig. 2). Recovery experiments, which were made by adding pure vasisine to a *G. officinalis* extract, gave a mean value of 96.7%. The quantitative results agree with the literature values[16] also in the case of *P. harmala*.

With respect of the presence of vasicine in *G orientalis*, no quantitative data have previously been presented. Its occurrence was verified by typical fragments (Table I, Fig. 2), the concentration remaining at ppm level in all the samples from this plant (Table III). It is obvious that such a low vasicine content cannot be responsible for lowering the fodder value of goat's rue. However, larger amounts of material need to be screened to evaluate the variations in alkaloid production and especially the proportion of the variations due to colder environmental conditions.

The results show that vasicine and its related alkaloids can be reliably determined using the GC–MS–SIM technique. The method is rapid and sensitive and therefore highly suitable for controlling the vasicine content in goat's rue.

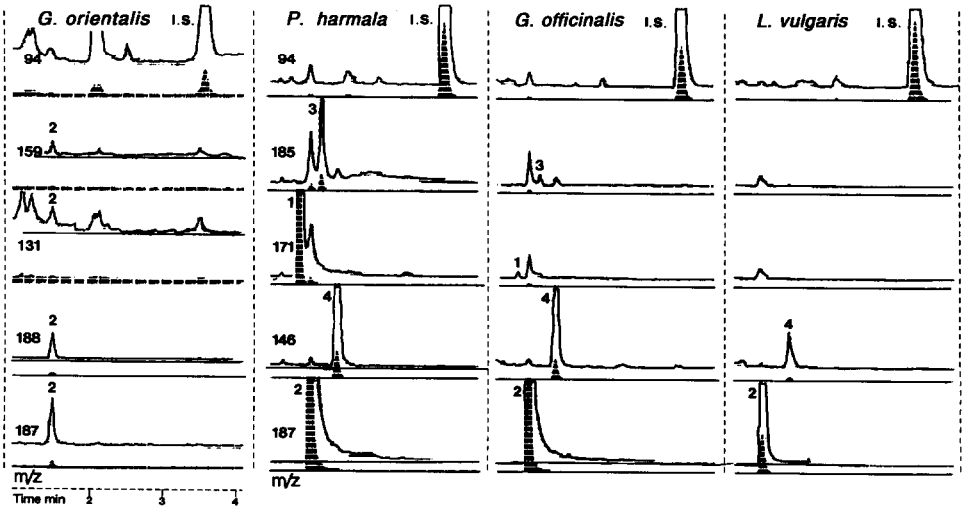


Fig. 2. GC–MS–SIM of quinazoline alkaloids. Peak numbers refer to constituents in Table I.

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