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Note

Determination of alkaloids of *Fumaria parviflora* and *Fumaria capreolata* by high-performance liquid chromatography and capillary isotachophoresis

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The plants *Fumaria parviflora* (Lam.) and *F. capreolata* (L.) have been employed in folk medicine for a variety of therapeutic purposes[1,2]. Their biological activity is predominantly associated with the presence of isoquinoline alkaloids. Several methods have been used for the determination of these alkaloids in plant extracts, *e.g.*, thin-layer and high-performance thin-layer chromatography[3–5], gas chromatography[6], enzyme immunoassay and radioimmunoassay[6,7] and particularly high-performance liquid chromatography (HPLC)[8–10]. We have previously reported the isotachophoretic analysis of some alkaloids of *F. parviflora* from the Varna region of Bulgaria[11] and of various isoquinoline alkaloids in pharmaceutical preparations[12]. This paper describes the determination of five common alkaloids (Fig. 1) in various extracts of *F. parviflora* and *F. capreolata* by HPLC and capillary isotachophoresis (ITP) and compares the results obtained.

EXPERIMENTAL

Plant materials and extraction

The plants were collected in northern Algeria in June 1984. The air-dried whole plants were powdered and extracted with methanol in a Soxhlet extractor. The meth-

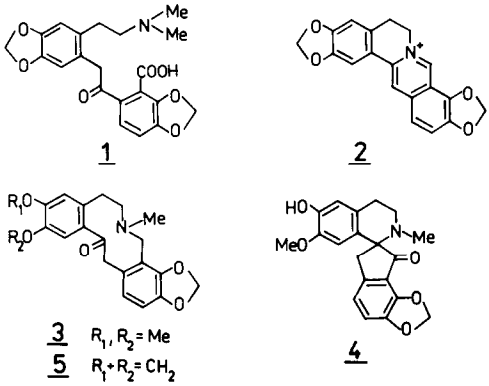

Fig. 1. Structures of alkaloids referred to in Table I. Me = methyl.

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anolic extract was concentrated *in vacuo*, acidified with acetic acid and washed with diethyl ether. Adjustment of the pH of the aqueous phase to 8 with solid sodium carbonate and extraction with diethyl ether gave fraction A. The aqueous layer was then adjusted to pH 12 with sodium hydroxide and extracted with diethyl ether to yield fraction B. The aqueous phase was acidified to pH 5 with hydrochloric acid, solid potassium iodide was added and the mixture was extracted with chloroform to yield fraction C.

Fraction A was separated into the non-phenolic (A_1) and phenolic (A_2) parts in the usual manner[13]. With *F. parviflora* the aqueous layer after separation was still alkaloid-positive and was subsequently extracted with chloroform (A_2Ch).

The yields were as follows. *F. parviflora* (757 g dry weight): A_1 267 mg (0.035% dry weight), A_2 198 mg (0.026%), A_2Ch 194 mg (0.025%), B 526 mg (0.069%), C 421 mg (0.056%). *F. capreolata* (284 g): A_1 176 mg (0.062%), A_2 6 mg (0.002%), B 651 mg (0.229%), C 185 mg (0.065%).

Analytical methods

HPLC determinations were performed on a Spectra-Physics liquid chromatograph (Model SP 8700 pump, SP 8440 variable-wavelength UV detector and SP 4100 computing integrator) equipped with a Rheodyne 7125 injection valve (10-μl sample loop). A Separon SGX C_{18} (7 μm) column (250 × 4 mm I.D.) was used. The mobile phases were (A) methanol–water–triethylamine, from 50:50:0.1 (v/v/v) to 80:20:0.1 in 10 min and (B) 50 m*M* triethylammonium phosphate buffer (pH 4.0) in methanol–water (52:48, v/v). Mobile phase A was used for the analyses, except for coptisine. The flow-rate was 1 ml/min and detection was at 357 nm for coptisine and at 280 nm for the other alkaloids. Quantification was carried out using external standards of the alkaloids, as follows. A weighed sample of about 1 mg was dissolved in 2 ml of methanol and the solution was allowed to pass through a C_{18} pre-separation column (Separcol) to prevent deterioration of the analytical column. After elution with 3 ml of methanol, the solvent was evaporated under a stream of nitrogen, the sample was reconstituted in 500 μl of mobile phase and 10 μl were injected.

The isotachophoretic measurements were carried out on an instrument with coupled columns and a conductivity detector constructed at the Palacký University. The driving current on the pre-separation capillary (230 × 0.8 mm I.D.) was 250 μA and on the analytical capillary (250 × 0.3 mm I.D.) it was 50 μA. The leading electrolyte was acetate buffer of pH_L 5.0 with K^+ as the leading ion (10 m*M*) and poly(vinyl alcohol) (Gohsenol GM-14 L, 0.5 g/l) as an additive. The terminating electrolyte was acetic acid (10 m*M*). Aliquots of individual fractions were dissolved in dilute acid (1 mg in 1 ml of 5 m*M* hydrochloric acid) and 5 or 10 μl of the resulting solution were injected. The amounts of the alkaloids were determined by means of calibration graphs. The time required for the analysis of one sample was about 20 min.

Thin-layer chromatography (TLC) was performed on silica gel G_{254} with cyclohexane–diethylamine (80:20) and benzene–ethyl acetate–diethylamine (50:40:10).

TABLE I

ISOTACHOPHORETIC AND CHROMATOGRAPHIC BEHAVIOUR OF THE ALKALOIDS STUDIED

Relative step height of the terminator = 100.

Alkaloid	*Relative step height*	*Retention time (min)**
Adlumidiceine (1)	–**	2.7
Coptisine (2)	49	6.7***
Cryptopine (3)	75	11.2
Parfumine (4)	85	7.9
Protopine (5)	68	12.3

* In mobile phase A.
** Adlumidiceine not determined isotachophoretically.
*** In mobile phase B.

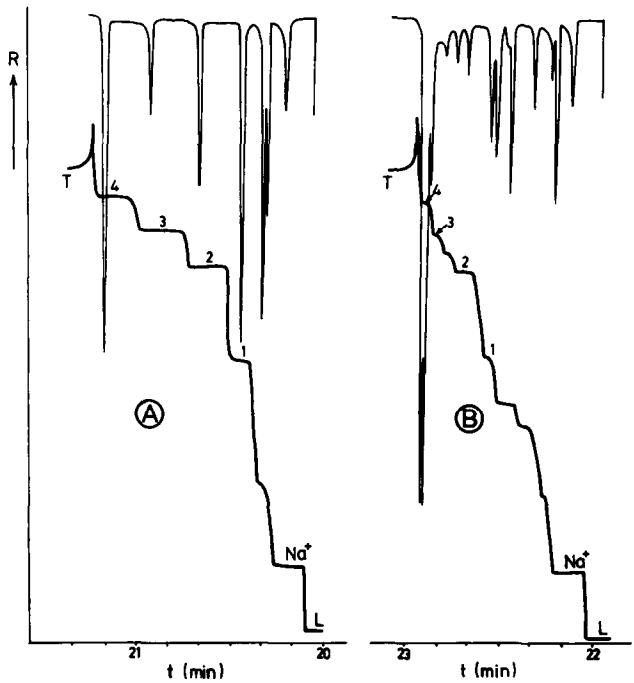

Fig. 2. Isotachophoretic separation of the alkaloids studied. (A) Mixture of standards of alkaloids: 1 = coptisine; 2 = protopine; 3 = cryptopine; 4 = parfumine. Concentration of each alkaloid, 0.1 m*M*; 10 µl injected. (B) Fraction B of *F. parviflora* (2 mg/ml, 5 µl injected): 1 = coptisine; 2 = protopine; 3 = cryptopine; 4 = parfumine. *R* = resistance of conductometric detector; L, T = zones of leading and terminating electrolytes, respectively; Na^+ = zone of sodium ions.

TABLE II

CONTENTS OF ADLUMIDICEINE, PARFUMINE, CRYPTOPINE, PROTOPINE AND COPTISINE IN *F. PARVIFLORA* AND *F. CAPREOLATA*

Plant	*Fraction*	*Method*	*Content of alkaloid [% (w/w) of fraction]**				
			*Adlumidiceine***	*Parfumine*	*Cryptopine*	*Protopine*	*Coptisine*
Fumaria parviflora	A_1	HPLC	0.9 ± 0.27 (30.5)	16.2 ± 0.01 (0.6)	22.3 ± 0.56 (2.3)	8.5 ± 0.18 (2.1)	–
		ITP		14.5 ± 0.80 (5.6)	21.5 ± 0.25 (1.2)	10.0 ± 0.38 (3.8)	–
	A_2	HPLC	9.8 ± 0.17 (1.7)	1.7 ± 0.075 (4.5)	20.2 ± 0.48 (2.2)	40.7 ± 0.58 (1.4)	–
		ITP		1.9 ± 0.22 (11.6)	21.0 ± 0.28 (1.3)	38.8 ± 0.28 (0.73)	–
	A_2Ch	HPLC	0.4 ± 0.13 (33.5)	70.4 ± 1.55 (2.2)	3.4 ± 0.03 (9.5)	1.0 ± 0.086 (8.6)	–
		ITP		67.2 ± 0.44 (0.65)	3.6 ± 0.44 (12.2)	1.26 ± 0.24 (20.0)	–
	B	HPLC	–	2.1 ± 0.06 (2.9)	3.2 ± 0.16 (5.0)	7.7 ± 0.32 (4.2)	3.1 ± 0.18 (5.6)
		ITP		2.6 ± 0.23 (8.9)	2.1 ± 0.23 (11.2)	7.4 ± 0.29 (4.0)	2.6 ± 0.16 (6.2)
	C	HPLC	44.3 ± 0.66 (1.5)	0.8 ± 0.045 (5.6)	–	–	–
		ITP		1.1 ± 0.12 (11.9)	–	–	–
Fumaria capreolata	A_1	HPLC	Traces	1.15 ± 0.065 (4.2)	–	72.9 ± 2.26 (3.1)	–
		ITP		0.95 ± 0.10 (10.5)	–	73.5 ± 1.8 (2.5)	–
	B	HPLC	Traces	–	–	–	4.9 ± 0.15 (3.8)
		ITP		–	–	–	4.4 ± 0.43 (9.9)
	C	HPLC	1.9 ± 0.07 (3.7)	–	–	–	–
		ITP		–	–	–	–

* Relative standard deviation (n = 3) (%) in parentheses.

** Adlumidiceine not determined isotachophoretically.

RESULTS AND DISCUSSION

On the basis of literature data[14] and the results of the TLC separation, the main components of the fractions, adlumidiceine (1), coptisine (2), cryptopine (3), parfumine (4) and protopine (5), were selected for detemination. The fraction A_2 of *F. capreolata* was not included in the determination owing to its small amount.

Optimal conditions for the isotachophoretic separation and identification of the alkaloids were as described previously[11]. Relative step heights of the alkaloids with respect to the step height of the terminator are given in Table I.

The isotachopherograms of a model mixture of alkaloids and of fraction B of *F. parviflora* are shown in Fig. 2. The isotachophoretic zones of the alkaloids were sharp and allowed their precise determination. The calibration graphs were linear over the whole range of amounts injected (0.25–10 nmol) and the correlation coefficients between the zone lengths and amounts injected were better than 0.999. The ITP determination of adlumidiceine (1) failed, because its effective mobility was lower than that of the terminator owing to the negative charge of the carboxylate anion in adlumidiceine.

Retention times of the alkaloids in the HPLC determination are given in Table I. Satisfactory separation of tertiary [adlumidiceine (1), cryptopine (3), parfumine (4) and protopine (5)] and quaternary [coptisine (2)] bases was not obtained using only one mobile phase. Separation of the tertiary alkaloids was achieved with mobile phase A, but coptisine showed tailing under those conditions. Therefore, mobile phase B was used for its determination; detection at 357 nm improved the selectivity.

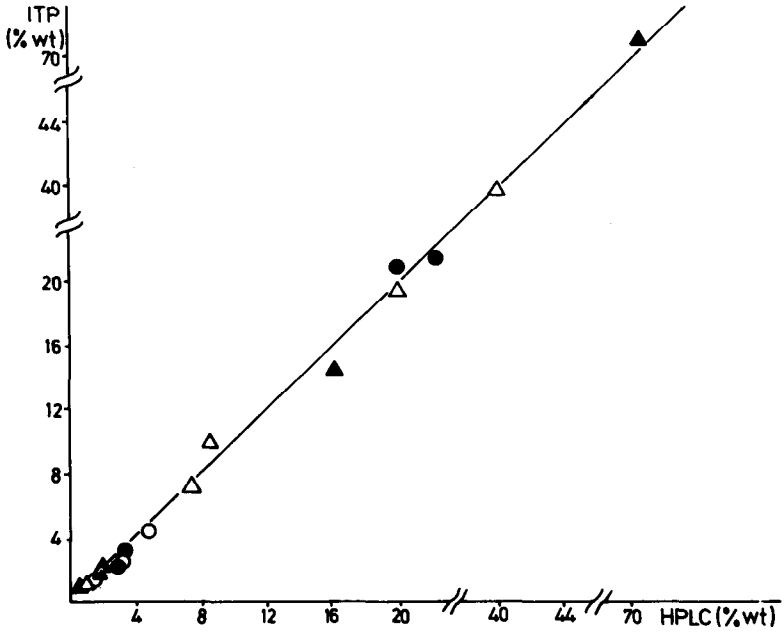

Fig. 3. Correlation between ITP and HPLC results. ITP = content of alkaloids in % (w/w) of fraction determined by ITP. HPLC = content of alkaloids in % (w/w) of fraction determined by HPLC. Each value is the mean of three measurements. ○, Coptisine; ●, cryptopine; △, protopine; ▲, parfumine.

The results obtained in the determination of the alkaloid content of fractions of *F. parviflora* and *F. capreolata* by both methods are given in Table II. HPLC and ITP yielded comparable results ($y_{ITP} = 0.031 + 0.979x_{HPLC}$; $r = 0.9989$), as can be seen from Fig. 3. The precisions of both methods were found to be comparable. For HPLC, the relative standard deviation for the determination of protopine (5) was 0.85% ($n = 10$, within-day, 1 m*M* standard solution) and 1.82% ($n = 10$, between-day); for ITP, the corresponding values were 0.92 and 2.05% under identical conditions.

Isotachophoresis appears to be a sufficiently selective and rapid method for the analytical screening of alkaloids in plant extracts. For the problem studied, ITP is less expensive, allows simpler sample preparation and is advantageous for the simultaneous determination of tertiary and quaternary bases. On the other hand, the simultaneous application of the two methods increases the reliability of the identification of individual alkaloids.

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