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Note

High-performance liquid chromatographic separation of quaternary alkaloids of *Chelidonium majus* L. roots

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Quaternary alkaloids are common in several plants of the family Papaveraceae[1] and have also been isolated from other families, such as Rutaceae[2], Berberidaceae[3], Magnoliaceae[2], Anonaceae[2,4,5], Menispermaceae[6–9], Fumariaceae[10,11] and Ranunculaceae[12–14].

A number of procedures have been developed for the analysis of such alkaloids. A colorimetric method was reported for *Bocconia cordata*[15], circular thin-layer chromatography (TLC) for *Jatrorrhiza palmata* and *Chasmanthera dependens*[6] and column chromatography or semipreparative TLC for *Glaucium squamigerum*[16] and *Stylophorum diphyllum*[17]. Quaternary alkaloids were separated from *Fumaria parviflora* by capillary isotachophoresis[18].

The liquid chromatographic separation of quaternary alkaloids is rather difficult, due to their high polarity and strong interaction with the absorption sites of the stationary phase. Normal-phase ion-pair chromatography has been employed for the separation of such alkaloids [19–21] as has reversed-phase ion-pair chromatography with various solvents at different pH [22–24] or reversed-phase[25] or ion-exchange high-performance liquid chromatography (HPLC)[26–27]. The HPLC of quaternary alkaloids has been summarized by Verpoorte and Baerheim Svendsen[28].

However, for *Chelidonium majus* L. alkaloids these techniques result in incomplete separations and/or imprecise determination. Quaternary alkaloids from this plant were detected by direct remission measurement of TLC plates[29]; an isotachophoretic separation has also been described[30]. The single berberine alkaloid has been quantitated by HPLC in plant material[31] and also analyzed using ion-pair HPLC in crude drugs and in pharmaceutical preparations[32,33]. Berberine was separated from a test mixture of alkaloids by using pH-gradients in droplet counter-current chromatography[34].

In this paper we report a method of HPLC separation of quaternary alkaloids from roots of *Chelidonium majus* L.

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EXPERIMENTAL

Apparatus

A Waters Assoc. liquid chromatograph, equipped with a Model 6000 A solvent pump and an U6K universal injector, was employed. Detection was performed with a Perkin-Elmer Model LC 75 variable wavelength detector, linked to a Perkin-Elmer Sigma 10 computing integrator. The column was an Alltech silica (250 mm × 4.6 mm I.D.) particle size 10 μm.

Chemicals

LiChrosolv reagent grade solvents were used (Merck, Darmstadt, F.R.G.). Sanguinarine, chelerythrine and berberine were obtained from Sarsynthex (Mérignac, France).

Plant material

Chelidonium majus L. roots were collected in the Botanical Garden of the University of Milan, during spring 1986.

Preparations of standards

A set of ten standard solutions of a mixture of sanguinarine, chelerythrine and berberine (1:1:1, w/w/w) was prepared containing between 0.01 and 0.25 mg/ml of each alkaloid. The solutions were stored in airtight flasks at 25°C in the dark.

Extraction and separation of quaternary alkaloids

Fresh roots (5 g), thinly minced in a blender (60 mesh), were extracted in a Soxhlet apparatus with 70% ethanol, according to the commonly used procedure[35]. High-performance liquid chromatography of the crude quaternary alkaloids was performed with 0.005 *M* sodium acetate in methanol–1,4-dioxane–acetic acid (88:10:2, v/v/v) degassed by sonication just before use, at a flow-rate of 1.5 ml/min; detection was performed at 280 nm. In order to obtain the best separation of the above cited alkaloids, we used several eluent systems ranging from 0.1 to 0.005 *M* sodium acetate in methanol. The alkaloids, after separation by analytical HPLC, were compared with authentic samples and found to be identical[36–38].

RESULTS AND DISCUSSION

The quaternary alkaloids of *Chelidonium majus* L. show very similar chromatographic properties; we have separated these compounds by HPLC on silica gel column with sodium acetate in methanol as the eluent.

Fig. 1 shows a representative chromatogram of a root extract from *Chelidonium majus* L. Three major quaternary alkaloids, sanguinarine, chelerythrine and berberine, were obtained pure by separation under direct isocratic conditions. Their structures were confirmed by physical and spectral data, compared with those of authentic samples (see Experimental). Under our experimental conditions the retention times of sanguinarine, chelerythrine and berberine were 4.40, 8.73 and 12.32 min respectively and they were dependent on the composition of the mobile phase used. As shown in Fig. 2, the retention time increases with decreasing molarity of sodium acetate in the mobile phase.

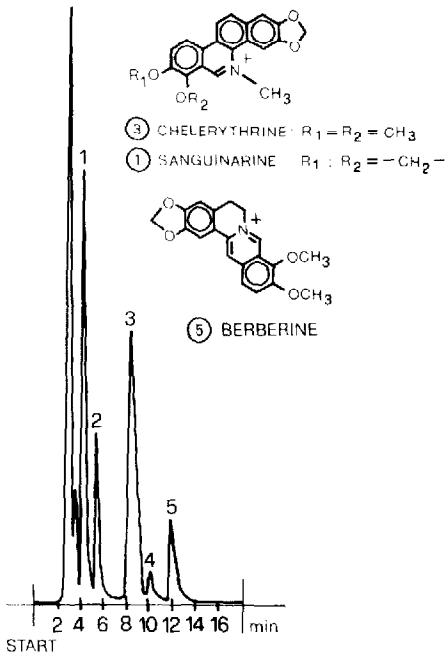

Fig. 1. Representative chromatogram of *Chelidonium majus* L. extract. Peaks: 1 = sanguinarine; 2 = chelidonine; 3 = chelerythrine; 4 = unknown; 5 = berberine. Conditions: Alltech silica column (250 mm × 4.6 mm I.D.); mobile phase, 0.005 *M* sodium acetate in methanol–1,4-dioxane–acetic acid (88:10:2); flow-rate, 1.5 ml/min; detection, UV at 280 nm; injection volume, 10 μl.

The determination of alkaloids was successively accomplished by means of a calibration graph of the peak height *versus* the amount of standard injected, found to be linear in the range 0.01–0.50 mg/ml (regression coefficients: sanguinarine, 0.90; chelerythrine, 0.86; berberine, 0.32).

In Table I are reported the concentrations (% dry weight) of the alkaloids

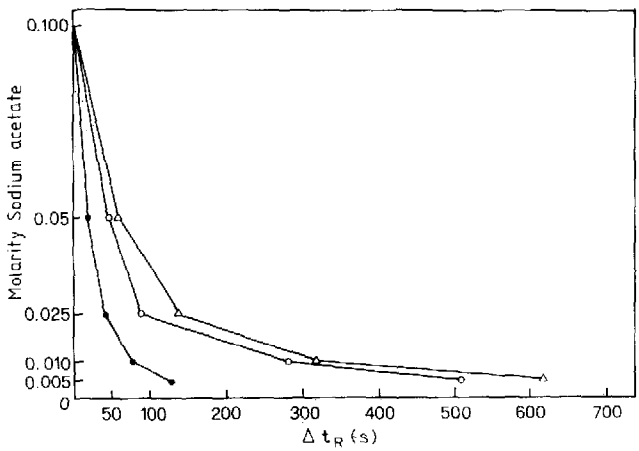

Fig. 2. Variation of the retention time with the concentration of sodium acetate for sanguinarine (●), chelerythrine (○) and berberine (△).

TABLE I

REPRODUCIBILITY OF THE DETERMINATION OF SANGUINARINE, CHELERYTHRINE AND BERBERINE IN *CHELIDONIUM MAJUS* L. ROOTS

	Sanguinarine	*Chelerythrine*	*Berberine*
% Dry weight (average of 10 analyses)	0.388	0.33	0.07
Standard deviation	0.0035	0.0105	0.0034
Coefficient of variation (%)	0.93	3.22	4.69

sanguinarine, chelerythrine and berberine in a sample of 5 g of fresh roots of *Chelidonium majus* L.; the method was quantitative and reproducible, based on ten measurements. The concentrations of sanguinarine, chelerythrine and berberine were found to be 0.38, 0.33 and 0.07% respectively, the corresponding coefficients of variation ($n = 10$) being 0.93%, 3.22% and 4.69%.

The reversed-phase or ion-pair chromatography[20,23,24] tested by us on the crude drug of *Chelidonium majus* L. gave unsatisfactory peak shapes and the quaternary alkaloids sanguinarine, chelerythrine and berberine were not well resolved. In an attempt to obtain improved separations, various solvent systems in reversed-phase HPLC were evaluated using as mobile phase either aqueous methanol and acetonitrile or aqueous methanol and dioxane, both modified or not by the addition of sodium hexanesulphonate, sodium octanesulphonate or sodium lauryl sulphate. The best results obtained were a resolution between sanguinarine and chelerythrine of 0.91 and of 2.09 between chelerythrine and berberine; however, in the latter case berberine does not appear to be clearly resolved from other non-quaternary alkaloids present in the crude extract.

On the contrary by adopting the normal-phase method as described in this paper we obtained a better separation with sharp peaks; resolution between sanguinarine and chelerythrine, 1.37; between chelerythrine and berberine, 1.84; analysis time less than 15 min. The alkaloids were detectable with good sensitivity at 280 nm. The reported method for qualitative and quantitative determination of quaternary alkaloids of *Chelidonium majus* L. is very simple and rapid.

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