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Reversed-phase high-performance liquid chromatographic separation of tertiary and quaternary alkaloids from *Chelidonium majus* L.

LIANG-FENG HAN, WASSYL NOWICKY and VIKTOR GUTMANN*

Institute of Inorganic Chemistry, Technical University of Vienna, Getreidemarkt 9, A-1060 Vienna (Austria)

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ABSTRACT

A reversed-phase high-performance liquid chromatographic procedure is described for the determination of the alkaloids in *Chelidonium majus* L. extracts. For the analysis of tertiary and quaternary alkaloids, replacement of the commonly applied organic amine modifiers with potassium iodide led to a considerable improvement in resolution. By using a gradient elution programme more than twenty peaks were resolved and thirteen tertiary and quaternary alkaloids were identified.

INTRODUCTION

Chelidonium majus L., a medicinal plant of the Papaveraceae family, has stimulated much interest owing to its chemically and pharmacologically interesting alkaloids. By classical methods more than twenty alkaloids have been detected in this plant [1], but no systematic studies have been carried out by high-performance liquid chromatography (HPLC). Three quaternary alkaloids from this plant, sanguinarine, chelerythrine and berberine, and a non-quaternary one, chelidonine, have been separated by normal-phase chromatography [2]. Reversed-phase ion-pair chromatography using different ion-pair agents for the separation of chelidonine, chelerythrine and sanguinarine [3] and for the separation of allocryptopine, protopine and chelidonine [4] have been reported. However, little attention has been paid to the alkaloids present in very small amounts, probably because the available HPLC techniques were unsuitable for their identification.

In this paper, an HPLC method for the separation and identification of tertiary and quaternary alkaloids from *Chelidonium majus* L. is described.

EXPERIMENTAL

Apparatus

A Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 1090 liquid chromatograph was used, equipped with an HP Model DR5 solvent-delivery system and an HP 1090

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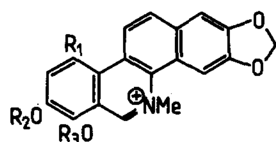
option 044 auto-injector. Detection was performed with an HP 1090 option 080 diode-array UV detector.

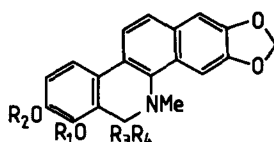
Chemicals

Acetonitrile (LiChrosolv; Merck, Darmstadt, Germany), methanol (reinst, Merck) and water (Chromasolv für Gradientenelution, Riedel-de Haën, Seelze, Germany) were used. Potassium iodide (Merck) was of analytical-reagent grade.

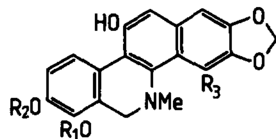
The following standard alkaloids were kindly supplied: sanguinarine and allocryptopine by L. Jusiak (Lublin, Poland); methoxychelidonine, oxysanguinarine, dihydrosanguinarine, chelerythrine, dihydrochelerythrine, chelilutine, chelirubine, corysamine and (−)-stylopine by J. Slavík and L. Slavíkova (Brno, Czechoslovakia); berberine and protopine by W. Debska (Poznan, Poland); and chelidonine, homochelidonine and sparteine by F. Kuffner (Vienna, Austria). Their structures are given in Fig. 1.



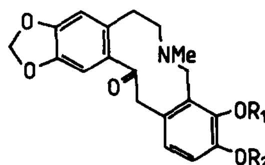
sanguinarine, $R_3+R_2=CH_2$, $R_1=H$
chelerythrine, $R_3=R_2=CH_3$, $R_1=H$
chelilutine, $R_3=R_2=CH_3$, $R_1=OCH_3$
chelirubine, $R_3+R_2=CH_2$, $R_1=OCH_3$



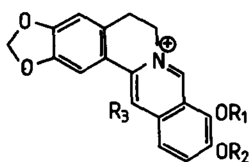
oxisanguinarine, $R_1+R_2=CH_2$, $R_3+R_4=O$
dihydrochelerythrine, $R_3=R_4=H$, $R_1=R_2=CH_3$
dihydrosanguinarine, $R_1+R_2=CH_2$, $R_3=R_4=H$



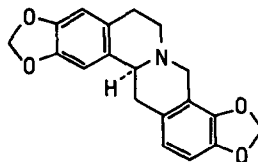
chelidonine, $R_1+R_2=CH_2$, $R_3=H$
homochelidonine, $R_1=R_2=CH_3$, $R_3=H$
methoxychelidonine, $R_1+R_2=CH_2$, $R_3=OCH_3$



protopine, $R_1+R_2=CH_2$
allocryptopine, $R_1=R_2=CH_3$



corysamine, $R_1+R_2=CH_2$, $R_3=CH_3$
berberine, $R_1=R_2=CH_3$, $R_3=H$



(−)-stylopine

Fig. 1. Structures of alkaloids from *Chelidonium majus* L. Me = Methyl.

All of the standard alkaloids were dissolved in methanol containing 0.1 *M* ammonia and stored in air-tight flasks in the dark. The plants of *Chelidonium majus* L. were collected from fields near Vienna.

Extraction of alkaloids

The plants were dried at room temperature and extracted with ethanol. Ethanol from the extract was distilled off and the residue was transferred into 1% sulphuric acid and filtered. The acidic aqueous filtrate was neutralized with sodium hydroxide and extracted with chloroform. The pH of the solution was then increased to 8 and again extracted with chloroform. The pH was increased again by 1 unit, the solution was extracted, and these procedures were repeated until a pH of 14 was established. The extracts were combined, filtered and evaporated to dryness under vacuum. The residue was dissolved in 1% hydrochloric acid, filtered and the filtrate evaporated to dryness under vacuum. After dissolution in methanol containing 0.1 *M* ammonia, the solution was subjected to HPLC.

Separation and identification method

A Hypersil ODS column (RP C_{18}, Hewlett-Packard) (100 × 4.6 mm I.D., 5 μm particle size) was used. The mobile phase was water–acetonitrile–methanol. The water was adjusted to pH 7.5 with propylamine and the methanol contained 0.15 *M* potassium iodide. Isocratic and gradient elution were used, as given in the captions of Figs. 2 and 4. The column temperature was 30°C. The chromatograms were monitored at 285 nm with a bandwidth of 30 nm. The reference wavelength was 400 nm with a bandwidth of 100 nm. Retention time data were measured directly from chromatograms based on five measurements. Other HPLC conditions are given in the captions of Figs. 2 and 4.

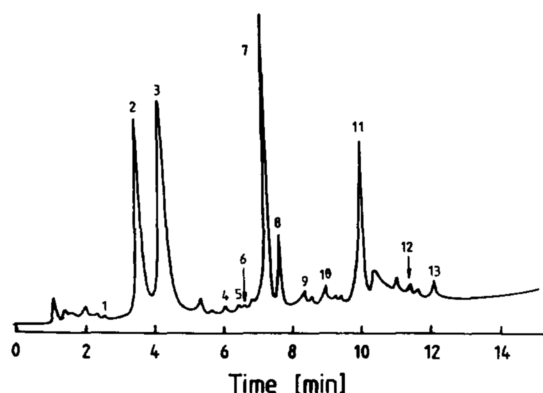


Fig. 2. HPLC of *Chelidonium majus* L. extract using gradient elution. Mobile phase: water–acetonitrile–methanol, from 50:20:30 to 15:55:30 in 15 min. The water was adjusted to pH 7.5 with propylamine and the methanol contained 0.15 m*M* potassium iodide. Flow-rate: increased from 0.8 to 1.5 ml/min in 15 min. Injection volume: 10 μl.

RESULTS AND DISCUSSION

Reversed-phase HPLC is ineffective in many instances for compounds with structures containing basic nitrogen atoms owing to interactions between the basic compounds and the acidic silanol groups on the stationary phase surface [5–10]. Practical experience has shown that in such instances the addition of organic amines to the mobile phase is essential in order to achieve good separations. The general effect of such amines is to decrease retention and improve peak shape in the analysis of basic compounds. Additions of organic amines to the mobile phase have also been reported to alter the selectivity of the stationary phase [11].

In an attempt to obtain improved separations for the crude drug of *Chelidonium majus* L., propylamine and tetrabutylammonium salts were added to the mobile phase with pH < 7.5. However, reversed-phase HPLC with these amines gave unsatisfactory separations. Considerable improvement in the peak shape and resolution of the extract of *Chelidonium majus* L. was achieved by applying potassium iodide as modifier. As shown in Fig. 2, thirteen alkaloids were detected in this way and several

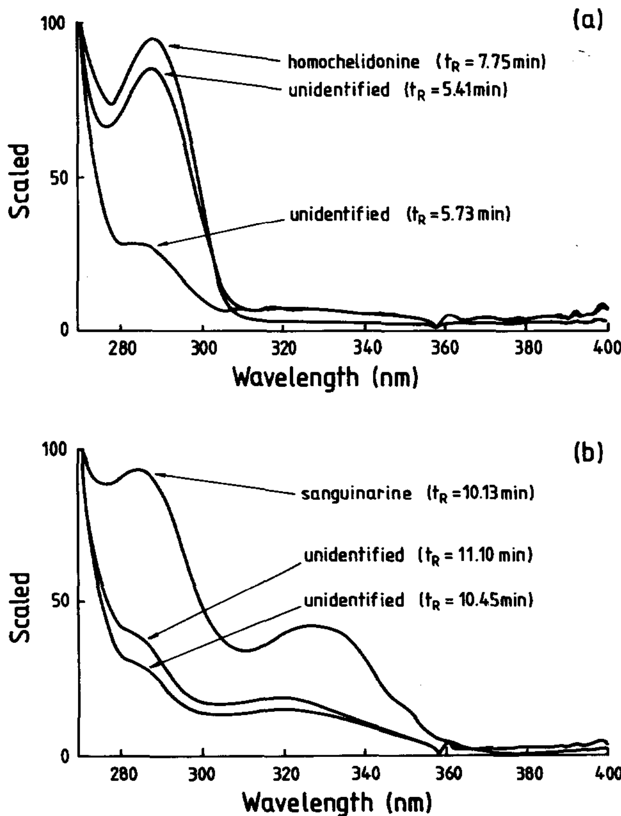


Fig. 3. Examples of spectral comparison between the peaks: (a) spectra of homochelidonine (retention time, t_R = 7.75 min, peak 8 in Fig. 2) and two unknowns at t_R = 5.41 and 5.73 min; (b) spectra of sanguinarine (t_R = 10.13, peak 11 in Fig. 2) and two unknowns at t_R = 10.5 and 11.1 min.

TABLE I

RETENTION TIMES OF THE ALKALOIDS

Chromatographic conditions are given in Fig. 2.

Alkaloid	t_R (min)	Peak No.
Corysamine	2.63	1
Methoxychelidonine	3.61	2
Allocryptopine	4.30	3
Protopine	6.13	4
Chelerythrine	6.52	5
Berberine	6.71	6
Chelidonine	7.34	7
Homochelidonine	7.75	8
Oxysanguinarine	8.28	9
(−)-Stylopine	9.05	10
Sanguinarine (chelitutine, chelirubine)[a]	10.13	11
Dihydrochelerythrine	11.52	12
Dihydrosanguinarine	12.22	13

[a] Alkaloids with the same retention time as Sanguinarine.

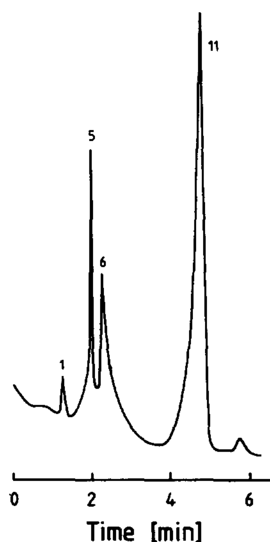


Fig. 4. HPLC of a standard mixture of four quaternary alkaloids. Alkaloids denoted by numbers as in Table I. Mobile phase: water–acetonitrile–methanol (30:40:30); the water was adjusted to pH 7.5 with propylamine and the methanol contained 0.15 *M* potassium iodide. Isocratic elution. Flow-rate: 0.8 ml/min. Injection volume: 10 µl (sample solution concentration about 0.1–0.5 mg/ml of each alkaloid). Other conditions as in Fig. 2.

small peaks were identified by comparing both their retention times and their UV spectra with those of standards. The comparision of the spectra as a second criterion was found useful for the identification, because most of the alkaloids were present in fairly low concentrations. Apart from the identification of thirteen alkaloids (denoted by 1–13 in Fig. 2), several other small peaks may also be due to the presence of alkaloids, because the spectra are typical of them (Fig. 3). In the absence of appropriate standards, their identification has not been possible. It may be noted that according to our results, summarized in Table I, sparteine [12] was not found.

Another difficulty in the separation of quaternary alkaloids arises from their strong interactions with the stationary phase [13]. Our results have shown that these interactions may be weakened by the use of potassium iodide as a modifier. Sharp peaks were achieved for corysamine, chelerythrine and sanguinarine. The separations between corysamine, chelerythrine and sanguinarine are also satisfactory, but berberine has not been resolved clearly from chelerythrine (peaks 5 and 6 in Fig. 2). Better separations with almost symmetrical peaks and good resolution between corysamine, chelerythrine, berberine and sanguinarine were obtained using other gradient conditions, as outlined in legend of Fig. 4. Under these conditions the tertiary alkaloids, especially those present in small amounts, were not well resolved.

Increased interest has recently been shown in the alkaloids from *Chelidonium majus* L. because of the potential use of their derivatives in antitumour therapy [14]. It seems likely that the method described here may be applied in further studies directed towards the isolation and identification of alkaloids of biological and clinical interest.

ACKNOWLEDGEMENTS

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