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Short Communication

Analytical studies of *dl*-stylopine in *Chelidonium majus* L. using high-performance liquid chromatography

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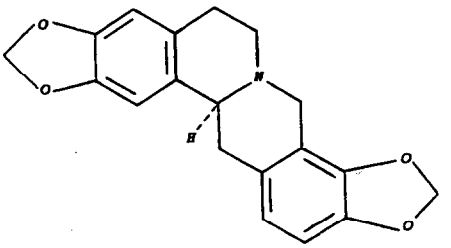
ABSTRACT

A low-pressure liquid chromatographic method using silica gel 60 with hexane–chloroform–ethyl acetate proportions varying from 80:20:0 to 20:20:8 (v/v/v) as eluent is described for the isolation of *dl*-stylopine from the flowered aerial parts of *Chelidonium majus* L. An isocratic high-performance liquid chromatographic (HPLC) method using a Superspher Si 60 column with chloroform–methanol (90:10, v/v) containing 0.1% trifluoroacetic acid as mobile phase and UV detection at 292.5 nm that allows the determination of *dl*-stylopine in greater celandine is described. The total alkaloid fraction is 0.34% for the flowered aerial parts and 2.17% for the underground parts and the amount of *dl*-stylopine is 61.3 and 4.9% of the total alkaloid fraction, respectively.

INTRODUCTION

Further to our studies of the application of high-performance liquid chromatography (HPLC) to the analysis of medicinal plants with sedative and spasmolytic properties, we have examined the components of *Chelidonium majus* L. There have been many investigations into the alkaloidal constituents of this plant [1–8], and almost twenty isoquinoline alkaloids have been isolated. It appears that greater celandine is variable in its alkaloid content and that many differences exist between different parts of the plant. The major alkaloids proved to be chelidonine, chelerythrine, sanguinarine and *dl*-stylopine [2–4]. The minor alkaloids are mainly protopine, berberine, coptisine, chelidamine and chelamidine [3].



DL-STYLOPINE

Numerous studies on the benzo[*c*]phenanthridine group of *C. majus* L. have been undertaken using thin-layer chromatography (TLC) [9–15], isotachophoretic analysis [16] and HPLC

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[14,17–19], but no work has been reported on the determination of *dl*-stylopine in greater celandine using HPLC. In this paper, we describe a simple method for the isolation of this alkaloid and a normal-phase HPLC method for the determination of *dl*-stylopine which can be used as a specific tracer in *C. majus* L.

EXPERIMENTAL

Chemicals

Chloroform and methanol were of HPLC quality from Rathburn (Walkerburn, UK). All other solvents were of analytical-reagent grade from Labosi (Paris, France).

High-performance liquid chromatography

A Varian Model 5000 chromatograph was used, equipped with a Rheodyne Model 7125 injector and a Merck L 3000 photodiode-array detector under computer control (Merck HPLC Manager). Analyses were conducted at 20°C.

Analytical HPLC was carried out on a normal-phase Superspher Si 60 column (125 × 4 mm I.D., particle size 4 μm) (Merck) used with a LiChrospher Si 60 precolumn (4 × 4 mm I.D., particle size 5 μm) (Merck). The mobile phase was chloroform–methanol (90:10, v/v) containing 0.1% trifluoroacetic acid (TFA) at a flow-rate of 1 ml/min. The injection volume was 10 μl and UV detection at 292.5 nm was applied.

Alkaloid extraction

A 500-g amount of flowered aerial parts of *Chelidonium majus* L. (harvested in Maine et Loire, France, May 1992), dried at room temperature and finely powdered, was moistened with dilute ammonia solution and kept for 2 h before Soxhlet extraction with chloroform (5 l). The organic solution was evaporated under reduced pressure at 40°C to a final volume of about 100 ml, and then extracted with 5 × 50 ml of 0.25 *M* sulphuric acid. The acidic layers were mixed and filtered. After alkalinization with ammonia (pH 10), they were extracted with 4 × 50 ml of chloroform. The organic layers were washed with 70 ml of distilled water, filtered and evaporated under reduced pressure, affording a residue (1.68 g) corresponding to the total alkaloid fraction (0.34% dry material).

An alkaloid extraction was also applied to the underground parts of the same plant material harvested at the same date. The extraction procedure was the same as above (total alkaloid fraction = 2.17% dry material).

Isolation of dl-stylopine

dl-Stylopine was isolated using low-pressure liquid chromatography preparative separation (Fig. 1). ^{1}H NMR spectrometry (Brucker AC 200 P NMR spectrometer), mass spectrometry (MS) (Nermag R 1010 C mass spectrometer), melting point determination and UV spectrophotometric analysis confirmed the identification of the isolated pure compound as *dl*-stylopine: m.p. 220–221°C [2,4,20]; ^{1}H NMR spectra identical with the literature [5]; electron

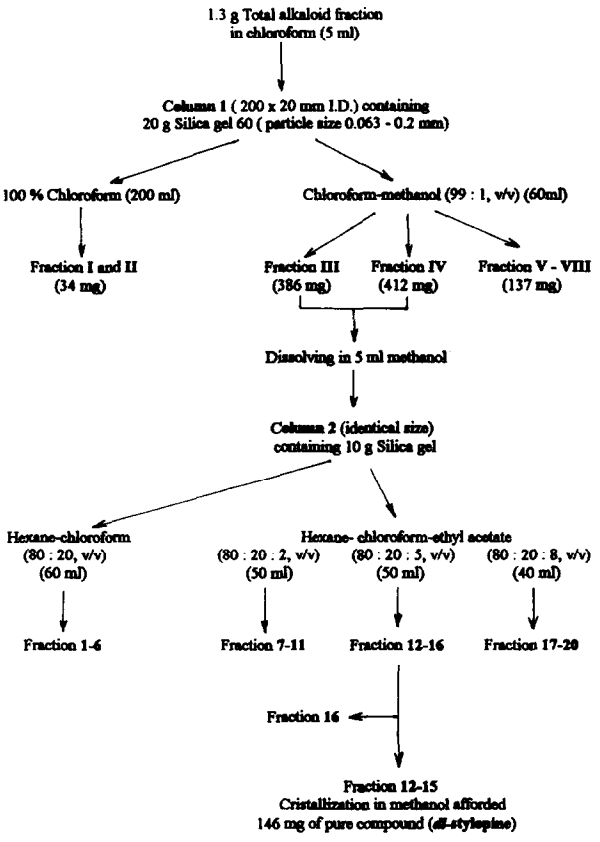

Fig. 1. Low-pressure liquid chromatography preparative separation of *dl*-stylopine.

impact MS, m/z 323 ($M^+ \cdot$), 148 (100), 91 (75) [2]; UV λ_{max} [chloroform–methanol–TFA (90:10:0.1, v/v/v)] 246 and 292.5 nm (log(ε) 2.4 and 3.9) [3].

RESULTS AND DISCUSSION

In contrast to the separation of the alkaloids of *Chelidonium majus* L. using reversed-phase HPLC [18,19] or ion-pair chromatography [17,18], and in comparison with the separation of chelidonine, chelerythrine and sanguinarine using a LiChrosorb Si 60 column with toluene–methanol (96:4, v/v) [17], we made use of normal-phase HPLC on Superspher Si 60 with chloroform–methanol (90:10, v/v) containing 0.1% TFA, which resulted in a considerable improvement in the chromatographic profile. TFA was chosen because of its low absorbance in the UV region. Moreover, it increases the column efficiency and improves the column resolution in both the reversed-phase [21] and normal-phase modes.

For quantitative analysis, the calibration graph shows a linear correlation from 0.1 to 2 mg/ml between the amounts of *dl*-stylopine injected and the intensity of the absorption at 292.5 nm [correlation coefficient (r^2) = 0.9983]. Five determinations were carried out on each sample of greater celandine, in order to test the precision of the method. The determination of *dl*-stylopine was attempted on the total alkaloid fraction of flowered aerial parts (Fig. 2) and underground parts of *Chelidonium majus* L.

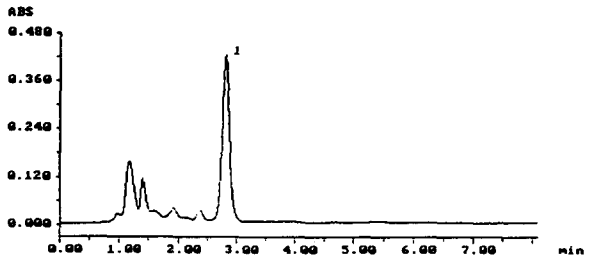

Fig. 2. HPLC of a total alkaloid fraction of flowered aerial parts of *Chelidonium majus* L. Peak: 1 = *dl*-stylopine. Conditions: column, Superspher Si 60 (125 × 4 mm I.D.; particle size 4 μm); precolumn, LiChrospher Si 60 (4 × 4 mm I.D.; particle size 5 μm); mobile phase, chloroform–methanol (90:10, v/v) containing 0.1% TFA; flow-rate, 1 ml/min; UV detection at 292.5 nm.

The results obtained (*dl*-stylopine = 61.3% of the total alkaloid fraction whose extraction efficiency is 0.34% dry material) for the flowered aerial parts harvested in May, in comparison with those observed for the underground parts of the same plants harvested at the same date (*dl*-stylopine = 4.9% of the total alkaloid fraction whose extraction efficiency is 2.17% dry material), show the importance of seasonal changes in alkaloid contents in greater celandine [12]. Moreover, this is correlated with the high percentage of *dl*-stylopine in the siliques [2,4] and with the variation in the qualitative and quantitative composition of alkaloids from *Chelidonium majus* L. during its growth [22].

Nevertheless, we must consider that the alkaloid extraction described does not permit the extraction of quaternary compounds. In fact, a tetrahydroprotoberberine alkaloid such as *dl*-stylopine should be oxidized to form coptisine under these conditions. Thus this possibility cannot be excluded.

In conclusion, the proposed method allows the isocratic separation of *dl*-stylopine in greater celandine and can be used in its routine determination in drugs and in medicinal plant extracts containing other Papaveraceae species [23].

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