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Analysis of aporphine and quinolizidine alkaloids from *Caulophyllum thalictroides* by densitometry and HPLC

Tibebe Z. Woldemariam [a,*], Joseph M. Betz [b], Peter J. Houghton [a]

[a] *Department of Pharmacy, King's College London, Manresa Road, London SW3 6LX, UK*
[b] *Division of Natural Products, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, Washington, DC 20204, USA*

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Abstract

High-performance liquid chromatographic (HPLC) and densitometry procedures were developed to determine the principal alkaloids in the roots of *C. thalictroides*. In both techniques the alkaloids content was assessed using cytisine as an internal standard. The purity and identity of the peaks of the alkaloids was examined by diode array detection and by comparison with the standards. The content of individual alkaloids was found to be in the range 0.02–1.1% w/w. © 1997 Elsevier Science B.V.

Keywords: Caulophyllum thalictroides; Berberidaceae; Blue Cohosh; HPLC; Densitometry; Alkaloids

1. Introduction

Caulophyllum thalictroides (L.) Mich. (Berberidaceae), known as 'Blue Cohosh', is a perennial herb with a thick, crooked and horizontal rhizome. The mature plant is a peculiar bluish green color and bears dark blue fruits. It is commonly found in eastern North America where the roots are used in diuretic, uterine antispasmodic and laxative preparations. However, concern has been expressed over the potential toxicity of the alkaloids and glycosides present [1].

The quinolizidine alkaloids, *N*-methylcytisine, baptifoline and anagyrine and the aporphine alkaloid magnoflorine have been reported from this plant [2]. The quinolizidine alkaloids have been implicated as teratogens [3] in higher animals and their toxicity to range animals is well documented [4] sheep appear to be especially susceptible to intoxication with symptoms including incoordination, convulsions and even death through respiratory paralysis.

This study was undertaken because dietary supplement products containing this plant are considered foods under US law, and a rapid, reliable method for determination of these potentially hazardous natural toxins was needed inorder to evaluate the safety of these products.

Flom et al. (1967) [2] employed column chromatography on silica gel and alumina for the separation and isolation of the lupin alkaloids and magnoflorine, respectively, from the ethanol ex-

* Corresponding author.

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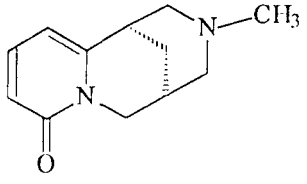
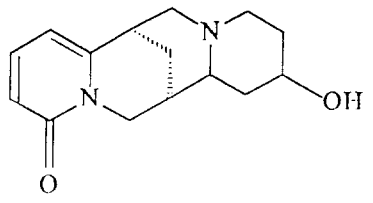


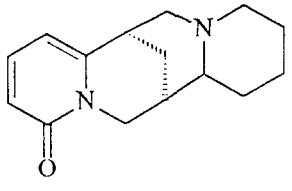
N-Methylcytisine

1



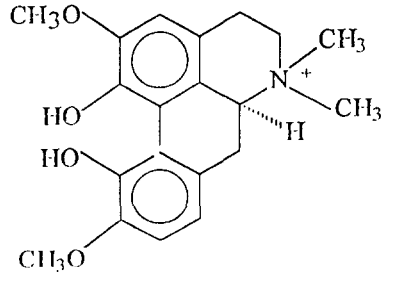
Baptifoline

2



Anagyrine

3



Magnoflorine

4

Scheme 1. Structures of the major alkaloids in the roots of *C. thalictroides*.

tract of the root and rhizomes of *Caulophyllum thalictroides*.

In the present work the alkaloids *N*-methylcytisine (1), baptifoline (2), anagyrine (3) and magnoflorine (4) (Scheme 1) which are major components, were chosen for quantitative measurement. Densitometry following TLC separations and reversed-phase HPLC methods were developed for analysis and quantitation in the crude methanolic extract of *Caulophyllum thalictroides*.

2. Materials and methods

2.1. Plant material

The roots of *Caulophyllum thalictroides* (L.) Michx. used in this investigation were obtained from a commercial supplier (Penn Herb, Philadelphia, PA, USA).

2.2. Reagents and reference materials

The analytical reference standards for *N*-methylcytisine, anagyrine, baptifoline and magnoflorine were previously isolated in Pharmacognosy Laboratories, Department of Pharmacy, King's College London, UK. Cytisine was purchased from Aldrich, UK. All solvents and reagents were of AnalaR grade.

2.3. Extraction and isolation

The powdered root (400 g) of *C. thalictroides* was percolated with 70% EtOH (2 l × 3). Evaporation of the solvent at reduced pressure afforded a residue (50 g). The residue was divided into two parts (Extract A and B). Extract A (45 g) was chromatographed on silica gel column (Merck, type 60, 230–400 mesh, 400 g, 2.5 × 54 cm) and gradient elution using MeOH in CH_2Cl_2–28%

NH_4OH (100:1) as reported previously [5]. Fractions (20 ml each) were monitored by TLC on silica gel using CH_2Cl_2–MeOH–28% NH_4OH (90:9:1) as the mobile phase. The CH_2Cl_2–MeOH–28%NH_4OH (100:10:1) eluates were mixed together to give the lupin alkaloids *N*-methylcytisine, anagyrine and baptifoline after preparative TLC on silca gel using CH_2Cl_2–MeOH–28%NH_4OH as the mobile phase. The fractions rich in magnoflorine were eluted with MeOH–28%NH_4OH (70:30). Pure magnoflorine was obtained by preparative TLC on silica gel using MeOH–28%NH_4OH (70:30) as the mobile phase.

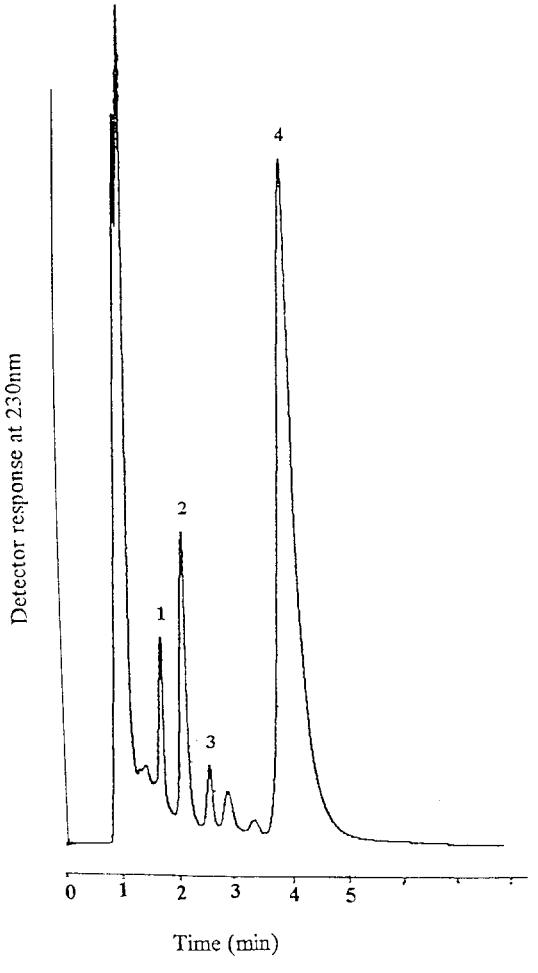


Fig. 1. LC separation of (1) baptifoline, (2) *N*-methylcytisine, (3) anagyrine, and (4) magnoflorine from the roots of *C. thalictroides* on Econosphere-ODS column.

2.4. Densitometry

Quantitative analysis was carried out by conventional procedures using cytisine as an internal standard, and the appropriate reference samples in the concentration range 10–50 μg ml^{-1}. An aliquot (10 μl) of each solution and the extract obtained as described above (Extract B), were applied to a precoated silica gel GF254 layer (0.25 mm, Merck) which was developed in CH_2Cl_2–MeOH–28%NH_4OH (90:9:1) and MeOH–28%NH_4OH (70:30) (double development) and sprayed with Dragendorff's reagent. The intensity of the visualised zones was measured using a Camag (Switzerland) Densitometer equipped with TLC scanner (Turner, Palo Alto, CA, USA) with 1 mm slit width and a light filter of 495 nm. Peak areas were obtained for the internal standard (cytisine; R_f 0.55–0.59) and anagyrine (R_f 0.75–0.78); *N*-methylcytisine (R_f 0.65–0.69); baptifoline (R_f 0.45–0.49), and magnoflorine (R_f 0.34–0.40).

2.5. Sample treatment for densitometry and HPLC

Extract B (5 g) was dissolved in 10 ml methanol. An aliquot (0.5 ml) of the extract was passed through a C-18 Bond-Elut (Analytichem International, Harbor City, USA) extraction column, and methanol was used to elute the alkaloidal components from the column. The methanol eluates (60 ml) were collected and the column was eluted with a further 20 ml methanol. The combined methanol eluates were taken to dryness under reduced pressure and the residue dissolved in 10 ml of the cytisine internal standard solution for HPLC or 2 ml of the cytisine solution for densitometry analysis.

2.6. Analytical HPLC procedure

The equipment used comprised a Perkin-Elmer 410 LC pump (Norwalk, CT, USA), a Rheodyne 7125 injection valve (Cotati, Berkeley, CA, USA) fitted with a 10-μl sample loop. A variable UV detector (Perkin- Elmer LC-290) coupled with a Perkin-Elmer integrator and printer was used for recording the chromatograms. The chromato-

Table 1
Concentration of alkaloids measured by HPLC and densitometry in roots of *C. thalictroides*

	Content (mg/g dry wt)			
	N-methylcytisine	Baptifoline	Anagyrine	Magnoflorine
HPLC	0.90	0.75	0.18	11.00
Densitometry	0.91	0.71	0.19	11.08

graphic separation was achieved with an Econosphere ODS column (150 × 4.6 mm i.d.; 5 μm; Alltech Associates, Deerfield, IL, USA). The purity of the peaks were examined by a Varian 9065 diodearray detector (Varian Associates, Walnut Creek, CA, USA). An isocratic mobile phase consisting of methanol–10 mM sodium dihydrogen phosphate buffer pH 7.5 (75:25, v/v) developed at a rate of 1.0 ml/min. The injection volume was 10 μl. Detection was effected at 230 nm.

3. Results and discussions

The identity of the isolated alkaloids *N*-methylcytisine, baptifoline, anagyrine and magnoflorine was confirmed UV, IR, MS and NMR spectroscopy and by comparison with literature data [6,7] while confirmation of the separated compounds in the analytical determination was by retention time, spiking, and photodiode array detector. Because of the structural differences between the quinolizidine alkaloids and the aporphine alkaloid magnoflorine, it was not possible to analyze them simultaneously using normal-phase HPLC or gas chromatographic methods on the crude extract.

The quinolizidine alkaloids were well separated under normal phase conditions (CH_2Cl_2–MeOH (100:10, v/v)), but magnoflorine seemed to be irreversibly bound to the HPLC column and no peak was observed for this compound.

GC analysis of the aporphine alkaloids has not been previously reported, although that of the quinolizidine alkaloids in general has been discussed by other workers [8,9]. In this study, when the crude methanol extract of *C. thalictroides* was analyzed using an OV-3 column, the quinolizidine alkaloids eluted within 12 min, but no peak corresponding to magnoflorine was seen. These observations indicate that magnoflorine probably decomposes under the GC conditions used (inj = 250°; oven = 300°) or, alternatively, it may be retained on the column due to irreversible binding to the stationary phase.

Because of the failure of the GC and normal phase HPLC methods to detect magnoflorine and the need for a rapid and reliable assay that could be used for the analysis of the alkaloids in the crude extract, reversed-pase HPLC and normal-phase TLC systems were developed. Both were found to be effective for the simultaneous determination of the major alkaloids found in crude methanolic extracts of *C. thalictroides*.

3.1. HPLC assay

The separation of the alkaloids in the crude extract of *C. thalictroides* is presented in Fig. 1. The purity and identity of the peaks were examined by diode array detection and by comparison with standards. The UV spectra of the peaks associated with the alkaloids and the internal standard, cytisine as determined by the diode array detector agreed with the wavelength and relative heights of absorbance maxima of spectra for the pure compounds determined on a Perkin-Elmer Lambda 2 spectrometer. The spectra recorded for the leading and trailing edges of each peak were superimposable. It is unusual that magnoflorine, the most polar of the alkaloids examined, has the greatest retention time in this reverse-phase system and there does not appear to be a ready explanation for this.

Quantitative analysis was carried out by conventional calibration procedures with cytisine (R_t

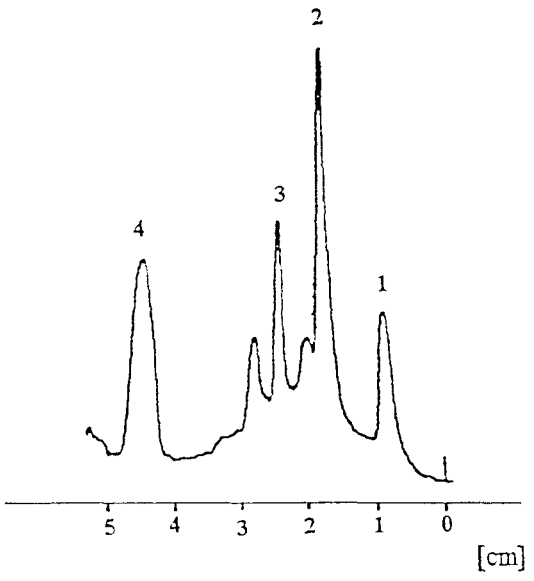


Fig. 2. Densitogram curve of EtOH extract of roots of *C. thalictroides*: (1) anagyrine, (2) *N*-methylcytisine, (3) baptifoline, and (4) magnoflorine.

5.56 min) as an internal standard using the appropriate reference standards in the range 5–50 μg ml^{-1}. The results of quantitative HPLC analysis of the alkaloids in the crude extract is presented in Table 1. Detection limits for the analyzed compounds ranged between 5 and 25 ng ml^{-1}. The ratios of the peak areas of the calibration solutions were plotted against the ratios of concentrations and gave a straight line (correlation coefficient 0.997).

3.2. TLC assay

The TLC separation of the alkaloids using the scanning densitometer is presented in Fig. 2. The results of quantitative analysis of the alkaloids is given in Table 1. Detection limits ranged between 0.5 and 2.5 μg ml^{-1}. The calibration graph gave a straight line with a correlation coefficient of 0.994.

Given these results, both the isocratic reversed-phase HPLC and normal-phase TLC methods employed were found to be simple and reliable methods for the determination of the alkaloids in *C. thalictroides*. These methods are suitable for the determination of these alkaloids in other families.

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