Journal of Chromatography, 587 (1991) 314–317
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 610

Short Communication

Analytical and quantitative studies of californin and protopin in aerial part extracts of *Eschscholtzia californica* Cham. with high-performance liquid chromatography

Jean-Pierre Rey*, Joël Levesque and Jean-Louis Pousset

Laboratoire de Pharmacognosie, Faculté de Médecine et de Pharmacie, 34 Rue du Jardin des Plantes, 86034 Poitiers Cedex (France)

François Roblot

Laboratoire de Pharmacognosie, Faculté de Pharmacie, Rue J. B. Clément, 92296 Chatenay-Malabry (France)

(First received April 26th, 1991; revised manuscript received July 2nd, 1991)

ABSTRACT

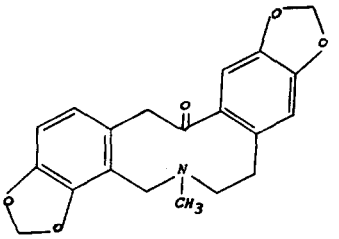
In the medicinal plant *Eschscholtzia californica* Cham., californin and protopin are mainly responsible for the sedative and spasmolytic effects. A selective high-performance liquid chromatographic method for the determination of these two alkaloids in plant extracts on a normal-phase column is described that allows their characterization and determination in medicinal plant drugs containing *Eschscholtzia californica* Cham. The most appropriated process for conceiving an *Eschscholtzia* liquid remedy seems to be a weak aqueous alcoholic preparation (30% ethanol) containing 1% of tartaric acid (total alkaloid efficiency = 0.405% dry material).

INTRODUCTION

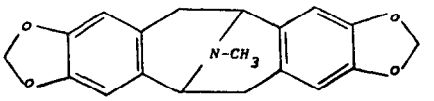
As part of a study of the application of high-performance liquid chromatography (HPLC) to the analysis of medicinal plant extracts with sedative properties, we have examined the components of *Eschscholtzia californica* Cham. This plant contains many alkaloids, the main ones being protopin ($C_{20}H_{19}NO_5$), first isolated in 1871 by Hesse from opium, then later identified among various Fumariaceae species (fumarin) and other Papaveraceae [1], and californin (=eschscholtzin or crychin) ($C_{19}H_{17}NO_4$), belonging to the pavin group, isolated in 1964 by Gertig [first named alkaloid ("Fz")] [2] and then by Manske and Shin, who named it eschscholtzin [3]. The latter alkaloid is specific in the *Eschscholtzia* genus.

Yellow poppy has been thoroughly studied for its sedative power [1,4–8], which is attributed to the alkaloids present acting with synergistic effects. Numerous studies on protopin and also on *Fumaria* and *Eschscholtzia* spp., have been made using thin-layer chromatography (TLC) [2,9–13], no work has been reported on the determination of these alkaloids with HPLC. We describe here a quantitative HPLC method for the determination of protopin and californin, which can be used as specific tracers in flowered aerial parts of the plant.

0021-9673/91/$03.50



Protopin (= Fumarin)



Californin (= Eschscholtzin ; Crychin)

EXPERIMENTAL

TLC

Silica gel Si 60 F_{254} plates were obtained from Merck (Darmstadt, Germany). The mobile phase was chloroform–methanol–acetic acid (80:15:5, v/v/v) and detection was effected with Dragendorff's reagent [14].

HPLC

A varian model 5000 chromatograph was used, equipped with a Rheodyne Model 7161 injector and a photodiode-array detector (Merck L3000) under computer control (Merck HPLC Manager). Analyses were conducted at 20°C.

Preparative HPLC was carried out with a Lichrosorb Si 60 column (250 × 10 mm I.D.; film thickness 7 μm) (Merck). The mobile phase was chloroform–methanol (99:1, v/v), at a flow-rate of 4 ml/min, and UV detection at 254 nm was applied.

Analytical HPLC was carried out on two columns. A normal-phase Lichrosorb Si 60 column (250 × 4 mm I.D., film thickness 7 μm) (Merck) was used with a Lichrosorb Si 60 precolumn (4 × 4 mm I.D. film thickness; 5 μm) (Merck). The mobile phase was chloroform–methanol (90:10, v/v) containing 0.1% of 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. The second column was a reversed-phase Lichrosorb RP-18 (250 × 4 mm I.D. film thickness 7 μm) (Merck) with a LiChrosorb RP-18 precolumn (25 × 4 mm I.D.; film thickness 5 μm) (Merck). The mobile phase was acetonitrile–water (65:35, v/v) containing 0.1% of 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 harvested in Maine et Loire (France), 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 (2.18 g) corresponding to the total alkaloid fraction (0.456% dry material).

Californin separation

A 1-g amount of the total alkaloid fraction diluted with chloroform was placed on a column containing 20 g of silica gel 60 (grain size 0.063–0.2 mm; 70–230 mesh ASTM) for column chromatography (Merck) and 100-ml fractions were collected. Elution was effected with pure chloroform for fractions I–VII (18 mg) and with chloroform–methanol (95:5, v/v) for fraction VIII (513 mg), IX (170 mg) and X (9.6 mg). The whole of fraction VIII was diluted with 1.5 ml of chloroform and then injected on to the preparative HPLC column. Fractions of 4 ml were recovered according to the following scheme: chloroform–methanol (99:1, v/v), fractions 1–25; chloroform–methanol (98:2, v/v), fractions 26–48; chloroform–methanol (95:5, v/v), fractions 49 to completion.

The product was checked using analytical HPLC. Fractions 9–24, mixed and evaporated to dryness, gave 224 mg of pure compound. ^{1}H and ^{13}C NMR

spectrometry (Bruker AC 200 P), mass spectrometry (MS) (Nermag R1010 C), melting point determination, UV spectrophotometric analysis and TLC allowed its identification as californin: m.p. 128°C (methanol); ^1H and ^{13}C NMR spectra identical with the literature [3]; electron impact m/z 323 ($M^{+\cdot}$), 188 (100%); UV λ_{max} [chloroform–methanol–TFA (90:10:0.1, v/v/v)], 245 and 292.5 nm; TLC, R_F (californin) = 0.58 and R_F (protopin) = 0.14.

RESULTS

We started to investigate reversed-phase HPLC (RP-18) with acetonitrile–water as the mobile phase containing from 35 to 70% of acetonitrile, and in addition several kinds of acids such as 1% phosphoric acid or acetic acid. The best results, obtained with acetonitrile–water (65:35, v/v) containing 0.1% of TFA were insufficient for a quantitative study.

We then tried normal-phase HPLC on Si 60 with chloroform–methanol (90:10, v/v) containing 0.1% of TFA, which resulted in 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 [15] and normal-phase modes.

The calibration graphs show a linear correlation between the amounts of the two alkaloids injected and the intensity of the absorption at 292.5 nm [correlation coefficient (r^2) 0.9978 for protopin and 0.9942 for californin]. Identification and determination of the two alkaloids were attempted (three consecutive times) on the total alkaloid fraction of different samples: fresh flowered aerial parts (Fig. 1); spray-dried fresh flowered aerial parts; dried flowered aerial parts; dried plant aqueous alcoholic extract (30% ethanol containing 1% of tartaric acid); and dried plant aqueous acidic extract (1% tartaric acid).

Quantitative analysis gave the results summarized in Table I.

DISCUSSION

This study has shown the simplicity, speed and reliability of HPLC for the determination of californin and protopin in *Eschscholtzia californica* Cham. extracts. The method is suitable for the systematic monitoring of sedative phytotherapeutic medicines. A quantitative study on various samples demonstrated the existence of chemotypes in this genus, correlated with a variable alkaloid ratio. The most appropriate process for obtaining an *Eschscholtzia* liquid remedy seems to be a weak aqueous alcoholic preparation (30% ethanol) containing 1% of tartaric acid.

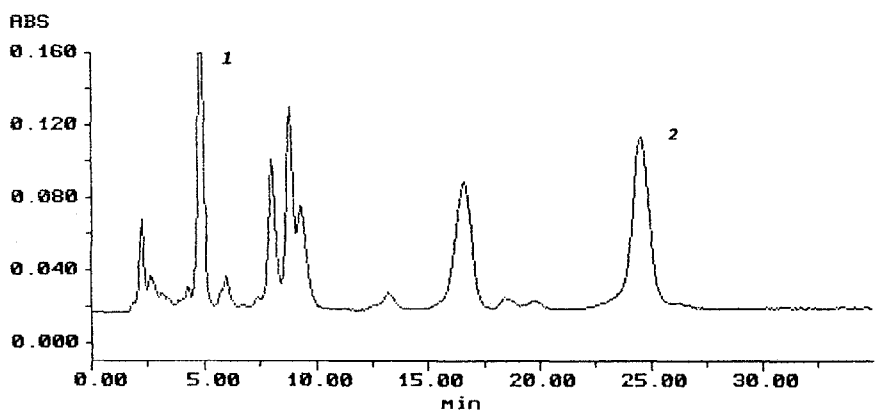


Fig. 1. Chromatogram of a total alkaloid fraction of fresh aerial parts of *Eschscholtzia californica* Cham. Peaks: 1 = californin; 2 = protopin. Conditions: column, LiChrosorb Si 60 (250 × 4 mm I.D.; film thickness 7 μm); precolumn, LiChrosorb Si 60 (4 × 4 mm I.D.; film thickness 5 μm); mobile phase, chloroform–methanol (90:10, v/v) + 0.1% TFA; flow-rate, 1 ml/min; UV detection at 292.5 nm.

TABLE I

VARIATION OF ACTIVE PRINCIPALS CALIFORNIN AND PROTOPIN WITHIN EXTRACTION PROCESSES

Extract	Total alkaloid efficiency (% dry material)	Amount of californin (mg per 100 g total alkaloids)	Amount of protopin (mg per 100 g total alkaloids)	Protopin: californin ratio
Fresh plant	0.471	23.5 ± 0.5	17.5 ± 1.3	0.74
Spray-dried	0.483	17.4 ± 0.3	16.5 ± 1.8	0.94
Dried plant	0.456	34.9 ± 0.2	10.1 ± 0.05	0.29
Aqueous alcoholic acidic extract	0.405	19.9 ± 0.8	2.2 ± 0.1	0.11
Aqueous acidic extract	0.273	11.3 ± 1.7	1.3 ± 0.2	0.11

ACKNOWLEDGEMENTS

Thanks are due to Laboratoire Pharmaceutique Florina (Valanjou) and Agrocinq (Toulouse) for providing pure protopin and financial support.

REFERENCES

1 R. H. Cheney, *Q. J. Crude Drug. Res.*, 3 (1963) 413.
2 H. Gertig, *Acta Pol. Pharm.*, 21 (1964) 59 and 127; 22 (1965) 271, 443, 462 and 473.
3 R. H. F. Manske and K. H. Shin, *Can J. Chem.*, 43 (1965) 2180 and 2183.
4 F. F. Vincieri, S. Celli, N. Mulinacci and E. Speroni, *Pharmacol. Res. Commun.*, 20 (1988) 41.
5 P. Delaveau, *Acta Pharm.*, 208 (1984) 33.
6 S. S. Lamba and R. W. Trottier, *Q. J. Crude Drug Res.*, 15 (1977) 25.
7 A. H. Dil, *Thérapie*, 28 (1973) 767.
8 R. Badacci, *Phytotherapy*, 9 (1984) 31.
9 B. Sener, *Int. J. Crude Drug. Res.*, 21 (1983) 135 and 272.
10 M. E. Popova, V. Simanek, J. Nouak, L. Dolejs, P. Sedmera and V. Preininger, *Planta Med.*, 48 (1983) 272.
11 V. B. Pandey, K. K. Seth and B. Das Gupta, *Pharmazie*, 37 (1982) 453.
12 M. E. Popova, A. N. Boeva, L. Dolejs, V. Preininger, V. Simanek and F. Santavy, *Planta Med.*, 40 (1980) 156.
13 J. Suspulgas, M. Lalaurie, G. Privat and R. Got, *Trav. Soc. Pharm. Montpellier*, 21 (1961) 28.
14 A. Baerheim Svendsen and R. Verpoorte, *Chromatography of Alkaloids, Part A: Thin-layer Chromatography (Journal of Chromatography Library*, Vol. 23A), Elsevier, Amsterdam 1983, pp. 11 and 502.
15 M. B. Radosevich and N. E. Delfel, *J. Chromatogr.*, 368 (1986) 443.