PHYTOCHEMICAL ANALYSIS
Phytochem. Anal. **10**, 264–267, (1999)

A Comparative Investigation on Alkaloid Composition in Different Populations of *Eschscholtzia californica* Cham.

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The alkaloid compositions of different populations of *Eschscholtzia californica* Cham. grown under controlled conditions and of some samples of the commercial drug have been examined using a reversed-phase HPLC method. Seven compounds have been detected (O-methylcaryachine, protopine, α-allocryptopine, eschscholtzine, californidine, sanguinarine and chelerytrine). In all samples the major components were the pavine alkaloids eschscholtzine and californidine, although their content varied to a large extent (from 1 to 10-fold). The commericial drug contained a significantly lower concentration of alkaloids. Copyright © 1999 John Wiley & Sons, Ltd.

Keywords: Eschscholtzia californica Cham.; isoquinoline alkaloids; alkaloid contents; HPLC.

INTRODUCTION

Eschscholtzia californica Cham. (Papaveraceae) is a native of the west coast of the United States and is described in the flora of New Mexico, Arizona, Nevada and Utah; from these regions the plants diffused to Central America and to Chile. It was introduced into Europe in the middle of nineteenth century as an ornamental plant. The local names for the plant (California Poppy, Globe du Soleil, Dedal de oro, Kalifornisher Mohn) arise from the cream-coloured, orange and golden yellow flowers of this plant (Cheney, 1963). Its preferred habitat is dry, sandy soil, although it may also be present in more damp localities. It is a polymorphic species with an interesting ecological success: it grows at altitudes from sea-level to 1900–2000 m and is herbaceous and annual in arid climates but becomes suffruticous and perennial when growing in rainy zones and on wet soils (Fedde, 1909; Hegnauer, 1969).

E. californica contains isoquinoline alkaloids of the pavine type (mainly eschscholtzine and californidine), of the benzophenanthridine type (sanguinarine and chelerytrine) and, in a lesser amount, some protopine derivatives (Slavik and Slavikova, 1986; Guédon *et al.*, 1990). Recently, (+) and (−)-cheilantifoline, hunnemarine and norsanguinarine have been detected and isolated (Jain *et al.*, 1996a). Among the flavonoids, rutin has previously been reported from this species (Sando and Bartlett, 1920). Two new isoflavones, together with quercitrin, have been isolated from the whole plant; the structures of these two isoflavones are 2′-methoxy-formononetin and 7-methoxy-2′,4′-dihydroxyisoflavone (Jain *et al.*, 1996b). Furthermore, flavonols and anthocyanidins have been isolated from the aqueous fraction of the flowers (Bilia *et al.*, 1996).

West coast Amerindians eat the leaves boiled or roasted on hot stones and occasionally smoke the leaves and petals. *E californica* is also an ancient traditional medicinal plant used by the rural populations of California for its spasmolytic properties, to reduce milk flow, as a poultice for sores and ulcers and, in addition, the plant mixed with black pepper has been used for ague, jaundice and skin ailments (Cheney, 1963; Duke, 1985). The tincture or dry extract of *E. californica* is used in Europe, mainly in Germany and France, for its sedative and anti-neuralgic properties. Pharmacological investigations have shown the sedative and anxiolytic action of extracts of *E. californica* in the absence of toxic effects, thus validating the traditional uses (Rolland *et al.*, 1991).

The hypnotic, analgesic and sedative properties are the main reasons that the use of this plant has spread widely. Probably, as a recent work shows, this activity can be explained by the effect of the extracts on catecholamine metabolism (Kleber *et al.*, 1995). There are no indications of the compounds responsible for the physiological effects of extracts from *E. californica*, although it is reasonable to suppose that the alkaloid content has a role in the pharmacological action (Vincieri *et al.*, 1988).

The purpose of the present study was to comparatively evaluate, using a reversed-phase HPLC method, the alkaloid composition of plants of *E. californica* obtained from seeds of different origin (i.e. different European botanical gardens) and grown under controlled conditions; furthermore, the alkaloid contents of some samples of the commercial drug have also been examined.

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Contract/grant sponsor: Ministero dell'Università e della Ricerca Scientifica (MURST).

CCC 0958–0344/99/050264–04 $17.50

Received 2 April 1998
Revised 22 September 1998
Accepted 24 September 1998

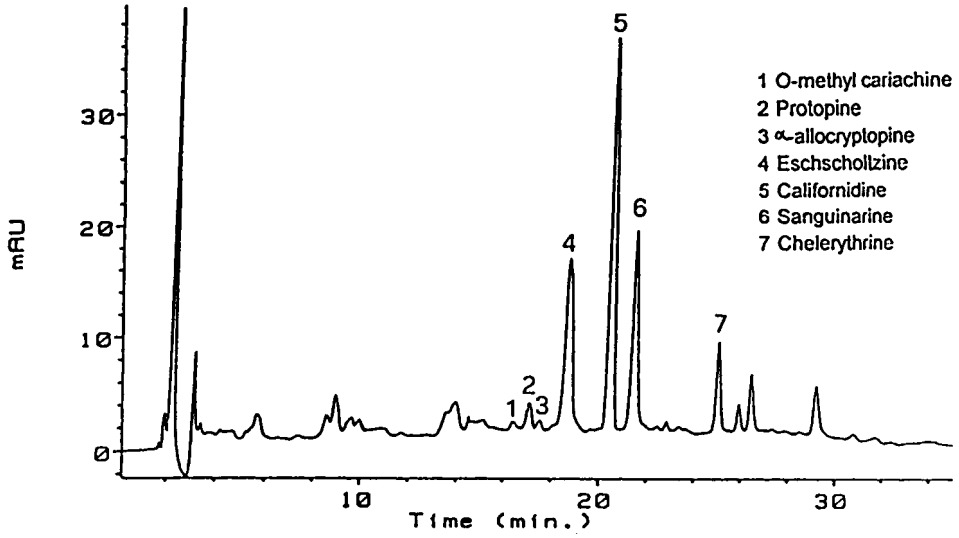

Figure 1. Representative reversed-phase HPLC chromatogram of extracts of *Eschscholtzia californica* Cham. (for details of the HPLC analytical protocol see the Experimental section).

EXPERIMENTAL

Plant material. Plants of *Eschscholtzia californica* were grown from seeds provided by the European Botanical Gardens of Milan (Italy), Budakalasz (Hungary), Tübingen (Germany), Dijon (France), Coimbra (Portugal), and Meise (Belgium). The seeds were germinated in Petri plates, and further plant growth was carried out in 14 cm i.d. pots (10 plants per pot) in controlled chambers (day length, 14 h; light level, 47.5 cm^2s^{-1}; temperature, 24 ± 1°C; relative humidity, 60%). Commercially available dried aerial parts and dry alcohol extracts were also examined.

Extraction and analysis of alkaloids. The fresh plant material (4–5 g), usually randomly collected from 10 plants, or the commercial dried material (3 g), was exhaustively extracted in a Soxhlet apparatus with 70% ethanol, and the alkaloid fraction isolated by ion-pair partition as described elsewhere (Bugatti *et al.*, 1991). The dried alcoholic extract (3 g) was subjected to the same method, omitting the first step of the extraction procedure. Both commercial and fresh plant material were extracted in triplicate.

Alkaloids were separated by reversed-phase HPLC using a chromatographic system consisting of a Merck-Hitachi (Darmstadt, Germany) L-6200 intelligent pump, a LiChrospher C8 column (250×4 mm i.d.; 5 μm; Merck) and a Merck-Hitachi L-4200 UV–VIS detector set at 280 nm. The mobile phase solvents were water:-acetonitrile (80:20) containing 10 mM aqueous octylsulphonic acid (sodium salt) and 0.15 M triethylamine adjusted to pH 3 with phosphoric acid (eluent A), and water:acetonitrile (60:40) containing 10 mM aqueous octylsulphonic acid (sodium salt) and 0.15 M triethylamine adjusted to pH 3 with phosphoric acid (eluent B). The elution profile was: 0–5 min, isocratic elution with 100% A; 5–25 min, linear gradient from 0 to 100% B; 25–35 min, isocratic elution with 100% B. The flow rate was 1 mL/min. Following return to 100% A, the system was left to stabilize for 15 min with this eluent between consecutive injections.

In order to determine the peak identity and purity, alkaloid extracts were analysed by means of diode array detection (Hewlett Packard, Cernusco sul Naviglio (MI), Italy; model 1040 M series II detector) monitored from 200 to 600 nm and by comparison with authentic standards. The reference compounds, protopine, sanguinarine and chelerythrine were purchased from Sigma (Milan, Italy), whilst eschscholtzine, californidine and *O*-methyl-caryachine were isolated in our laboratory and identified by spectroscopic methods. The quantitative determination of alkaloid content was performed by the method of external standard.

RESULTS AND DISCUSSION

The comparative analyses of the alkaloid compositions in populations of *E. californica* of different origins were carried out using the aerial parts of plants grown in a phytotron with a long-day photoperiod and harvested in the pre-blooming phase 45 days after sowing. Reversed-phase HPLC separations of the alkaloid extracts revealed the presence of seven compounds (Fig. 1): *O*-methylcaryachine [**1**; retention time (R_t), 16.4 ± 0.2 min]; protopine (**2**; R_t, 17.1 ± 0.2 min); α-allocryptopine (**3**; R_t, 17.5 ± 0.2 min); eschscholtzine (**4**; R_t, 18.6 ± 0.3 min); californidine (**5**; R_t, 20.5 ± 0.2 min); sanguinarine (**6**; R_t, 21.4 ± 0.4 min); and chelerythrine (**7**; R_t, 24.9 ± 0.3 min).

The quantitative determinations of the alkaloids were accomplished by means of calibration graphs comparing peak areas with amounts of standards injected. These graphs were found to be linear within the range 0.01–0.50 mg/mL with the following regression coefficients: *O*-methylcaryachine, 1600; protopine, 481; α-allocryptopine, 535; eschscholtzine, 708; californidine, 571; sanguinarine, 2600; and chelerythrine, 2644. Data were obtained from the average of at least three measurements performed on at least three samples. The correlation coefficients for the linear regressions were highly significant (r values were all higher than 0.987). For each chromatographic peak the purity of the compound

Table 1. The alkaloid content of extracts of the fresh aerial parts of *Eschscholtzia californica* Cham.

	Content % dry weight (±standard deviation)					
Alkaloid	Sample 1, Milan (Italy)	Sample 2, Budakalasz (Hungary)	Sample 3, Tübingen (Germany)	Sample 4, Dijon (France)	Sample 5, Coimbra (Portugal)	Sample 6, Meise (Belgium)
O-methylcaryachine	$0.55 \pm 4.9 \times 10^{-2}$	Traces	$0.07 \pm 1.9 \times 10^{-2}$	$0.09 \pm 3.1 \times 10^{-3}$	$0.09 \pm 2.2 \times 10^{-2}$	$0.03 \pm 2.3 \times 10^{-3}$
Protopine	$0.01 \pm 1.8 \times 10^{-3}$	Traces	Traces	Traces	Traces	Traces
α-Allocryptopine	$0.04 \pm 4.9 \times 10^{-3}$	$0.04 \pm 7.3 \times 10^{-3}$	Traces	Traces	Traces	Traces
Eschscholtzine	$0.88 \pm 6.2 \times 10^{-2}$	$1.08 \pm 1.2 \times 10^{-1}$	$0.37 \pm 4.8 \times 10^{-2}$	$0.98 \pm 3.5 \times 10^{-2}$	$0.19 \pm 2.2 \times 10^{-2}$	$0.48 \pm 3.6 \times 10^{-2}$
Californidine	$0.47 \pm 5.8 \times 10^{-2}$	$0.98 \pm 7.3 \times 10^{-2}$	$0.11 \pm 3.7 \times 10^{-2}$	$0.51 \pm 5.1 \times 10^{-2}$	$0.41 \pm 3.3 \times 10^{-2}$	$0.17 \pm 1.5 \times 10^{-2}$
Sanguinarine	$0.02 \pm 1.1 \times 10^{-3}$	$0.04 \pm 6.1 \times 10^{-3}$	$0.04 \pm 7.1 \times 10^{-3}$	$0.11 \pm 1.2 \times 10^{-2}$	$0.04 \pm 4.3 \times 10^{-3}$	Traces
Chelerythrine	$0.02 \pm 4.7 \times 10^{-3}$	$0.03 \pm 6.8 \times 10^{-3}$	$0.04 \pm 0.8 \times 10^{-2}$	$0.07 \pm 4.4 \times 10^{-3}$	$0.06 \pm 7.9 \times 10^{-3}$	Traces

Table 2. The alkaloid content of extracts of commercial samples of *Eschscholtzia californica* Cham.

	Content % dry weight (±standard deviation)		
Alkaloid	Sample A, aerial parts	Sample B, aerial parts	Sample C, hydroalcoholic extract
O-methylcaryachine	$0.002 \pm 4.7 \times 10^{-4}$	$0.043 \pm 5.1 \times 10^{-3}$	Traces
Protopine	$0.032 \pm 4.1 \times 10^{-3}$	$0.032 \pm 2.3 \times 10^{-3}$	$0.004 \pm 5.8 \times 10^{-4}$
α-Allocryptopine	$0.031 \pm 5.2 \times 10^{-3}$	$0.025 \pm 3.7 \times 10^{-3}$	$0.005 \pm 3.4 \times 10^{-4}$
Eschscholtzine	$0.101 \pm 1.1 \times 10^{-2}$	$0.351 \pm 2.1 \times 10^{-2}$	$0.031 \pm 3.1 \times 10^{-3}$
Californidine	$0.302 \pm 9.7 \times 10^{-3}$	$0.301 \pm 1.4 \times 10^{-2}$	$0.172 \pm 1.8 \times 10^{-2}$
Sanguinarine	$0.021 \pm 3.8 \times 10^{-3}$	$0.025 \pm 3.2 \times 10^{-3}$	$0.002 \pm 1.6 \times 10^{-4}$
Chelerythrine	$0.007 \pm 1.6 \times 10^{-3}$	$0.027 \pm 4.9 \times 10^{-3}$	$0.001 \pm 1.1 \times 10^{-4}$

was evaluated according to the Hewlett Packard software system (Kohn, 1994). The HPLC method was found to be quantitative and reproducible (based on 10 replicate measurements). The recovery values of the extraction assays were in the range from 98.4% to 99.7%.

The alkaloid profiles were qualitatively similar in all populations of *E. californica* independent of the origin of the seed material. Significant differences, however, were detected in the quantitative pattern. The alkaloid contents of the various populations are reported in Table 1. The major components were the pavine alkaloids eschscholtzine and californidine. The former varied from values near to 1% dry weight (samples 1, 2 and 4) to a minimum value of 0.2% dry weight (sample 5). Californidine was found to be present in a range between 1% dry weight (sample 2) and 0.1% dry weight (sample 3). The trend of the variation was not, however, similar for the two alkaloids: the eschscholtzine: californidine ratio varied from 0.47 for the Coimbra sample to 3.70 for that from Tübingen. The protopine alkaloids protopine and α-allocryptopine were always present (except in sample 1) in trace amounts, whilst the benzophenanthridine type sanguinarine and chelerythrine did not exceed 0.1% dry weight.

The present study was extended to evaluate the alkaloid content of some commercial samples (Table 2). The components detected in such material (namely, dried aerial parts or dry alcohol extract) were the same as previously mentioned. The quantitative patterns showed that, whilst levels of the minor compounds were rather stable, the major alkaloids, especially eschscholtzine, were significantly lower than in the samples obtained from seeds.

The above results show that in *E. californica*, the alkaloid content may vary to a great extent, especially with respect to eschscholtzine and californidine. For this reason, owing to the extensive use of *E. californica* and, consequently, to the increasing demand and economic interest in the plant material, great attention must be given to the origin of the drug. In addition studies are also required to be directed to clarify the true active constituents of the plant.

Acknowledgements

This work was financially supported by MURST (Ministero dell'Università e della Ricerca Scientifica).

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