Journal of Chromatography, 596 (1992) 276–280
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 046

Short Communication

Analytical studies of isorhoeadine and rhoeagenine in petal extracts of *Papaver rhoeas* L. using 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, B.P. 199, 86005 Poitiers Cedex (France)

François Roblot

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

(First received October 29th, 1991; revised manuscript received January 28th, 1992)

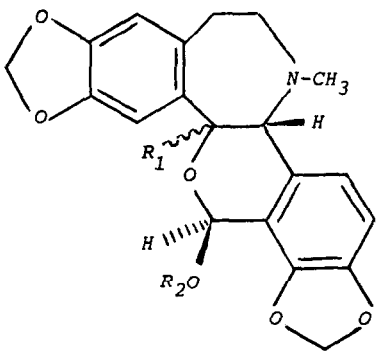
ABSTRACT

A low-pressure liquid chromatographic method on silica gel 60, with chloroform containing 0–1% (v/v) of methanol as eluent, is described that allows the isolation of isorhoeadine from the total alkaloids of petals of *Papaver rhoeas* L. A preparative high-performance liquid chromatographic (HPLC) method with a LiChrosorb Si 60 column, using chloroform containing 0–5% (v/v) of methanol (isocratic and then linear gradient) as mobile phase, is described that allows the isolation of rhoeagenine from a few fractions issuing from the previous low-pressure liquid chromatographic run. Finally, a selective analytical HPLC method with a Superspher Si 60 column using chloroform–methanol (90:10, v/v) containing 0.1% of trifluoroacetic acid as mobile phase and UV detection at 292.5 nm is described that allows the determination of isorhoeadine and rhoeagenine in red poppy extracts. In comparison with a classical chloroformic alkaloid extraction of petals from Maine et Loire (France) (total alkaloid efficiency = 0.203% dry material), a weak aqueous alcoholic acidic extract (30% ethanol) (0.216%) and an aqueous acidic extract (0.123%) of the same material, the amount of isorhoeadine is 71.1, 42.4 and 10.5 mg/g total alkaloids, respectively, and the amount of rhoeagenine is 629.7, 424.2 and 117.3 mg/g total alkaloids, respectively. Hence, the aqueous alcoholic acidic extract seems to be the most appropriate for conceiving a red poppy remedy.

INTRODUCTION

Further to our 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 *Papaver rhoeas* L. There have been numerous investigations into the alkaloidal constituents of this plant [1] and over 30 alkaloids have been isolated. It appears that *Papaver rhoeas* L. is variable in its alkaloid content and that different chemotypes exist [2]; the major alkaloid isolated proved to be rhoeadine [3,4], N-methylasimilobine [5] or rhoeagenine [6], depending on the plant source. The minor alkaloids are mainly protopine, isorhoeadine, isorhoeagine and papaverrubines [7]. Numerous studies on the rhoeadine–papaverrubine group of *P. rhoeas* L. have been made using thin-layer chromatography (TLC) [4,8–12], but no work has been reported on the determination of these alkaloids

0021-9673/92/$05.00



Rhoeagenine : R_1 = —H ; R_2 = H

Isorhoeadine : R_1 = ····H ; R_2 = CH_3

using HPLC. We describe here a quantitative HPLC method for the determination of rhoeagenine and isorhoeadine, which can be used as specific tracers in petals of the plant.

EXPERIMENTAL

TLC

Silica gel Si 60 F_{254} plates were obtained from Merck (Darmstadt, Germany). The mobile phase was cyclohexane–diethylamine (80:20, v/v) and detection was effected with Dragendorff's reagent [12].

HPLC

A Varian Model 5000 chromatograph was used, equipped with a Rheodyne Model 7125 injector and a photodiode-array detector (Merck L 3000) 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., particle size 7 μm) (Merck). Two solvents were used, (A) chloroform and (B) methanol. The elution profile was as follows: 0–40 min, 100% A (isocratic); 41–46 min, 1% B in A (isocratic); 47–51 min, 2% B in A (isocratic); 52–61 min, 2–5% B in A (linear gradient). A flow-rate of 4 ml/min and UV detection at 280 nm were applied.

Analytical HPLC was carried out on a Superspher Si 60 normal-phase 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% 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.

Petal alkaloid extraction

A 500-g amount of petals [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 (1.015 g) corresponding to the total alkaloid fraction A (0.203% dry material).

An alkaloid extraction was also applied to another batch of plant material [harvested in Vienne (France)]. The extraction procedure was the same as above (total alkaloid efficiency = 0.237% dry material).

Petal extract preparation

Aqueous alcoholic acidic extract. A 25-g amount of dried petals (Maine et Loire batch) were heated under reflux with 250 ml of 30% ethanol containing 250 mg of tartaric acid for 2 h and then filtered to afford the extract. For the extraction of the alkaloid of this extract, the ethanol was evaporated under reduced pressure until condensation of water. After alkalinization with ammonia (pH 10), the solution was extracted with 4 × 50 ml of chloroform. The organic layers were washed with 70 ml of distilled water, filtered and evaporated to dryness under reduced pressure (total alkaloid efficiency = 0.216% dry material).

Aqueous acidic extract. A 25-g amount of dried petals (Maine et Loire batch) was heated under reflux with 250 ml of distilled water containing 250 mg of tartaric acid for 2 h and then filtered to afford

the extract. This extract was directly alkalinized with ammonia (pH 10) and then extracted with 4 × 50 ml of chloroform. The organic layers were washed with 70 ml of distilled water, filtered and evaporated to dryness under reduced pressure (total alkaloid efficiency = 0.123% dry material).

Isorhoeadin isolation

A 500-mg amount of the total alkaloid fraction A diluted with chloroform (5 ml) was placed on a column (200 × 20 mm I.D) containing 20 g of silica gel 60 (particle size 0.063–0.2 mm) for low-pressure column chromatography (Merck) and 50-ml fractions were collected. Elution was effected with pure chloroform (100 ml) for fractions I and II (3 mg) and with chloroform–methanol (99:1, v/v) (450 ml) for fractions III (18.6 mg), IV–X (324 mg) and XI (8 mg).

The product of fraction III, crystallized in ethanol, afforded 11 mg of pure compound. ^{1}H NMR spectrometry (Brucker AC 200 P NMR spectrometer), mass spectrometry (MS) (Nermag R 1010 C mass spectrometer), melting point determination, UV spectrophotometric analysis and TLC allowed its identification as isorhoeadine: m.p. 161°C [11, 13]; ^{1}H NMR spectra identical with the literature [13,14]; electron impact MS, *m/z* 383 ($M^{+\cdot}$), 368 (100), 352 (10), 177 (96) [15]; UV, λ_{max} [chloroform–methanol–TFA (90:10:0.1, v/v/v)] 244 and 292.5 nm; TLC, R_F = 0.65.

Rhoeagenin isolation

The whole of fraction IV–X 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: pure chloroform (160 ml), fractions 1–40; chloroform–methanol (99:1, v/v) (24 ml), fractions 41–46; chloroform–methanol (98:2, v/v) (20 ml), fractions 47–51; chloroform–methanol (95:5, v/v) (40 ml), fractions 52–61.

The product was checked using analytical HPLC. Fractions 13–43, mixed, evaporated to dryness and crystallized in ethanol, gave 96 mg of pure compound. ^{1}H NMR spectrometry, melting point determination, UV spectrophotometric analysis and TLC allowed its identification as rhoeagenine: m.p. 236°C [11,13,16]; ^{1}H NMR spectra identical with the literature [13,17]; electron impact MS, *m/z* 369 ($M^{+\cdot}$, 10), 206 (100), 192 (79), 177 (12), 163 (100) [13,15,17]; UV, λ_{max} [chloroform–methanol–TFA (90:10:0.1, v/v/v) 245 and 292.5 nm; TLC, R_F = 0.36.

RESULTS AND DISCUSSION

In contrast to our work on the separation of alkaloids using reversed-phase HPLC [18], we adopted normal-phase HPLC on Superspher Si 60 with chloroform–methanol (90:10, v/v) containing 0.1% of TFA, which resulted in a considerable improvement in the chromatographic profile.

For quantitative analysis, the calibration graphs show a linear correlation from 0.1 to 2 mg/ml between the amounts of isorhoeadine and rhoeagenine injected and the intensity of the absorption at

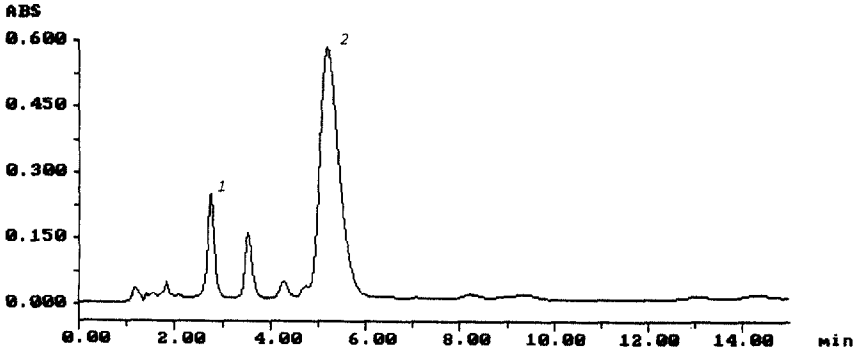


Fig. 1. Chromatogram of a total alkaloid fraction of petals of *Papaver rhoeas* L. (Maine-et-Loire). Peaks: 1 = isorhoeadine; 2 = rhoeagenine. Conditions: column, Superspher Si 60 (125 × 4 mm I.D.; particle size 4 μm); precolumn, LiChrospher Si 60 (4 × 4 mm I.D.; particle size 5 μm); mobile phase, chloroform–methanol (90:10, v/v) containing 0.1% TFA; flow-rate, 1 ml/min; UV detection at 292.5 nm.

TABLE I

VARIATION OF ISORHOEADINE AND RHOEAGENINE CONTENTS WITHIN DIFFERENT PETAL EXTRACTS OF *PAPAVER RHOEAS* L.

Quantitative parameters: $\bar{x}$ = mean (n = 5); S.D. = standard deviation; R.S.D. = relative standard deviation.

Extract	Total akaloid efficiency (% dry material)	Isorhoeadine				Rhoeagenine				Isorhoeadine/ rhoeagenine ratio
		$\bar{x}$ (mg/g total alkaloids)	S.D. (mg/g total alkaloids)	R.S.D. (%)	$\bar{x}$ (mg per 100 g dry material)	$\bar{x}$ (mg/g total alkaloids)	S.D. (mg/g total alkaloids)	R.S.D. (%)	$\bar{x}$ (mg per 100 g dry material)	
Petals (Vienne)	0.237	123.4	2.8	2.26	29.25	754.2	5.6	0.74	178.74	0.16
Petals (Maine et Loire)	0.203	71.1	1.9	2.67	14.43	629.7	3.9	0.62	127.83	0.11
Aqueous alcoholic acidic extract	0.216	42.4	0.9	2.12	9.16	424.2	2.8	0.66	91.63	0.09
Aqueous acidic extract	0.123	10.5	0.2	1.9	1.29	117.3	2.8	2.38	14.43	0.09

292.5 nm [correlation coefficient (r^2) = 0.9968 for isorhoeadine and 0.9837 for rhoeagenine]. Five determinations were carried out on different extracts in order to test the accuracy and precision of the method in terms of standard deviation and relative standard deviation. The determination of the two alkaloids was attempted on the total alkaloid fraction of petals [Vienne and Maine et Loire (Fig. 1)], aqueous alcoholic acidic and aqueous acidic extract (Maine et Loire). Quantitative analysis gave the results summarized in Table I.

If we consider that the classical procedure for the extraction of the alkaloids (involving Soxhlet extraction with chloroform) gives the real amount (100%) of isorhoeadine and rhoeagenine contained in the red poppy petals, we can calculate that during the preparation of the aqueous alcoholic acidic extract we extracted 63.5% of the total amount of isorhoeadine and 71.7% of the total amount of rhoeagenin in the petals. Also, during the preparation of the aqueous acidic extract, we extracted only 8.9% of the total amount of isorhoeadine and 11.3% of the total amount of rhoeagenine in the petals. Hence the most efficient process for conceiving a red poppy liquid remedy seems to be a weak aqueous alcoholic preparation (30% ethanol) containing 1% of tartaric acid (dry material).

Quantitative studies on various samples of petals from different sources (Vienne and Maine-et-Loire) demonstrated the existence of chemotypes in this genus, correlated with a variable alkaloid ratio (0.16 and 0.11).

In conclusion, the proposed method allows the simultaneous isocratic separation of isorhoeadine and rhoeagenine in petals of *Papaver rhoeas* L. and can be used in their routine determination in drugs.

ACKNOWLEDGEMENTS

Thanks are due to Laboratoire Pharmaceutique Florina (Valanjou), Sevres-flore (Chemillé) and Agrocing (Toulouse) for providing financial support.

REFERENCES

1 F. Santavy, *Alkaloids (N.Y.)*, 12 (1970) 339 and 17 (1979) 395.
2 A. Nemeckova, F. Santavy and D. Walterova, *Collect. Czech. Chem. Commun.*, 35 (1970) 1733.
3 Y. N. Kalav and G. Sariyar, *Planta Med.*, 55 (1989) 488.
4 J. Slavik, L. Slavikova and J. Bochorakova, *Collect. Czech. Chem. Commun.*, 54 (1989) 1118.
5 S. El-Masry, M. G. El-Ghazooly, A. A. Omar, S. M. Khafagy and J. D. Philipson, *Planta Med.*, 41 (1981) 61.
6 V. Preininger, V. Simanek, O. Gasic, F. Santavy and L. Dolejs, *Phytochemistry*, 12 (1973) 2513.
7 V. Preininger, *Alkaloids (N.Y.)*, 29 (1986) 1.
8 A. Nemeckova, F. Santavy and D. Walterova, *Collect. Czech. Chem. Commun.*, 35 (1970) 1733.
9 F. Santavy, J. Hrbek and K. Blaha, *Collect. Czech. Chem. Commun.*, 32 (1967) 4452.
10 J. D. Philipson, O. O. Thomas, A. I. Gray and G. Sariyar, *Planta Med.*, 41 (1981) 105.
11 M. Maturova, D. Pavlaskova and F. Santavy, *Planta Med.*, 14 (1966) 22.
12 A. Baerheim Svendsen and R. Verpoorte, *Chromatography of Alkaloids, Part A: Thin-Layer Chromatography (Journal of Chromatography Library*, Vol. 23A), Elsevier, Amsterdam, 1983, pp. 181, 209 and 214.
13 C. T. Montgomery, B. K. Cassels and M. Shamma, *J. Nat. Prod.*, 46 (1983) 441.
14 F. Santavy, J. L. Kaul, L. Hruban, L. Dolejs, V. Hanus, K. Blaha and A. D. Cross, *Collect. Czech. Chem. Commun.*, 30 (1965) 3479.
15 L. Dolejs and V. Hanus, *Tetrahedron*, 23 (1967) 2997.
16 S. Pfeifer and I. Mann, *Pharmazie*, 23 (1968) 82.
17 G. Sariyar and J. D. Philipson, *Phytochemistry*, 19 (1980) 2189.
18 J. P. Rey, J. Levesque, J. L. Pousset and F. Roblot, *J. Chromatogr.*, 587 (1991) 314.