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Short Communication

Extraction and high-performance liquid chromatographic methods for the γ-lactones parthenolide (*Chrysanthemum parthenium* Bernh.), marrubiin (*Marrubium vulgare* L.) and artemisinin (*Artemisia annua* L.)

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)

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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 80:20:6 (v/v/v) as eluent is described as a simple and inexpensive process for the isolation of the γ-lactones parthenolide, marrubiin and artemisinin from the aerial parts of feverfew, white horehound and quinghao, respectively. A selective high-performance liquid chromatographic (HPLC) method using a Superspher Si 60 column with hexane–dioxane (85:15, v/v) for elution of parthenolide and marrubiin and (90:10, v/v) for artemisinin and UV detection at 210 nm for parthenolide and artemisinin and 225 nm for marrubiin is described that allows the determination of these γ-lactones in plants. Compared with the HPLC method, the extraction yield of the large-scale process was calculated to be 72.3, 78.4 and 74.1% of the total amount of parthenolide, marrubiin and artemisinin, respectively, contained in each plant.

INTRODUCTION

Further to our study of the application of high-performance liquid chromatography (HPLC) to the analysis of medicinal plants with anti-inflammatory, antispasmodic and antimalarial properties, we have examined the components of *Chrysanthemum parthenium* Bernh. (feverfew), *Marrubium vulgare* L. (white horehound) and *Artemisia annua* L. (quinghao). Feverfew contains almost 30 sesquiterpene lactones [1] and mainly parthenolide in the European species [2]. This compound is mainly responsible of the therapeutic efficiency of this drug against migraine [3]. White horehound contains many diterpenic lactones [4], the main one being marrubiin [5], whose sodium salt has a pronounced action on biliary secretion [6]. Quinghao, used for many centuries in chinese folk medicine, contains artemisinin [7,8], a sesquiterpenic endoperoxide lactone used in the treatment of chloroquine-resistant and cerebral malarias [9–11].

Numerous studies have been reported on the isolation of parthenolide [1,12,13], marrubiin [14,15] and artemisinin [16–19], but all of them involved expensive solvents (acetonitrile, diethyl ether and acetone) and time-consuming methods. Moreover,

Correspondence to: Dr. J.-P. Rey, Laboratoire de Pharmacognosie, Faculté de Médecine et de Pharmacie, 34 Rue du Jardin des Plantes, B.P. 199, 86005 Poitiers Cedex, France.

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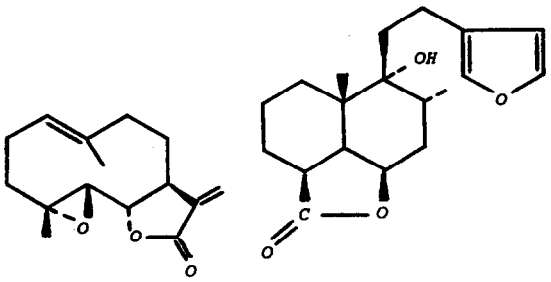
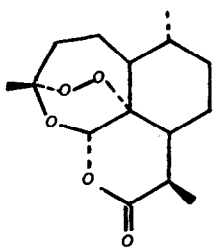


PARTHENOLIDE **MARRUBIIN**



ARTEMISININ

whereas many determinations have been achieved by infrared spectroscopy [13], thin-layer chromatography (TLC) [20,21] and reversed-phase HPLC [22,23], there have been no reports of the determination of parthenolide, marrubiin and artemisinin by normal-phase HPLC. In this paper we describe a rapid and inexpensive process for the isolation of these pure lactones which can be extrapolated to an industrial scale. We also describe a normal-phase HPLC method for their determination in the aerial parts of plants.

EXPERIMENTAL

Chemicals

Hexane and 1,4-dioxane were of HPLC quality from Rathburn Chemicals (Walkerburn, UK). All other solvents were of analytical-reagent grade quality from Labosi (Paris, France).

Thin-layer chromatography

Silica gel Si 60 F_{254} plates were obtained from Merck (Darmstadt, Germany). The mobile phase was chloroform–acetone (90:10, v/v). All TLC separations were performed at room temperature (20°C). The mobile phase was allowed to remain in the closed glass tank for 1 h before chromatography. The inside of the tank was lined with filter-paper. A distance of 15 cm was used for the development of the chromatograms. Detection was effected with a 2% methanolic solution of resorcinol mixed with an equal volume of 2% sulphuric acid (reagent No. 1 [20]) and with a 1% ethanolic solution of vanillin mixed with 2% concentrated sulphuric acid (reagent No. 2 [21]). Dried chromatograms treated with reagent 1 or 2 were kept for 2–4 min at 110°C.

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 hexane–dioxane (85:15, v/v) at a flow-rate of 1 ml/min for parthenolide and marrubiin elution and hexane–dioxane (90:10, v/v) at a flow-rate of 2 ml/min for artemisinin elution. The injection volume was 10 μl and UV detection was at 210 nm for parthenolide and artemisinin and 225 nm for marrubiin.

Isolation of artemisinin

Extraction of the lactonic fraction. A 1.7-kg amount of flowered aerial parts of *Artemisia annua* L. (harvested in China and received dried), finely powdered, was subjected to a Soxhlet extraction with chloroform (4 l) for 4 h. The solvent was evaporated under reduced pressure at 40°C to give a residue of 105 g (R_1, 6.2% dry material).

Purification with liquid–liquid extraction. This residue was dissolved in warm methanol (1 l), then 200 ml of distilled water were added. The mixture was kept cool, filtered, and extracted with 4 × 500 ml of hexane. We monitored by TLC that artemisinin was present in the methanolic layer [purple spot at R_F = 0.72 (reagent No. 2)], but not in the hexane phases, which were discarded.

Low-pressure column chromatography. The meth-

anolic layer was evaporated under reduced pressure and the residue (R_2, 52 g) was diluted with chloroform (250 ml) and washed out on a column (700 × 40 mm I.D.) containing 150 g of silica gel 60 (Merck) (particle size 0.063–0.2 mm) for low-pressure column chromatography with chloroform (until eluate remained colourless) to remove polar compounds. The eluate was evaporated (R_3, 35 g) and chromatographed on an identical-sized column containing 350 g of silica gel 60. Fractions of 500 ml were recovered according to the following elution profile: hexane–chloroform (80:20, v/v) (solvent A, 1 l) for fractions I–II; hexane–chloroform–ethyl acetate (80:20:2, v/v/v) (solvent B, 2 l) for fractions III–VI; hexane–chloroform–ethyl acetate (80:20:4, v/v/v) (solvent C, 3 l) for fractions VII–XII; and hexane–chloroform–ethyl acetate (80:20:6, v/v/v) (solvent D, 2 l) for fractions XIII–XVI. The product was checked using TLC. Fractions VIII–XI, mixed, evaporated to dryness and crystallized from cyclohexane, afforded 2.74 g of pure compound (0.16% dry material). ^{13}C and ^{1}H NMR spectroscopy (Brucker AC 200 P), melting point determination, UV spectrophotometric analysis and TLC confirmed its identification as artemisinin: m.p. 154°C [7,17,18]; ^{13}C and ^{1}H NMR spectra identical with the literature [24]; UV λ_{max} [hexane–dioxane (90:10, v/v)], 210 nm; TLC, R_F = 0.72.

Isolation of parthenolide

The latter process was applied to 1.7 kg of flowered aerial of *Chrystanthenum parthenium* Bernh. [harvested in Maine et Loire (France) and dried at room temperature]. We obtained the following results: R_1 = 132 g (7.78% dry material); cherry-coloured spot in the methanolic layer at R_F = 0.60 (reagent No. 1); R_2 = 5.47 g; R_3 = 15.7 g; fractions X–XIII, mixed, evaporated to dryness and crystallized from cyclohexane, afforded 4.08 g of pure compound (0.24% dry material). ^{1}H NMR spectroscopy (Bruker AC 200 P), melting point determination, UV spectrophotometric analysis and TLC confirmed its identification as parthenolide: m.p. 115°C [25]; ^{1}H NMR spectra identical with the literature [25]; UV λ_{max} [hexane–dioxane (85:15, v/v)], 210 nm; TLC, R_F = 0.60 [20].

Isolation of marrubiin

The latter process was applied to 1.7 kg of flowered aerial parts of *Marrubium vulgare* L. [harvested in Maine et Loire (France) and dried at room temperature]. We obtained the following results: R_1 = 77 g (4.53% dry material); purple spot in the methanolic layer at R_F = 0.66 (reagent No. 2); R_2 = 3.17 g; R_3 = 11.8 g; fractions IX–XI, mixed, evaporated to dryness and crystallized from ethanol, afforded 2.13 g of pure compound. (0.12% dry material). ^{1}H NMR spectroscopoy (Brucker AC 200 P), melting point determination, UV spectrophotometric analysis and TLC confirmed its identification as marrubiin: m.p. 160°C [15,26,27]; ^{1}H NMR spectra identical with the literature [4,5,26,28]; UV λ_{max} [hexane–dioxane (85:15, v/v)], 225 nm; TLC, R_F = 0.66.

HPLC sample preparation method

Amounts of 10 g of flowered aerial parts of each plant from the same batch processed via the large-scale method were subjected to a Soxhlet extraction with chloroform (500 ml) for 4 h. The solvent was evaporated under reduced pressure at 40°C and sample washed out on a column (200 × 20 mm I.D.) containing 10 g of silica gel 60 (particle size 0.063–0.2 mm) for low-pressure column chromatography (Merck), with chloroform (400 ml) until the eluate remained colourless. The eluate was evaporated to dryness and then diluted with 5 ml of chloroform (quinghao and white horehound samples) or 20 ml of chloroform (feverfew sample) accurately before HPLC analysis.

RESULTS AND DISCUSSION

In comparison with the separation of sesquiterpene lactones [23] and particularly of parthenolide [22] by reversed-phase HPLC, we adopted normal-phase HPLC on silica gel 60, which resulted in good-quality chromatographic profiles. For quantitative analysis, the calibration graphs show a linear correlation from 0.2 to 5 mg/ml between the amounts of the three injected lactones and the intensity of the absorption at 210 nm [correlation coefficient (R^2) 0.9938 for artemisinin and 0.9975 for parthenolide] and at 225 nm (R^2 = 0.9956 for marrubiin). Five determinations were carried out on each sample of feverfew, white horehound and quinghao, in order to test the precision of the method. The determination of each lactone was attempt-

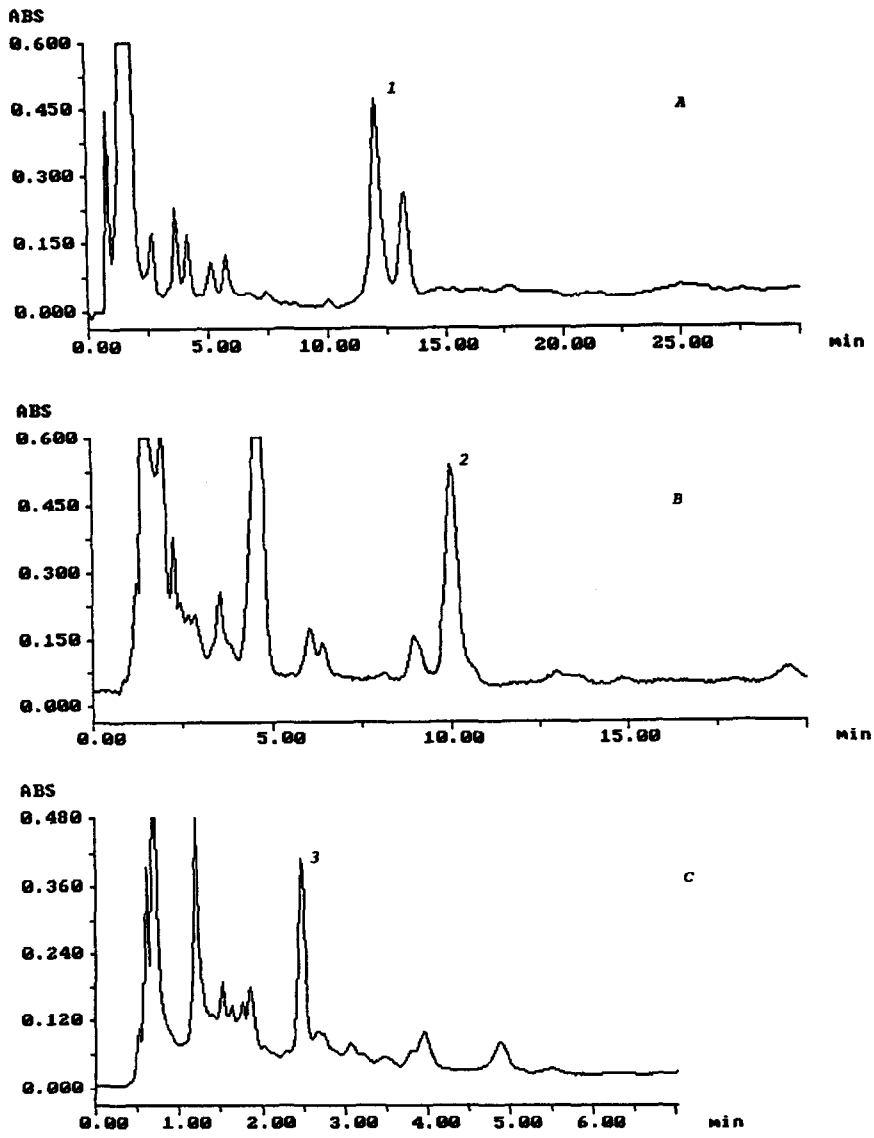


Fig. 1. HPLC of the total lactonic fraction of flowered aerial parts of (A) *Chrysanthemum parthenium* Bernh., (B) *Marrubium vulgare* L. and (C) *Artemisia annua* L. Peaks: 1 = parthenolide; 2 = marrubiin; 3 = artemisinin. Conditions: column, Superspher Si 60 (125 × 4 mm I.D.; particle size 4 μm); precolumn, LiChrospher Si 60 (4 × 4 mm I.D.; particle size 5 μm); mobile phase (A and B) hexane–dioxane (85:15, v/v) and (C) (90:10, v/v); flow-rate (A and B) 1 ml/min and (C) 2 ml/min; UV detection at (A and C) 210 nm and (B) 225 nm.

ed on the total lactonic fraction of flowered aerial parts of *Chrysanthemum parthenium* Bernh. (Fig. 1a), *Marrubium vulgare* L. (Fig. 1b) and *Artemisia annua* L. (Fig. 1c). The results indicated that with this new extraction process, we extracted 72.3, 78.4 and 74.1% of the total amount of parthenolide, marrubiin and artemisinin, respectively, contained in each plant.

In conclusion, the proposed process allows the use of inexpensive solvents (chloroform, hexane and ethyl acetate) and of a simple low-pressure liquid chromatographic method in general for the

isolation of γ-lactones, which can be extrapolated to an industrial scale. Moreover, the extraction yields with this process are better than those mentioned in the literature [13,14,17]. Finally, the proposed HPLC method allows the isocratic separation of parthenolide, marrubiin and artemisinin and can be used in their routine determination in drugs.

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