Journal of Chromatography, 634 (1993) 329-333
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 804

Determination of quaternary alkaloids from Phellodendri Cortex by capillary electrophoresis

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(First received November 5th, 1992; revised manuscript received December 9th, 1992)

ABSTRACT

A simple and rapid method for the simultaneous determination of six quatemary alkaloids (berberine, palmatine, jatrorrhiiine, magnoflorine, phellodendrine and berberrubine) in the Chinese herbal drug Phellodendrine Cortex by capillary electrophoresis was developed. A buffer solution composed of 0.5 M sodium acetate solution (pH 4.6, adjusted with acetic acid)-acetonitrile (1:1) was found to be the most suitable electrolyte for this separation, whereby the contents of the quaternary alkaloids in crude and processed samples of Phellodendri Cortex could be easily determined. The differences in alkaloid contents of various Phellodendri Cortex growing in different locations (China, Taiwan, Japan) were also investigated.

INTRODUCTION

Phellodendri Cortex is a commonly used Chinese herbal drug having gastric, intestinal alternative, astringent and antiphlogistic effects and contains five quaternary ammonium salts (berberine, palmatine, jatrorrhizine, phellodendrine and magnoflorine) (Fig. 1) as its major bioactive components [1–4]. In addition, berberrubine, obtained from heat-treated Phellodendron bark, has been found to inhibit the growth of several tumour cell lines [5].

Several methods have been reported for the determination of some of these quaternary alkaloids, including spectrophotometry [6,7], thin-layer chromatography [7–10], electron microscopic analysis [11] and high-performance liquid chromatography (HPLC) [12–16]. However, none of these methods is entirely adequate because their resolution is limited to at the most four of the quaternary alkaloids [15]. So far, there have been no reports dealing with the determination of phellodendrine and berberrubine .

We describe here the development of a simple, rapid and simultaneous method for determining these quaternary alkaloids in crude and processed samples of Phellodendri Cortex by capillary electrophoresis.

EXPERIMENTAL

Reagents and materials

Berberine chloride was purchased from Sigma (St. Louis, MO, USA), sodium acetate from Osaka (Osaka, Japan) and brucine, acetonitrile and acetic acid from Merck (Darmstadt, Germany). Palmatine and magnoflorine were isolated from ***Phellodendron amurense*** Pupr. [3]. Jatrorrhizine was isolated from coptis rhizome [17]. Phellodendrine was provided by Brion Research Institute (Taiwan). Berberrubine was obtained by heating berberine chloride at 150°C for 30 min. ***P. chinense*** Schneid, P. ***amurense*** Pupr. var. ***sachalinense*** Fr. Schm. and ***P. wilsonii***

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0021-9673/93/$06.00

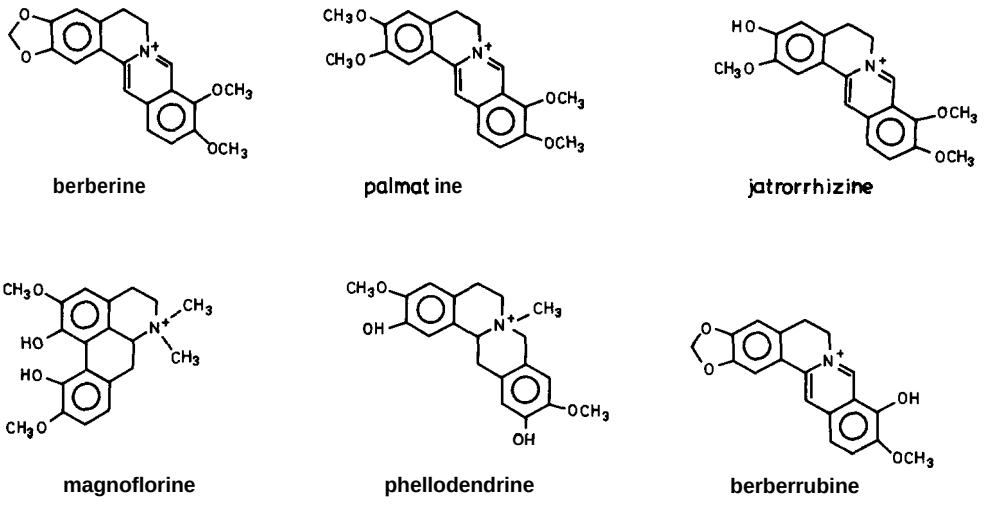

Fig. 1. Molecular structures of alkaloids in Phellodendri Cortex.

Hayata et Kanehira were purchased from Chinese herbal markets in China, Japan and Taiwan, respectively.

Preparation of Phellodendri Cortex extracts

A 0.5-g sample of pulverized Phellodendri Cortex was extracted with 70% methanol (7.5 ml) by stirring at room temperature for 30 min, then centrifuged at 1500 g for 10 min. Extraction was repeated three times. The extracts were combined and filtered through a No. 1 filter-paper. After the addition of 2.5 ml of internal standard solution (2 mg of brucine in 1 ml of 70% methanol), the Phellodendri Cortex extract was diluted to 25 ml with 70% methanol. This solution was passed through a 0.45-μm filter and *ca.* 1.1 nl (15-s hydrostatic sampling) of the filtrate were injected directly into the capillary electrophoresis system.

Apparatus and conditions

The analysis was carried out on a Waters Quanta 4000 capillary electrophoresis system equipped with a UV detector set at 280 nm and a 50 cm x *50* μm I.D. uncoated capillary (Millipore, Bedford, MA, USA) with the detection window placed at 42.5 cm. The conditions were as follows: sampling time, 15 s, hydrostatic; run time, 9 min; applied voltage, 15 kV (constant voltage, positive to negative polarity); and temperature, 24.5–25.0°C. The electrolyte was a buffer solution consisting of 0.5 *M* sodium acetate solution (pH 4.6, adjusted with acetic acid)–acetonitrile (1:1). The electrolyte was filtered through a 0.45-μm filter before use.

RESULTS AND DISCUSSION

The quaternary alkaloids of Phellodendri Cortex are mostly the same as those of Coptidis Rhizoma. Therefore, we first tried to use the same capillary electrophoresis conditions as for Coptidis Rhizoma [18]. However, the magnoflorine peak of Phellodendri Cortex is much higher than that of Coptidis Rhizoma and there was interference with the solvent peak, hence different conditions were necessary.

Both magnoflorine and berberrubine are easily deprotonated in basic or neutral solution owing to their acidic phenolic hydroxy groups and will be partially overlapped by the solvent peak. As carboxylate is a good counter ion for the positively charged nitrogen of the alkaloids [18], we finally chose sodium acetate solution as the buffer solution and increased its acidity with acetic acid. The peaks of magnoflorine and berberrubine then appeared before the solvent peak, but other peaks were still partly overlapped and the electric current of the capillary was too high. Increasing the concentration of

sodium acetate improved the resolution and the use of a 50 μm instead of 100 μm I.D. capillary reduced the electric current.

After a series of experiments, it was found that 0.5 *M* sodium acetate solution (pH 4.6, adjusted with acetic acid) could separate all the alkaloids well. At higher pH (4.8), the peaks of berberrubine and phellodendrine could not be separated. At lower pH (4.5), jatrorrhizine and berberrubine were found to be partially overlapped. Addition of acetonitrile to the buffer solution could make the peaks sharper, produce a better separation effect and reduce the capillary electric current. An acetonitrile concentration of 50% gave the best result.

An electrolyte consisting of 0.5 *M* sodium acetate solution (pH 4.6, adjusted with acetic acid)-acetonitrile (1:1) was found to give the best resolution. Fig. 2 is an electropherogram showing the separation of the six authentic quaternary alkaloids with the following migration times: 5.5 min, berberine; 5.9 min, palmatine; 6.2 min, jatrorrhizine; 6.3 min, berberrubine; 6.5 min, phellodendrine; 7.2 min, magnoflorine; and 7.6 min, internal standard (brucine). The measurement of all the constituents can be completed within 8 min. As the methanol-water extracts of Phellodendri Cortex were injected directly and analysed, the results were as good as those obtained with pure chemical samples without interference with each peak, as shown in Figs. 3-6.

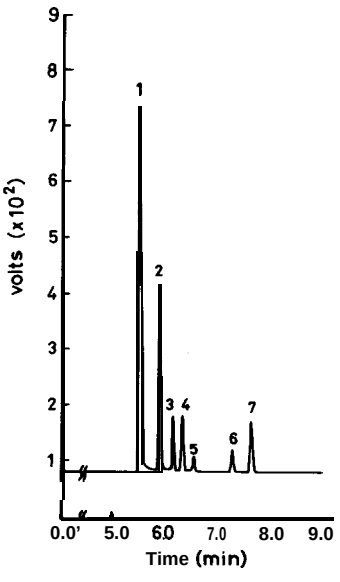

Fig. 2. Capillary electropherogram of a mixture of quaternary alkaloids usually present in crude and processed Phellodendri Cortex. Peaks: 1 = berberine, 0.600 mg/ml; 2 = palmatine, 0.200 mg/ml; 3 = jatrorrhizine, 0.040 mg/ml; 4 = berberrubine, 0.080 mg/ml; 5 = phellodendrine, 0.030 mg/ml; 6 = magnoflorine, 0.080 mg/ml; 7 = internal standard (brucine), 0.200 mg/ml.

Calibration graphs for quaternary alkaloids

Calibration graphs (peak-area ratio, y, vs. concentration, x, mg/ml) were constructed in the range 0.030-1.200 mg/ml for berberine, 0.010–0.300 mg/ml for palmatine, 0.002-0.060 mg/ml for jatrorrhizine, 0.003-0.090 mg/ml for berberrubine, 0.010-0.100 mg/ml for phellodendrine and 0.020-0.200 mg/ml for magnoflorine. The regression equations of these curves and their correlation coefficients were calculated as follows: berberine, y = 12.35~ + 0.07 (r = 0.9999); palmatine, y = 14.96x + 0.02 (r = 0.9999); jatrorrhizine, y = 21.64x + 0.01 (r = 0.9999); berberrubine, y = 15.84x + 0.00 (r = 0.9999); phellodendrine, y = 6.47x − 0.01 (r = 0.9997); and magnoflorine, y = 4.96x + 0.01 (r = 0.9997).

Determination of quaternary alkaloids in Phellodendri Cortex

When the test solutions of *P. wilsonii* Hayata et Kanehira, *P. chinense* Schneid, P. *amurense* Pupr. var. *sachalinense* Fr. Schm. and heat-treated *P. amurense* Pupr. var. *sachalinense* Fr. Schm. were analysed by capillary electrophoresis under the selected conditions, the graphs shown in Figs. 3-6 were obtained. The peaks were identified by comparison with those obtained from authentic samples of the alkaloids. By substituting the area ratios of the individual peaks for y in the above equations, the content of each quaternary alkaloid in the Phellodendri Cortex samples was obtained as shown in Table I. It was found that there were great differences in the alkaloid contents of Phellodendri Cortex collected from different locations and also the heat-treated sample. Further studies on the relationship between the origins of plants and the contents of alkaloids and also the processing of Phellodendri Cortex are in progress.

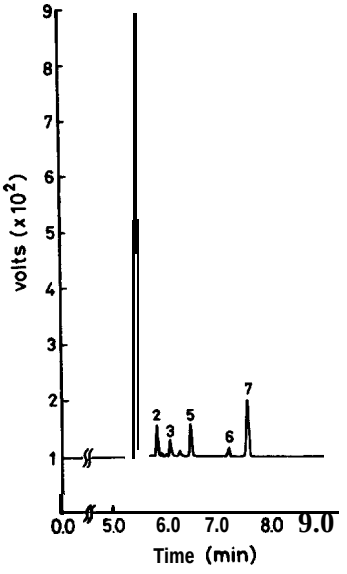

Fig. 3. Capillary electropherogram of the extract of a ***P. wilsonii*** Hayata et Kanehira sample (grown in Taiwan). Peak numbers as in Fig. 2.

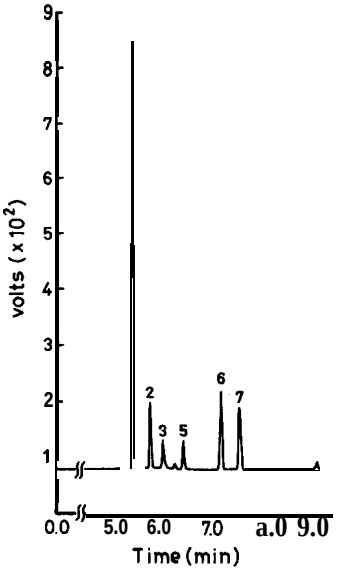

Fig. 5. Capillary electropherogram of the extract of a ***P. nmurense*** **Pupr . var.** ***sachalinense*** Fr. **Schm.** sample (grown in Japan). Peak numbers as in Fig. 2.

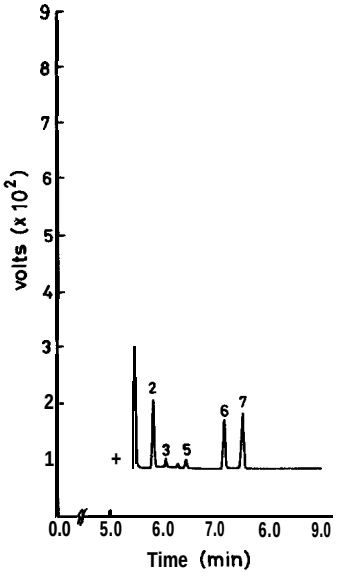

Fig. 4. Capillary electropherogram of the extract of a ***P. chinense*** Schneid sample (grown in China). Peak numbers as in Fig. 2.

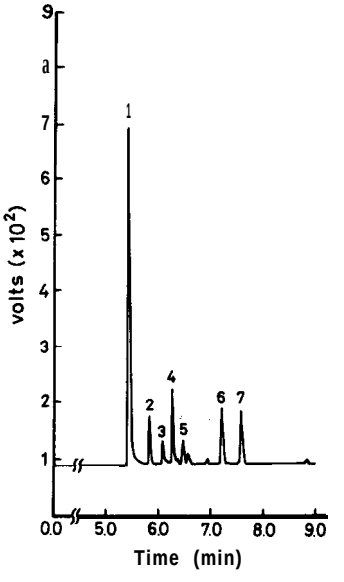

Fig. 6. Capillary electropherogram of the extract of a **heat-treated** ***P. amurense*** Pupr. var. ***sachalinense*** Fr. **Schm.** sample. Peak numbers as in Fig. 2.

Suitable amounts of the six quaternary alkaloids were added to a sample of Phellodendri Cortex of known alkaloid content and the mixture was extracted and analysed using the proposed procedure. The recoveries of the alkaloids were 98.5–103.3% with relative standard deviations of 1.2-2.1%.

From the above results, it can be concluded

TABLE I

CONTENTS OF ALKALOIDS IN PHELLODENDRI CORTEX

Sample[a]	Concentration (%)[b]						
	Berberine	Palmatine	Jatrorrhizine	Phellodendrine	Magnoflorine	Berberrubine	Total
1	4.621 ± 0.038	0.141 ± 0.011	0.067 ± 0.003	0.364 ± 0.011	0.144 ± 0.002	–	5.360 ± 0.048
2	0.624 ± 0.007	0.317 ± 0.003	0.031 ± 0.003	0.117 ± 0.004	0.809 ± 0.007	–	1.923 ± 0.011
3	2.993 ± 0.019	0.253 ± 0.004	0.105 ± 0.004	0.281 ± 0.011	1.141 ± 0.028	–	4.747 ± 0.167
4	2.420 ± 0.027	0.216 ± 0.004	0.044 ± 0.002	0.260 ± 0.008	0.940 ± 0.012	0.342 ± 0.007	4.222 ± 0.063

[a] 1 = *P. wilsonii* Hayata et Kanehira (grown in Taiwan); 2 = *P. chinense* Schneid (grown in China); 3 = *P. amurense* Pupr. var. *sachalinese* Fr. Schm. (grown in Japan); 4 = heat-treated *P. amurense* Pupr. var. *sachalinense* Fr. Schm.
[b] Mean ± standard deviation ($n = 5$).

that the method for the simultaneous determination of the quaternary alkaloids in Phellodendri Cortex by capillary electrophoresis as established in this study has the advantages of the need for only small amounts of sample, a short analysis time and simple electrolyte preparation.

ACKNOWLEDGEMENT

Financial support (NSC 82-0208-M-003-012) from the National Science Council, Taiwan, is gratefully acknowledged.

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