Journal of Chromatography A, 1208 (2008) 47–53

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

One-step purification of palmatine and its derivative *dl*-tetrahydropalmatine from *Enantia chlorantha* using high-performance displacement chromatography

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ARTICLE INFO

Article history:
Received 12 June 2008
Received in revised form 19 August 2008
Accepted 21 August 2008
Available online 5 September 2008

Keywords:
High-performance displacement chromatography
HPDC
Palmatine
dl-Tetrahydropalmatine
Protoberberine alkaloid
Enantia chlorantha

ABSTRACT

Palmatine and its reduced form, *dl*-tetrahydropalmatine are a group of isoquinoline alkaloids that have been reported to display a variety of biological and pharmacological activities. Both drugs are hydrophilic and are difficult to be purified by conventional purification methods of natural products. A high-performance displacement chromatography (HPDC) method successfully purified palmatine and its semi-synthetic derivative *dl*-tetrahydropalmatine from crude extract of the African medicinal plant *Enantia chlorantha*. The crude extract from the root bark of *E. chlorantha* was fractionated on an analytical reversed-phase C_{18} column by using 0.1% trifluoroacetic acid (TFA) or acetic acid/H_2O as a carrier and cetylpyridinium trifluoroacetate (or acetate) (1.9 mg/mL) in 0.1% TFA (or acetic acid)/H_2O as a displacer. Palmatine was quantitatively purified at >98% purity in the fully developed displacement mode. *dl*-Tetrahydropalmatine was semi-synthesized by $NaBH_4$ reduction from crude palmatine and directly purified by HPDC. Both palmatine and *dl*-tetrahydropalmatine were identified by high-resolution electrospray tandem mass spectrometry, ^{1}H NMR and ^{13}C NMR. This is the first report of one-step HPDC purification of natural and semi-synthetic products from a complex crude extract.

1. Introduction

Protoberberine alkaloids, which belongs to a class of the isoquinoline alkaloid, are widely distributed in the plant kingdom, e.g., in many species of the Berberidaceae, Annonaceae, Fumariaceae, Papaveraceae, Ranunculaceae, Rutaceae, and other plant families, encompassing a diverse class of secondary metabolites with many pharmacologically active members, berberine and palmatine [1]. Over the last decade, these alkaloids have attracted considerable attention due to a wide range of biochemical and pharmacological actions.

The medicinal plant, *Enantia chlorantha* Oliv., commonly known as the African yellow wood, which is a tropical rainforest tree widespread in central Africa, belongs to the family Annonaceae. Its root barks are locally used for the treatment of malaria, hepatic disorders, tuberculosis, etc. [2]. Phytochemical studies showed that several protoberberine-type quaternary alkaloids [2–4], of which palmatine (Fig. 1) is a major metabolite, were isolated from the stem bark of *E. chlorantha*. The stem bark extracts of this species and related protoberberine alkaloids displayed a great variety of biological and pharmacological activities such as anti-human immunodeficiency virus (HIV) activity [4], antitrypanosomal and antiplasmodial effects [5], hepatoprotection [6], anticandidal and antibacterial activities [7,8], anti-ulcer action [9,10], cytotoxicity [11,12], anti-monoamine oxidase activity [13], inhibitions of biosynthesis of catecholamine [14] and dopamine [15], anti-acetylcholinesterase activity [16], nerve growth factor-potentiating activity [17], inhibition of reverse transcriptase of tumor [18], antiradical and antioxidant effects [19,20], lipoxygenase inhibition [20], and rat lens aldose reductase inhibition [21], as well as anti-inflammatory, antinociceptive and antipyretic effects [22].

Besides palmatine was used as a natural pharmaceutical drug for treatment of some infectious diseases such as viral hepatitis

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0021-9673/$ – see front matter.
doi:10.1016/j.chroma.2008.08.112

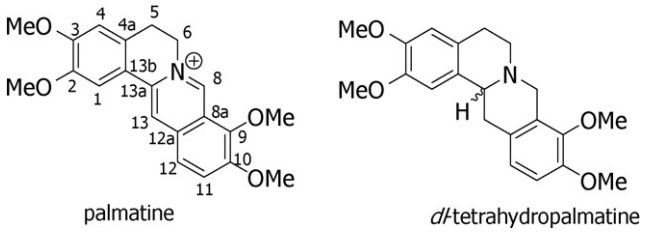

Fig. 1. Molecular structures of palmatine and *dl*-THP.

B [6], the reduced form *dl*-tetrahydropalmatine (*dl*-THP, Fig. 1) is also one of major bioactive components in *Corydalis yanhusuo* W.T. Wang that is well-known as a traditional Chinese herbal medicine. Recent studies have shown that *dl*-THP exerts remarkable analgesic without any addiction, sedative-tranquilizing, hypnotic [23] anxiolytic [24], hypotensive [25,26], inhibiting the aggregation of thrombocyles [27], hypo-locomotion [28], and neuroprotective [29] actions. Moreover, *dl*-THP has been shown to deplete the levels of dopamine, noradrenaline, serotonin, and monoamine in brain [30,31]. This compound also is a very effective antiepileptogenic and anticonvulsant agent [32].

To date, numerous methods for the analysis of protoberberine alkaloids from different medicinal plants have been reported, e.g., stepwise gradient in thin-layer chromatography (TLC) [33], optimum performance laminar chromatography (OPLC) [34], capillary electrophoresis (CE) [35] and capillary electrophoresis–mass spectrometry (CE–MS) [35,36], high-performance liquid chromatography (HPLC) [37,38], as well as HPLC–electrospray ionization tandem mass spectrometry [39,40]; however, few methods for the separation of these hydrophilic alkaloids have been achieved. One is the separation of four protoberberine alkaloids including palmatine and berberine from *Coptis chinensis* by high-speed counter-current chromatography (HSCCC) [41], and the other is the separation of four protoberberine alkaloids from *E. chlorantha* by high-performance centrifugal partition chromatography (HPCPC) [42].

Displacement chromatography (DC) is an independent mode of chromatography, where the more strongly adsorbed pigments displace the more weakly adsorbed ones. The method develops a displacement train which constitutes of consecutive rectangular zones of the pure compounds [43–46]. High-performance displacement chromatography (HPDC) works with conventional HPLC system and HPLC columns. It uses two mobile phases of the carrier and displacer. The carrier dissolves the sample and provides high affinity to the stationary phase. After loading the sample, the most strongly adsorbed displacer is loaded and displaces the sample components from the stationary phase, while the individual components also displace each other. Eventually, the displacer elutes out the sample components as it occupies all binding sites of the stationary phase.

Displacement chromatography has a few advantages over the conventional elution chromatography. One is the preparative scale of chromatography. Since an analytical HPLC column (250 mm × 4.6 mm) column can bind up to several hundred milligrams of substances, an analytical column in the conventional elution chromatography becomes a semi-preparative column when it uses in HPDC [47–49]. Another is the separation of homologous compounds as separations of geometrical and structural isomers, enantiomers, and even isotopes have been published [44,50,51].

In the past two decades, DC has mainly been applied to the isolation and purification of bio-molecules, or synthetic mixtures, such as peptides and proteins [49,52,53], and anthracycline antibiotics epirubicin and doxorubicin isomers [50], as well as soybean phospholipids [47]. However, there has been no report on purification of plant alkaloids such as palmatine and *dl*-THP from crude extracts, where conventional purification methods require several steps of purification with poor yields. Their high hydrophilic nature predominantly attributes to the inconveniences. The aim of the present work is to develop a HPDC method for the purification of major alkaloids (palmatine and *dl*-THP) in root bark extracts of *E. chlorantha*.

2. Experimental

2.1. Plant material

The stem barks of *E. chlorantha* Oliv. were collected and identified by Mr. Nana Victor in Edea, littoral Province, Cameroon. Voucher specimens are deposited at National Herbarium Yaounde Cameroon.

2.2. Reagents

HPLC-grade trifluoroacetic acid (TFA), acetonitrile and water were purchased from Baker (Phillipsburgh, NJ, USA). Cetylpyridinium chloride, dodecylpyridinium chloride hydrate, sodium borohydride ($NaBH_4$), sodium trifluoroacetate, sodium acetate hydrate, rimethobenzamide, benzydamine, amitriptyline and papaverine were supplied by Sigma–Aldrich (St. Louis, MO, USA). Other solvents used were of analytical grade.

All the solvents used in HPDC and HPLC were filtered through 0.22-μm Millipore filter and degassed by sparging with helium. Shiseido Capcell Pak C_{18} column (AQ S-5 μm, 250 mm × 4.6 mm) was obtained from JM Science (Grand Island, NY, USA).

2.3. Apparatus

HPDC was performed on an analytical column (250 mm × 4.6 mm), packed with Shiseido Capcell Pak C_{18} (AQ S-5 μm) from JM Science by using a Waters HPLC Millennium^{32} system. The system instrument is composed of two Model 303 pumps for the carrier and displacer solution, a Model 811 dynamic mixer, a Model 7000 injector (Rheodyne, Cotati, CA, USA) with a 10 mL injection loop, a Waters 2996 photodiode array detector (210–400 nm), and a Waters Fraction Collector II (Waters, Milford, MA, USA).

The purity of palmatine and *dl*-THP in the collected fractions were analyzed by a Waters liquid chromatography system, which is composed of a Waters 717 autosampler, a 600 model pump, and a 996 photodiode array detector (200–500 nm). The analyses were carried out with a Waters SymmetryShield RP_{18} column (50 mm × 4.6 mm, 3.5 μm) from Waters at room temperature. The mobile phase consisting of solvent A (0.1%, v/v, TFA in water) and solvent B (0.1%, v/v, TFA in acetonitrile) was eluted at a flow rate of 1.5 mL/min. The analysis was carried out by gradient elution with increasing solvent B concentration in solvent A from 0 to 90% in 10 min. Instrument management and data acquisition were performed using Waters Empower software.

The nuclear magnetic resonance (NMR) spectra were measured at 300 K on a Bruker Avance 500 (Bruker, Karlsruhe, Germany) spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), with [$^2H_6$]dimethyl sulfoxide (DMSO-d_6) (δ 2.49 and 39.5 ppm) and [$^2H_1$]chloroform ($CDCl_3$) (δ 7.26 and 77.0 ppm) solvent as internal reference. High-resolution electrospray ionization tandem mass spectrometry (HR-ESI-MS/MS) was performed on a Micromass Waters Q-TOF Global mass spectrometer (Micromass, Manchester, UK), a hybrid quadrupole time-of-flight mass spectrometer