Separation and Purification Technology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Separation and Purification Technology

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

One-step preparative isolation of aristolochic acids by strong ion-exchange centrifugal partition chromatography

Abdelgadir A. Abdelgadir [a,b], Leslie Boudesocque-Delaye [a,*], Isabelle Thery-Koné [a], Alain Gueiffier [c], Elhadi M. Ahmed [b], Cécile Enguehard-Gueiffier [c]

[a] UMR INRA 1282 Infectiologie et Santé Publique, Université de Tours, UFR des Sciences Pharmaceutiques, 31 avenue Monge, 37200 Tours, France
[b] Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Sudan
[c] Inserm UMR 1069 Nutrition, Croissance et Cancer, Université de Tours, UFR des Sciences Pharmaceutiques, 31 avenue Monge, 37200 Tours, France

ARTICLE INFO

Article history:
Received 15 July 2015
Received in revised form 13 October 2015
Accepted 14 October 2015
Available online xxxx

Keywords:
Aristolochic acids
Aristolochia bracteolata
Ion-exchange
Counter-current chromatography
Centrifugal partition chromatography

ABSTRACT

Aristolochic acids are biologically active and highly toxic secondary metabolites that recently became an important pharmacological tool for *in vivo* models of acute kidney disorders. Only aristolochic acid I and a mixture of aristolochic acids I and II are currently commercially available. A method based on the strong-ion exchange displacement mode in centrifugal partition chromatography was developed to extract and purify arictolochic acids, naturally present in *Aristolochia bracteolata* Lam. (*Aristolochiaceae*). The extraction and purification of aristolochic acids were performed at a flow rate of 2 mL min^{-1} in the descending mode by using the biphasic solvent system methyl-*ter*-butylether, acetone, methanol and water (3:1:1:3, v/v/v/v). Trioctylmethylammonium chloride (Aliquat 336®) was used as the anion exchanger in the organic stationary phase and sodium iodide as the displacer in the aqueous mobile phase. From 5 g of crude extract of *A. bracteolata*, 45.5 mg, 77.2 mg and 35.1 mg of aristolochic acids I, II and IIIa were obtained respectively with purities greater than 95%.

1. Introduction

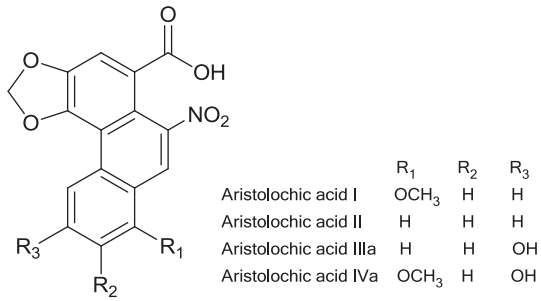
Aristolochic acids (AAs) are nephrotoxic secondary metabolites (Fig. 1) present in many *Aristolochia* species. "Chinese Herb Nephropathy" and endemic (Balkan) nephropathy are both major renal toxicities linked to the consumption of *Aristolochia* sp. in herbal remedies [1]. AA are responsible of progressive interstitial kidney fibrosis leading to the need for dialysis or transplant in most of the cases [2]. Moreover, AAs are also associated with high prevalence of urothelial carcinomas (up to 45%) [3]. Recently research has focused on the comprehension of mechanisms of AA-associated nephrotoxicity [4,5]. Reduced forms of AAs I and II form covalent adducts with DNA and RNA, which explains their important role in carcinomas. Nevertheless, the specific mechanisms by which AA-associated nephropathy develops remains unclear. AAs are now used to develop new nephrotoxicity models in zebrafish [6], in mice [7] or to cause acute kidney injury [8] in order to elucidate AA-specific mechanisms. Only AA I and a mixture of AAs I and II are currently commercially available even though four major AAs are commonly described in the literature (AA I, II, IIIa and IVa).

Isolation of those polar metabolites that exhibit closely-related structures by classical chromatography methods is usually time consuming and highly challenging, involving multistep process. Sequential solid/liquid extraction followed by two chromatographic steps of flash chromatography on silica gel [9], or on LH-20 resin [10] and then semi preparative HPLC [10] or preparative TLC [11] were usually reported to obtain pure AAs. To isolate milligrams of different AAs, support-free liquid–liquid chromatography techniques such as counter-current chromatography [12] and centrifugal partition chromatography (CPC) [13] could be an innovative option. These techniques are well adapted to the purification of polar secondary metabolites, when carried out either in the elution or in the pH-zone refining [14] and the ion-exchange displacement modes [15]. The Strong Ion eXchange mode (SIX-CPC), is particularly suitable for the purification of ionized or ionizable metabolites such as hydroxycinnamic acids [16], rosmarinic acid [17], glucosinolates [18,19], anthocyanins [20], saponosides [21] or peptides [22,23]. In SIX-CPC, the exchanger is permanently ionic and keeps its positive (cationic exchanger) or negative (anionic exchanger) charge at any pH value [24]. Anionic

Abbreviations: AAs, aristolochic acids; MP, mobile phase; Lc, liter of column; SIX-CPC, Strong Ion eXchange Centrifugal Partition Chromatography; SP, stationary phase.

* Corresponding author.
E-mail address: leslie.boudesocque@univ-tours.fr (L. Boudesocque-Delaye).

http://dx.doi.org/10.1016/j.seppur.2015.10.033
1383-5866/



	R_1	R_2	R_3
Aristolochic acid I	OCH_3	H	H
Aristolochic acid II	H	H	H
Aristolochic acid IIIa	H	H	OH
Aristolochic acid IVa	OCH_3	H	OH

Fig. 1. Chemical structures of aristolochic acids.

exchangers are principally quaternary ammonium salts, such as benzalkonium chlorides or methyltrioctylammonium chloride (Aliquat 336®). When using this mode, ionic analytes are directly captured into the stationary phase by forming stable ion-pairs with the exchanger. The displacer-free mobile phase is not able to elute the extracted analytes. However, when adding the displacer, analytes return to the mobile phase (Fig. 2). The separation mechanisms in SIX-CPC have been well described and modeled by Maciuk et al. [16] using separation of phenolic acid regioisomers as methodological support. All analytes moved along the column with the same velocity depending on displacer concentration. This typical column organization is called an isotachic train (Fig. 2). SIX-CPC provides high resolution purification based on competition between analytes to form ion-pairs with the exchanger.

The aim of this work is to demonstrate that SIX-CPC is an innovative tool for the preparative isolation of AA I, II and IIIa from the Sudanese *Aristolochia bracteolata* L.

2. Experimental

2.1. Chemicals and reagents

Acetone, acetonitrile, chloroform ($CHCl_3$), methanol (MeOH), methyl-*ter*-butylether (MtBE), trifluoroacetic acid (TFA) were purchased in chromatographic grade solvents from Carlo Erba (Rhodano, Italy). Methyltrioctylammonium chloride (Aliquat 336®), sodium iodide (NaI), sodium hydroxide (NaOH), mixture of aristolochic acids I and II standards were purchased from Sigma Aldrich (Saint Quentin, France). Water was purified by a Milli-Q-system (Millipore Corporation, Bedford, MA, USA).

A. bracteolata samples were collected during August 2013 from the Southern Gezira area (Sudan) and taxonomically identified in the Department of Pharmacognosy, University of Gezira. A voucher specimen is deposited in the Faculty of Pharmacy, University of Gezira.

2.2. Preparation of A. bracteolata crude extract

Crushed whole plants of *A. bracteolata* (289 g) were extracted by 1.25 L of MeOH in a Soxhlet apparatus during 20 h. MeOH was evaporated under reduced pressure and the extract was then freeze dried (71.2 g of freeze dried crude extract).

2.3. CPC apparatus

Centrifugal partition chromatography (CPC) separations were performed on a FCPC® Preparative 200 Kromaton Technologies apparatus (Rousselet Robatel, Annonay, France) using a rotor made of 20 circular partition disks (840 partition twin-cells; total column capacity: 205 mL, dead volume: 32.3 mL). Rotation speed could be adjusted from 200 to 3000 rpm, thus producing a centrifugal force field in the partition cells of about 120*g* at 1000 rpm and 480*g* at 2000 rpm. The solvents were pumped by a preparative Ecom Beta 50 Gradient pump binary low-pressure gradient pump (Praha, Czech Republic). The samples were introduced into the column through a PEEK dual mode preparative scale sample injector 3725i (Rheodyne, Rohnert Park, CA, USA) equipped with a 20 mL sample loop. Effluent content was monitored by an Ecom Flash 06 DAD 600 detector equipped with a preparative flow cell (45 μL internal volume, optical path 0.3 mm). Fractions were collected by an Advantec Super Fraction collector (Otowa, Japan).

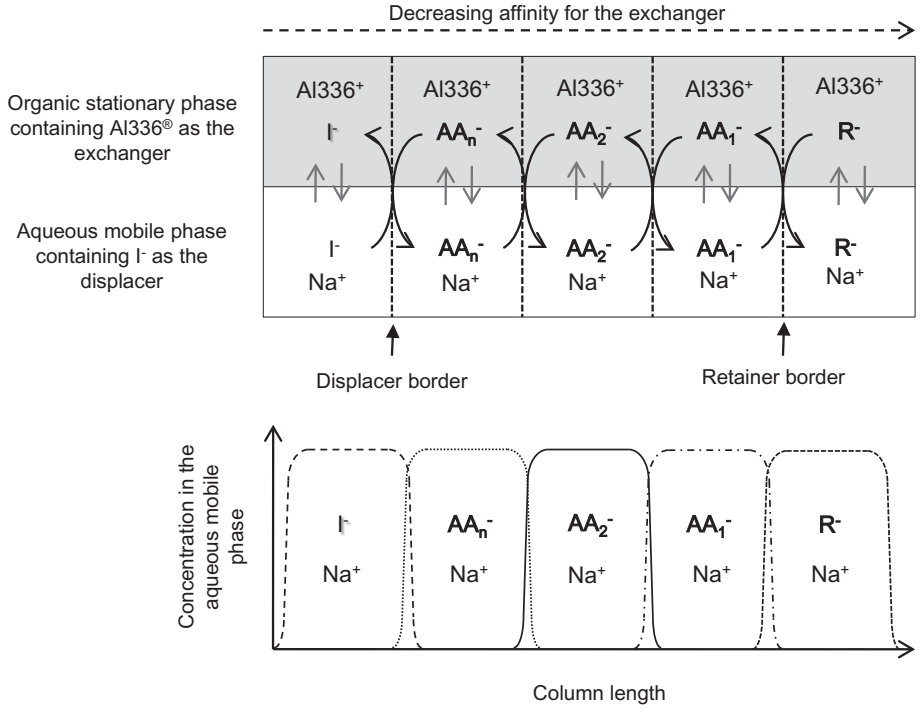


Fig. 2. The isotachic train in the strong ion-exchange displacement mode.