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Short communication

Preparative purification of antiamyloidogenic stilbenoids from *Vitis vinifera* (Chardonnay) stems by centrifugal partition chromatography

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ABSTRACT

Five stilbenoids, *E*-resveratrol, *E*-piceatannol, (+) *E*-(ε)-viniferin, (+)-ampelopsin A and vitisin C were isolated from methyl *tert*-butyl ether (M*t*BE) stem extract of *Vitis vinifera* (Chardonnay cv). Their purification on a preparative scale was obtained by centrifugal partition chromatography (CPC) using quaternary Arizona solvent systems composed of *n*-heptane/ethyl acetate/methanol/water. We tested 23 Arizona solvent systems to partition the extract and found that systems K and M (Hept/EtOAc/MeOH/water, 1:2:1:2 and 5:6:5:6, respectively; v/v) were the best to separate the stilbenes mentioned above. This support-free liquid–liquid chromatographic procedure made it possible to isolate ampelopsin A from *V. vinifera* for the first time. The antiamyloidogenic activity of the isolated stilbenes was evaluated versus β-amyloid fibrils. *E*-resveratrol and (+)-ampelopsin A were found to be the most active compounds with 63 and 46% inhibition at 10 μM, respectively. These findings suggest that *E*-resveratrol and (+)-ampelopsin A may function as attractive new candidates for protecting against brain cell dysfunction *in vivo* in AD by inhibiting the aggregation of Aβ.

1. Introduction

Stilbenoids constitute a class of secondary metabolites acting as phytoalexins and whose structure is derived from 1,2-diphenylethylene. They naturally occur in several plant families such as the Cyperaceae, Dipterocarpaceae, Gnetaceae, and Vitaceae [1,2]. Grapes (Vitaceae) and products manufactured from grapes are viewed as the most important dietary sources of these substances [3,4]. Among stilbenoids, hydroxystilbenes have raised much interest for their biological properties, such as their potential cancer-chemopreventive [5,6], antioxidative activity on human low-density lipoproteins (LDL) [7], and their neuroprotective activity [8].

However, studies on their bioactivities (*in vitro* and *in vivo* tests) require large amounts of stilbenoid reference standards, which may be obtained from grapevine and particularly stalk and stem extracts.

We investigated the purification of these polar compounds on a preparative scale using centrifugal partition chromatography (CPC). CPC is a particular kind of liquid–liquid countercurrent chromatography technique in which a biphasic solvent system is used to partition analytes between two immiscible liquid phases according to their partition coefficient. The process differs from conventional solid-support chromatography such as high-performance liquid chromatography (HPLC) in several ways. Higher selectivity can be attained by a skilled design of the solvent system, the quantity of solvent can be significantly reduced, scale-up is easily achieved and there is no irreversible adsorption onto a solid support [9]. In this study, we successfully used the quaternary Arizona solvent systems (*n*-heptane/ethyl actetate/methanol/water) [10] for the purification of *E*-resveratrol, *E*-piceatannol, (+) *E*-(ε)-viniferin, ampelopsin A, and vitisin C from *Vitis vinifera* stems (Fig. 1). In our previous studies, we found that stilbenes inhibit amyloid-β peptide (Aβ) aggregation *in vitro* [8,11]. Consequently, we investigated the inhibitory effects of the isolated stilbenes on the formation of amyloid-β peptide fibrils. *E*-resveratrol and (+)-ampelopsin A were found to be the most active inhibitors.

2. Experimental

2.1. Chemicals and reagents

All organic solvents were HPLC grade and purchased from Scharlau (Sentmenat, Spain). Water was bi-distilled. Peptide Aβ25–35

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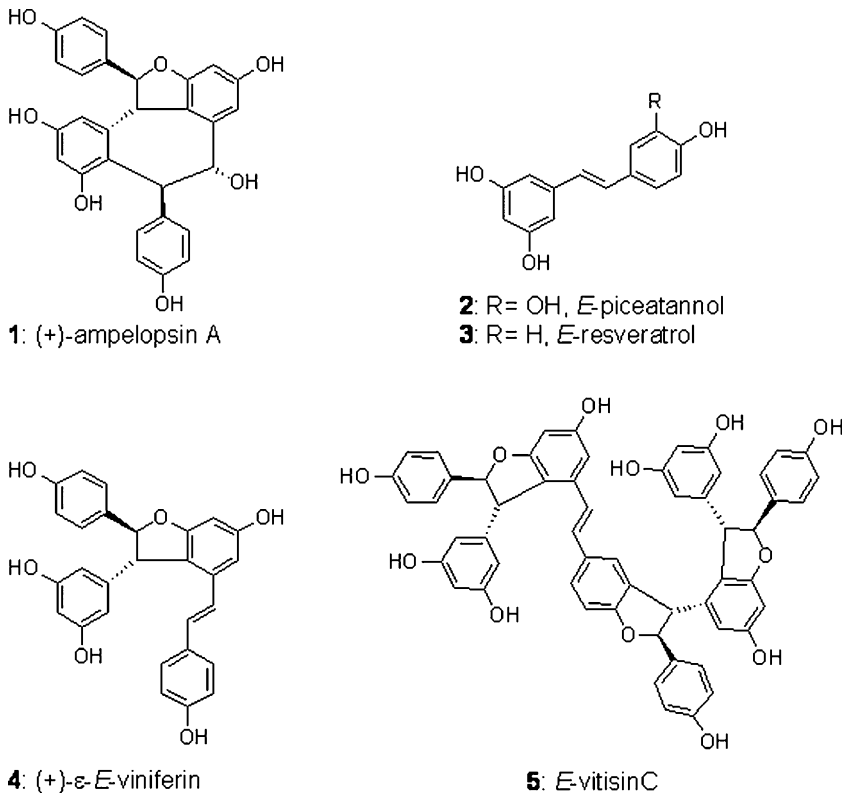

Fig. 1. Structure of stilbenoids isolated from *Vitis vinifera* (Chardonnay).

and curcumin were purchased from Bachem California (Torrance, CA, USA).

2.2. Plant material

Stems of *V. vinifera* Chardonnay *cv*. were collected in Champagne-Ardenne, France, in February 2005.

2.3. CPC apparatus

The laboratory CPC is a FCPC200® apparatus provided by Kromaton Technologies (Angers, France) that is fitted with a rotor made of 20 circular partition disks (1320 partition cells: 0.130 mL per cell; total column capacity of 204 mL; dead volume: 32.3 mL). Distance from the centre of the rotor to the centre of each cell is 105 mm. Rotation speed can be adjusted from 0 to 2000 rpm, thus producing a centrifugal force field in the partition cell of nearly 1200 m s^{-2} at 1100 rpm and 4200 m s^{-2} at 2000 rpm.

The solvents were pumped by a Gilson 321-H1 2-way binary high-pressure gradient pump. The samples were introduced into the CPC column via a high pressure injection valve (3725(i)038 Rheodyne) equipped with a 10 mL sample loop. The effluent was monitored with an ICS UV-Lambda 1010 detector equipped with a preparative flow cell. Fractions were collected by a Gilson FC 204 fraction collector. The experiments were conducted at room temperature.

2.4. Preparation of CPC solvents

The quaternary biphasic solvent systems were prepared by mixing *n*-Hept, EtOAc, MeOH, and water in the convenient proportion (1:2:1:2; v/v) for system K and (5:6:5:6, v/v) for system M at room temperature. The resulting two phases were separated just before use.

2.5. Preparation of crude stilbenoid extract

Dried and finely powdered stem of *V. vinifera* Chardonnay cv (300 g) was extracted with four times 3 L of water–acetone (3:2, v/v) at room temperature under agitation for 4 h. After filtration, the aqueous acetone solution was concentrated at 35 °C under reduced pressure. The residual aqueous phase (300 mL) was successively extracted with *n*-heptane (A1) (4 × 300 mL) and methyl *tert*-butyl ether (M*t*BE) (A2) (6 × 300 mL). A1 was discarded and A2 (M*t*BE extract) was reduced in vacuum at 35 °C and redissolved in water to be freeze-dried to yield 2.5 g of a crude stilbenoid extract (0.8% weight).

2.6. CPC separation procedure

The rotor was entirely filled with the aqueous stationary phase in the ascending mode without rotating. After injection of the 1.5 g extract (A2) initially dissolved in 8 mL of the organic/aqueous phase mixture (1:1), the organic mobile phase was pumped into the column in ascending mode at a flow-rate of 3 mL/min. Then, the rotation speed was increased from 0 to 1000 rpm. Fractions of 9 mL were collected every 3 min. The back pressure was 25 bars. The stationary phase retention at the end of the separation represented 75% of the column volume. The content of the outgoing organic phase was monitored by online UV absorbance measurement at λ = 280 nm.

2.7. Characterization of stilbenes

2.7.1. TLC analysis

All the fractions were checked by TLC pre-coated on silica gel 60 F_{254} plates (Merck) and developed with $CHCl_3$–methanol–acetic acid (85/15/3). Detection was achieved at 254 and 366 nm and by spraying with anisaldehyde sulphuric reagent contain-