Supporting Information

Inhibitory Activity of Plant Stilbenoids Against Nitric Oxide Production by Lipopolysaccharide-Activated Microglia

Merian Nassra¹, Stéphanie Krisa¹, Yorgos Papastamoulis¹, Gilbert Deceaux Kapche¹, Jonathan Bisson¹, Caroline André², Jan-Pieter Konsman², Jean-Marie Schmitter³, Jean-Michel Mérillon¹, Pierre Waffo-Téguo¹

Affiliations

¹Groupe d’Etude des Substances Végétales à Activité Biologique, Université de Bordeaux, Institut des Sciences de la Vigne et du Vin, Villenave d’Ornon, France
²Psychoneuroimmunologie, Nutrition & Génétique, Université de Bordeaux, Bordeaux, France
³Chimie et Biologie des Membranes et des Nanoobjets, Université de Bordeaux, Bordeaux, France

Correspondence

Dr. Stéphanie Krisa; Dr. Pierre Waffo-Téguo

Groupe d’Etude des Substances Végétales à Activité Biologique, EA 3675
Université de Bordeaux
Institut des Sciences de la Vigne et du Vin
210 Chemin de Leysotte, CS50008
33882 Villenave d’Ornon
France
Phone: +33 5 57 57 59 53
The stilbenoids (1-25) were isolated from *Milicia excelsa* (Moraceae), *Morus alba* (Moraceae), *Gnetum africanum* (Gnetaceae), and *Vitis vinifera* (Vitaceae) as follows:

The stem barks of *Milicia excelsa* known as Iroko were collected in Yaoundé Cameroon in 2006 and identified by Mr. Victor Nana at the National Herbarium, where a voucher specimen (N° 1702/RFK) is deposited. The air-dried and finely powdered stem barks (1.5 kg) were macerated in methanol for 48 h at room temperature, followed by filtration and concentration to yield a crude MeOH extract (70 g) which was washed with hexane and extracted with ethyl acetate. The ethyl acetate extract (25 g) was separated over silica gel (100.0 g, 0.040-0.063 mm) chromatographic column (ø = 4 cm) using a gradient of CHCl₃-MeOH as eluent. Fifty fractions of 200 mL each were collected, concentrated, monitored by TLC, and similar fractions were combined. Moracin M (1, 200 mg) was directly obtained after filtration as white amorphous powder from fractions (10-15) obtained with CHCl₃-MeOH 95/5. Other isolated compounds were identified as triterpenes and flavonoids and were not tested.

The roots of *Gnetum africanum* were collected in Cameroon in November 2006 and identified by Mr. Victor Nana at the National Herbarium, where a voucher specimen (N° 21165/SFRK) is deposited.

The air-dried and powdered roots (500 g) were extracted with MeOH for 48 h at room temperature. The methanolic extract was concentrated under reduced pressure to give a brown residue (40 g). This extract was successively extracted with *n*-hexane and ethyl acetate. Removal of the solvents gave 15.0 and 23.0 g of residues, respectively. 20 g of the EtOAc extract were subjected to chromatographic column (ø = 4 cm) over silica gel 60 (100.0 g, 0.040-0.063 mm) and eluted with CHCl₃-MeOH of increasing polarity. Forty fractions of 150 mL each were collected, concentrated, monitored by TLC, and similar fractions were combined. Fractions 1-10 (4.1 g, CHCl₃-MeOH 100/0 - 95/5, v/v) contained mostly hydrocarbons and were not investigated further. Fractions 11-15 (1.2 g, CHCl₃-MeOH
92.5/7.5 - 90/10, v/v) and 16-20 (1.0 g, CHCl₃-MeOH 87.5/2.55 - 85/15, v/v) were passed through Sephadex LH-20 (1.5 x 20 cm) and eluted with CHCl₃-MeOH (70/30) to give respectively E-gnetol (8, 100 mg, white amorphous powder) and gneaficanin A (12, 15 mg, brown oil). Fractions 21-35 (9.0 g, CHCl₃-MeOH 82.5/17.5 - 70/30) were subjected to chromatographic column (ø=4cm) over silica gel 60 (40.0 g, 0.025-0.040 mm) eluting with CHCl₃-MeOH (97/3, 94/6, 90/10, 85/15, 80/20, 75/25, v/v) to give 6 fractions (D₁-D₆). D₂ (1.2 g), D₃ (1.1 g), and D₆ (1.5 g) were purified on Sephadex LH-20 (1.5 x 20 cm) eluting with CHCl₃-MeOH (7:3) to give E-gnemonoside D (9, 150 mg, white amorphous powder) from D₂, E-gnemonoside C (10, 40 mg, white amorphous powder) from D₃, and E-gnemonoside A (11, 1.0 g, dark red oil) from D₆. Other fractions (36-40, 2.3 g) were found to contain mostly tannins and were not investigated further.

Compounds 2, 4, 12, and 16 were purified from *Vitis vinifera* cell suspension cultures [1, 2]. Compounds 3, 5, 13, 17, and 25 were isolated from the *Vitis vinifera* (Chardonnay) stem [3]. Compound 24 was obtained from the root of *V. riparia* x V [4].

Dried powdered stems of *Vitis vinifera* collected at Domaine de Merlet, Pessac-Léognan, were extracted with acetone/water as previously described [4, 5]. Aqueous mixture was partitioned with methyl tert-butyl ether (MtBE). The MtBE extract was then fractionated and purified by centrifugal partition chromatography. After testing the “Arizona” [5] solvent system, system K (n-heptane/ethyl acetate/methanol/water 1/2/1/2 v/v) was finally chosen for a first partition of the MTBE extract. After the CPC separation of MTBE extract (7.86 g), 16 fractions were obtained, eight in the ascending mode and eight in the descending mode [3]. Fraction 3 was fractionated with a second CPC step using Arizona solvent system L (n-heptane/ethyl acetate/methanol/water 2/3/2/3 v/v). The major stilbene *E*-piceatannol (5) and *E*-scirpusin A (15) were purified in ascending mode, whereas *E*-miyabenol C (21) was separated in descending mode and purified by semi-prep. HPLC using C18 reversed phase
column (Prontosil, 250 mm x 8.0 mm 5.0 μm; Bischoff) was used for purification. The mobile phase was composed of two solvents: A, 0.025% TFA in water and B, MeCN. The UV absorption was monitored with a Varian 345 dual wavelength UV-Vis detector simultaneously at 280 and 306 nm. The elution program at 3 mL/min was 20% B (0–5 min), 20–26% B (5–10 min), 26% B (10–22 min), 26-50% B (22–52 min), 50% B (52–55 min) and 50-100% B (55–56 min), 100% B (56–64 min), 100-20% B (64–65 min), 20% B (65–72 min). Fraction 14 (descending mode), issued from the first CPC separation, contained a multitude of phenolic compounds, especially stilbenes. The Arizona J system (n-heptane / ethyl acetate / methanol / water 2:5:2:5 v/v) proved to be the most adequate for this fraction’s purification. 131 tubes were collected, 117 in the ascending mode and 14 in the descending, grouped in ten fractions. From the tubes 84-107 of the ascending mode, flavanoids ((+) catechin, (-)-epicatechin, epicatechin-3-O-gallate, and astilbin) and stilbenoids (leachianol F (19) and G (20), quadrangularin A (18), hopeaphenol (22), and isohopeaphenol (23)) were purified by semi-preparative HPLC using the same conditions as above. The elution program at 3 mL/min was 5-10% B (0–5 min), 10% B (5–13 min), 10–20% B (13–26 min), 20% B (26–30 min), 20–26% B (30–37 min), 26% B (37–49 min), 26–100% B (49–56 min). The air-dried stem barks of Morus alba (registration number TAL 19910036) were extracted with acetone/water as above. The aqueous mixture was washed with CHCl₃ and extracted with MtBE. The MtBE extract was then purified by centrifugal partition chromatography using Arizona H system [5] in descending mode to give compound 7.


