SUPPLEMENTARY MATERIAL

Chemical composition of the pods of *Albizia polyphylla*

Clara Fredeline Rajemiarimoelisoa^{a,1}, Cédric Boyère^{b,1}, Léonie Pellissier^b, Marine Peuchmaur^b, Hanitra Ranjana Randrianarivo^c, Danielle Aurore Doll Rakoto^c, Victor Louis Jeannoda^c and Ahcène Boumendjel^{b,*}

^a*University of Antananarivo, Pharmacy Department, Faculty of Medicine, PO Box 375, Antananarivo 101, Madagascar*

^b*Université Grenoble Alpes, CNRS UMR 5063, Département de Pharmacochimie Moléculaire, BP 53, 38041 Grenoble Cedex 9, France*

^c*University of Antananarivo, Fundamental and Applied Biochemistry Department, Faculty of Sciences, PO Bo 906, Antananarivo 101, Madagascar*

¹Equal contributions to this work.

*Corresponding author. Email: Ahcene.Boumendjel@ujf-grenoble.fr

Abstract

In the present study we report for the first time the presence of alkaloids belonging to β -carboline type in the pods of the endemic *Albizia polyphylla* from Madagascar. Three major alkaloids were isolated and structurally identified as: 1-methyl- β -carboline, (+)-(R)-1-methyl-1,2,3,4-tetrahydro- β -carboline and (–)-(S)-1,2-dimethyl-1,2,3,4-tetrahydro- β -carboline.

Keywords: *Albizia polyphylla*, pods, alkaloids, β -carbolines.

Experimental

General procedures

Optical rotations were measured on a PerkinElmer 341 polarimeter. NMR spectra were recorded on a Bruker AVANCE-400 NMR spectrometer with TMS as an internal reference operating at 400 MHz for ^1H and 100 MHz for attached proton tested (APT) ^{13}C , respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS as internal standard or relative to the solvent [^1H : $\delta(\text{MeOD}) = 3.31$ ppm, $\delta(\text{CDCl}_3) = 7.26$ ppm; ^{13}C : $\delta(\text{MeOD}) = 49.00$ ppm, $\delta(\text{CDCl}_3) = 77.16$ ppm]. Electrospray ionization ESI and high resolution mass spectra HRMS mass spectrum were acquired respectively by the Analytical Department of Grenoble University on an Esquire 300 Plus Bruker Daltonis instrument with a nanospray inlet and on an ESI/TOF - Waters Xevo G2-S QToF spectrometer in positive ion mode. TLC analysis was carried out on silica gel plates (plates coated with silica gel 60 F254 from Merck) employing to UV light as the revelator then a H_2SO_4 -methanol mixture as contrasting agent. Automated Reveleris[®] X2 Flash Chromatography System with integrated ELS/UV/UV-Vis detection fitted with 4 g Reveleris[®] Flash Cartridges (40 or 20 μm silica) was used to purify crude products.

Plant material

Albizia polyphylla fruits were collected in Ampijoroa village (Majunga, western region of Madagascar), in June 2014. Voucher specimen (NNHN-P-P00458232) of this plant was deposited in the herbarium of Plant Biology and Ecology Department, Faculty of Sciences, University of Antananarivo, Madagascar. The fruits were washed and sun-dried. Then, the pods were separated from seeds and ground into a fine powder, using a blender.

Extraction and isolation

Two extraction methods were followed to extract alkaloids from pods.

a) Powder of *Albizia polyphylla* pods (10 g) was wetted with 20 mL of NH_4OH (20%), suspended in dichloromethane (300 mL) and the mixture was stirred for 24 h at room temperature. After filtration, the solution was evaporated in a rotary evaporator under reduced pressure at 40 °C. The residue was dissolved in water (20 mL) and acidified with H_2SO_4 (10%) to pH = 2-3 and then extracted with diethyl ether to remove lipophilic, acidic and neutral material. After basifying the aqueous solution to pH = 9-10 with NH_4OH (20%), it was extracted with dichloromethane (3x20 mL). The organic layer was washed three times with distilled water, dried over Na_2SO_4 and concentrated to dryness under reduced pressure to provide crude alkaloids as dark brownish liquid (0.11 g).

b) Powder of *Albizia polyphylla* pods (10 g) was wetted with 30 mL of diluted HCl (1 M) and extraction was carried out with methanol (100 mL) for 24 h at room temperature. After filtration, the solution was evaporated under vacuum, the aqueous acid solution was basified with NH₄OH (20%) to pH = 9-10, then extracted with dichloromethane (3x20 mL). The organic layer was dried over Na₂SO₄ and concentrated to dryness under reduced pressure to obtain crude alkaloids as dark brownish liquid (0.24 g). The TLC analysis using a solvent system CH₂Cl₂/MeOH in various proportions: 98:2; 95:5; 90:10 showed that the two extracts were similar and contain mainly three components.

The two alkaloid extracts were gathered together with CH₂Cl₂, then concentrated to dryness under vacuum to provide alkaloid extract. Purification by flash chromatography was performed on 0.34 g using CH₂Cl₂/MeOH, 90:10 as eluent. 112 fractions (fraction volume of 8.5 mL) were collected and the fractions having the same pattern of separated spots on the TLC were collected together. After evaporating solvent and concentrating the solution, three alkaloids compounds were determined: **Ap1** (fractions 12 – 21, 7 mg); **Ap2** (fractions 31 – 56, 14 mg) and **Ap3** (fractions 94 – 112, 11 mg).

Compounds characterisation

1-Methyl-pyrido[3,4-*b*]indole (**Ap1**)

Brown solid; *R_f* 0.23 (95:5 CH₂Cl₂/MeOH); ¹H NMR (400 MHz, CDCl₃): δ 9.23 (s, 1H, NH), 8.30 (d, *J* = 5.4 Hz, 1H, H3), 8.10 (d, *J* = 7.9 Hz, 1H, H5), 7.81 (d, *J* = 5.4 Hz, 1H, H4), 7.57-7.50 (m, 2H, H7, H8), 7.28 (ddd, *J* = 2.0, 6.0, 7.9 Hz, 1H, H6), 2.84 (s, 3H, -CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 141.6 (C, C1), 140.7 (C, C8a), 137.6 (CH, C3), 134.7 (C, C9a), 128.8 (C, C4b), 128.7 (CH, C7), 122.0 (CH, C5), 121.9 (C, C4a), 120.3 (CH, C6), 113.2 (CH, C4), 111.9 (CH, C8), 20.0 (CH₃). LRMS (ESI+) *m/z* (%) 183.1 (100) [M+H]⁺; HRMS (ESI+) *m/z* calcd. for C₁₂H₁₁N₂ 183.0922 [M+H]⁺, found 183.0924.

(+)-(R)-1,2-Dimethyl-1,2,3,4-tetrahydro-9H-pyrido[3,4-*b*]indole (**Ap2**)

Brown solid; [α]_D²⁰ +30° (c 1, CH₂Cl₂) {lit.[16] (*R*)-isomer ; [α]_D²⁰ +26° (c 0.72, CHCl₃)}; *R_f* 0.11 (95:5 CH₂Cl₂/MeOH); ¹H NMR (400 MHz, CDCl₃): δ 7.91 (s, 1H, NH), 7.49 (dd, *J* = 7.5, 0.9 Hz, 1H, H5), 7.31 (dd, *J* = 7.5, 1.3 Hz, 1H, H8), 7.15 (ddd, *J* = 1.3, 7.5, 7.5 Hz, 1H, H7), 7.10 (ddd, *J* = 0.9, 7.5, 7.5 Hz, 1H, H6), 3.60 (q, *J* = 6.6 Hz, 1H, CH-CH₃), 3.15 (dt, *J* = 4.9, 11.4 Hz, 1H, H3-1), 2.91-2.72 (m, 3H, H3-2, H4), 2.52 (s, 3H, N-CH₃), 1.45 (d, 3H, *J* = 6.6 Hz, CH-CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 136.2 (C, C8a), 135.8 (C, C9a), 127.2 (C, C4b), 121.4 (CH, C7), 119.3 (CH, C6), 118.1 (CH, C5), 111.0 (CH, C8), 107.4 (C, C4a), 55.6 (CH, C1), 50.7 (CH₂, C3), 42.0 (N-CH₃), 21.4 (CH₂, C4), 19.2 (CHCH₃). LRMS (ESI+) *m/z* (%) 201.1 (100) [M+H]⁺; HRMS (ESI+) *m/z* calcd. for C₁₃H₁₇N₂ 201.1392 [M+H]⁺, found 201.1401.

(-)-(*S*)-1-Methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**Ap3**)

Brown solid; $[\alpha]_{\text{D}}^{20} -83^{\circ}$ (c 1, MeOH) {lit.[17] (*S*)-isomer ; $[\alpha]_{\text{D}}^{20} -52^{\circ}$ (c 2, EtOH)} ; R_f 0.06 (95:5 CH₂Cl₂/MeOH); ¹H NMR (400 MHz, MeOD): δ 7.34 (dd, $J = 0.9, 7.8$ Hz, 1H, H5), 7.27 (dd, $J = 0.9, 8.0$ Hz, 1H, H8), 7.03 (ddd, $J = 0.9, 7.1, 8.0$ Hz, 1H, H7), 6.94 (ddd, $J = 0.9, 7.1, 7.8$ Hz, 1H, H6), 4.16 (q, $J = 6.7$ Hz, 1H, CH-CH₃), 3.31-3.24 (m, 1H, H3-1), 2.95 (ddd, $J = 5.1, 9.3, 14.3$ Hz, 1H, H3-2), 2.82-2.72 (m, 1H, H4-1), 2.66 (dddd, $J = 1.7, 5.3, 9.3, 15.6$ Hz, 1H, H4-2), 1.47 (d, $J = 6.7$ Hz, 3H, CH-CH₃). ¹³C NMR (100 MHz, MeOD): δ 137.9 (C, C8a), 134.9 (C, C9a), 128.1 (C, C4b), 122.5 (CH, C7), 120.0 (CH, C6), 118.8 (CH, C5), 112.1 (CH, C8), 107.4 (C, C4a), 49.9 (CH, C1), 43.1 (CH₂, C3), 21.4 (CH₂, C4), 20.0 (CH₃). LRMS (ESI+) m/z (%) 187.1 (100) [M+H]⁺; HRMS (ESI+) m/z calcd. for C₁₂H₁₅N₂ 187.1235 [M+H]⁺, found 187.1228.

NMR and Mass Spectrometry spectra of the isolated compounds

1-Methyl-pyrido[3,4-*b*]indole (Ap1)

Figure S1. ¹H and APT ¹³C NMR spectrum (CDCl₃, 400 MHz and 100 MHz respectively) of compound Ap1.

(+)-(R)-1,2-Dimethyl-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole (Ap2)

Figure S2. ^1H and APT ^{13}C NMR spectrum (CDCl_3 , 400 MHz and 100 MHz respectively) of compound **Ap2**.

(-)-(*S*)-1-Methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**Ap3**)

Figure S3. ^1H and APT ^{13}C NMR spectrum (MeOD , 400MHz and 100 MHz respectively) of compound **Ap3**.

Figure S4. ESI-MS and HRMS analyses of compound **Ap1**.

$[\text{C}_{12}\text{H}_{10}\text{N}_2+\text{H}]^+$ at $m/z = 183.0924$.

Figure S5. ESI-MS and HRMS analyses of compound **Ap2**.

$[\text{C}_{13}\text{H}_{16}\text{N}_2+\text{H}]^+$ at $m/z = 201.1401$.

Figure S6. ESI-MS and HRMS analyses of compound **Ap3**.

$[\text{C}_{12}\text{H}_{14}\text{N}_2+\text{H}]^+$ at $m/z = 187.1228$.