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Bioactive phloroglucinols from *Mallotus oppositifolius*

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ABSTRACT

The two new acylphloroglucinol derivatives, methylene-bis-aspidinol AB (1) and mallopposinol (2), together with the nine known compounds, aspidinol B (3), methylene-bis-aspidinol (4), (+)-α-tocopherol (5), lupeol (6), stigmasterol (7), phytol (8), bergenin (9), squalene (11) and methyl gallate (10) were isolated from the leaves of *Mallotus oppositifolius*. Their structures were elucidated by spectral analysis including MS, 1D and 2D-NMR spectroscopy. *In vitro* trypanocidal and antileishmanial activities of compounds 1–9 were evaluated. Mallopposinol (2) and aspidinol B (3) displayed weak antileishmanial activities against *Leishmania donovani* promastigotes, with EC_{50} values of 21.3 and 38.8 μM, respectively. Only the methylene-bis-aspidinol (4) exhibited trypanocidal activity against *Trypanosoma brucei brucei* trypomastigotes (LC_{100} = 0.8 μM) similar to the reference drug pentamidine (LC_{100} = 0.4 μM).

1. Introduction

Mallotus (Euphorbiaceae) are shrubs, trees and rarely climbers, with about 150 species distributed in tropical and subtropical areas of the Old World, mainly in Asia, Australia and the Pacific [1]. This genus is represented by five species in Africa and Madagascar tropical floras, including 3 endemics to Madagascar [2]. *Mallotus oppositifolius* (Geisler) Müll. Arg., known as "kisse kisse tree" is a diecious shrub growing in savanna and secondary forests of Africa and Madagascar. In traditional medicine, the leaves are used as analgesic, antibacterial, anthelmintic, hemostatic and antimalarial [2,3]. Extracts of *M. oppositifolius* have been described to possess anti-inflammatory, antioxidant [4–6], antidiabetic [6], antidiarrheal [7], antibacterial, antifungal [8,9], antitrypanosomial and antiplasmodial activities [10,11]. *Mallotus* are known for their richness in natural bioactive compounds [12,13], mainly diterpenoids, triterpenoids [14], cardenolides [15], benzopyrans [16], flavonoids [17], coumarinolignoids and phloroglucinol derivatives [18,19]. Phloroglucinol dimers have been isolated from *M. oppositifolius* [20]. In our ongoing research on bioactive molecules from Ivorian medicinal plants, an ethnobotanical study identified *M. oppositifolius* as a medicinal plant used against African trypanosomiasis [21,22]. We therefore examined the constituents of the leaves of *M. oppositifolius*. Eleven compounds were isolated, including two new dimeric phloroglucinols (1–2). In this paper, we present the isolation and structural determination of the new compounds, and the antiprotozoal activities of nine of these natural products.

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2. Material and methods

2.1. General

Optical rotations were measured on a PolAAR 32 polarimeter (Optical activity Ltd., Ramsey, UK) equipped with a sodium lamp (589 nm) and a 1 dm microcell. Melting points were determined using a Stuart SMP10 apparatus (Nemours, France) and were uncorrected. IR spectra were recorded on a Bruker Vector 22 (Champs-sur-Marne, France) spectrometer. UV spectra were recorded in MeOH using a Philips PU8720 spectrometer (Eindhoven, Netherlands). The chromatography columns were performed on silica gel (Merck, 70–230 mesh) or Sephadex® LH-20 (Pharmacia). Thin-layer chromatographies were carried out on aluminium plates coated with silica gel 60 F254 (Merck), and visualized with UV light then sprayed with vanillin-H_2SO_4 or Fast Blue B salt. The ^1H and ^{13}C NMR, as well as 2D spectra (COSY, HSQC, HMBC and NOESY), were recorded in $CDCl_3$ on a Bruker AC-400 spectrometer (Sarrebourg, France) operating at 400 MHz for ^1H spectra and 100 MHz for ^{13}C. A Bruker AM-300 spectrometer (Sarrebourg, France) was used for ^{13}C at 75 MHz. The EIMS spectra were recorded on a Hewlett-Packard Agilent device 6890 series equipped with a mass selective detector Agilent HP 5973 (EI mode, 70 eV) (Les Ulis, France). GC-MS analyses were performed on a Thermo Scientific Trace-GC Ultra gas chromatograph with mass detection performed on a Thermo Scientific ITQ900® (Courtabœuf, France). The injector was set with a split ratio of 1:10 at 230 °C. Compounds were separated with an Agilent Technologies DB5HT column (30 m × 0.250 mm × 0.1 μm) and carrier gas was high-purity helium at 1.1 mL min-1 flow. The oven temperature was initially held at 110 °C for 2 min, then raised to 360 °C at a rate of 7 °C min-1 and held for 5 min. Compounds were detected by Electronic Impact (EI) ionization, with the source temperature set at 200 °C. Data analysis was performed with Xcalibur™ software using NIST and a homemade database. APCIMS and ESIMS were acquired using a Bruker Esquire spectrometer (Champs-sur-Marne, France). HRESIMS spectra were registered with a Agilent 6530 Q-ToF spectrometer (Les Ulis, France). Leaves were ground using a Retsch apparatus (Eragny sur Oise, France).

2.2. Plant material

Leaves of *M. oppositifolius* were harvested twice, in May 2007 and July 2009, in Akoupé, Adzopé Department, south-east of Côte d'Ivoire. The plant samples were identified by Professor Aké ASSI of Centre National de Floristique (CNF), University of Cocody-Abidjan, where voucher specimens are deposited under the references MOK-07 (May 2007 sample) and MOT-09 (July 2009 sample). The samples were dried at room temperature, then ground.

2.3. Extraction and isolation

Leaves powder of the May 2007 sample of *M. oppositifolius* (3.0 kg) were extracted by maceration three times with 9 L of ethanol for 48 h. After filtration and solvent evaporation, a residue of 397 g (KE) was obtained. This residue was suspended in water/ethanol (1:1) (1.5 L) and extracted sequentially at room temperature with increasing polarity solvents to give after evaporation 116 g of *n*-hexane (KEH), 48 g of dichloromethane (KED) and 87 g of ethyl acetate (KEA) extracts. The isolation of pure compounds was completed by combination of different chromatographic techniques as described below.

A part of the hexane fraction (53 g) was first chromatographed on a silica gel column (Merck 60) using a gradient *n*-hexane/acetone (90:10–0:100), to give 21 fractions (F1–F21). Precipitated crystals from F11 (1.24 g) were purified on Sephadex® LH-20, using CH_2Cl_2/EtOH (2:1) as eluent to give an amorphous product, which after recrystallization from ethanol provided compound 4 (59 mg). The fraction F12 (1.64 g), treated by successive chromatographies on silica gel 60H columns (*n*-hexane/EtOAc, 75:25; CH_2Cl_2/EtOAc, 95:5), and on Sephadex® LH-20 (CH_2Cl_2/EtOH, 2:1) led to compound 6 (20 mg). Another part of KEH extract (50 g) was treated on a silica gel (Merck 60) column by VLC, using *n*-hexane/EtOAc as gradient (90:10–0:100), and resulted to 11 fractions (H1–H11). The fraction H6 (3.40 g) was chromatographed on a silica gel 60H column using *n*-hexane/petroleum ether/CH_2Cl_2 (20:10:80) to give six fractions (H61–H66). Fraction H61 was treated on a silica gel 60H column, using *n*-hexane/CH_2Cl_2 (70:30) to give compound 7 (20 mg). From the fraction H63, first chromatographed on a silica gel 60H (*n*-hexane/petroleum ether/CH_2Cl_2, 10:10:80) and then on Sephadex® LH-20 (CH_2Cl_2/EtOH, 2:1) columns, compound 1 was obtained (6.6 mg). The methylene chloride fraction KED (48 g) was subjected to column chromatography on silica gel (Merck 60) using a gradient *n*-hexane/EtOAc (90:10–0:100) to give three major fractions (D1–D3). The fraction D1 (8.54 g) was chromatographed on a silica gel 60H column using *n*-hexane/CH_2Cl_2 (10:90) to give two major fractions (D11 and D12). The fraction D11 was treated on Sephadex® LH-20 column (CH_2Cl_2/EtOH, 2:1) and preparative thin layer chromatography (Merck 60F_{254}, 20 × 20 × 0.5 cm, petroleum ether/CH_2Cl_2/EtOAc, 50:30:10) to provide compound 3 (9.6 mg). From fraction D12 compound 2 (46.7 mg) precipitated as crystals in *n*-hexane. From the ethyl acetate fraction KEA (87 g), compound 9 (140.4 mg) was obtained directly as crystals. The KEA remaining portion was successively chromatographed on a silica gel column 60H using CH_2Cl_2/EtOAc (50:50) and Sephadex® LH-20 columns eluted with CH_2Cl_2/EtOH (2:1), to provide compound 8 (9 mg).

The July 2009 sample of *M. oppositifolius* (4.8 kg) was treated as above to give after drying 447 g of ethanol residue (TE), 50 g of *n*-hexane residue (TEH), 49 g of dichloromethane residue (TED) and 36 g of ethyl acetate residue (TEA). The fraction TED (49 g) was chromatographed on a silica gel 60H column using a gradient cyclohexane/$CHCl_3$/EtOAc (90:10:0–0:0:100) to give 11 major fractions (D′1–D′11). The fraction D′4 (3.20 g) was subjected to column chromatography on silica gel 60H using $CHCl_3$ (100%) as eluent to give four fractions D′42–D′45. From the fraction D′42 compound 5 (4 mg) was obtained after treatment on a silica gel 60H column using $CHCl_3$ (100%) as eluent. The fractions D′43 and D′45 were treated on a silica gel 60H column, eluted with $CHCl_3$ (100%), and on Sephadex® LH-20 column, eluted with CH_2Cl_2/EtOH (2:1), to provide compound 10 (20 mg) and 11 (10 mg).

2.3.1. Methylene-bis-aspidinol AB (1)

Reddish spangle crystals; UV λ_{max} (MeOH) nm (log ε): 286 (3.441), 279 (3.354), 207 (3.457); IR ν_{max} (cm^{-1}): 3336, 2961, 2361, 1625, 1601, 1419, 1280, 1134, 1101, 1021, 906, 726, 648; For NMR ^1H and ^{13}C spectra, see Table 1; ESI-MS m/z (%): 431 $[M - H]^-$ (100), 223 (6.2), 195 (4.8); HREIMS m/z 431.1715 $[M - H]^-$ ($C_{23}H_{27}O_8$, calculated 431.1706).

2.3.2. Mallopposinol (2)

White amorphous powder; IR ν_{max} (cm^{-1}): 3260, 2990, 2950, 2400, 2350, 1670, 1600, 1550, 1280, 1350, 1300, 1200, 1150, 1050, 790; For ^1H and ^{13}C NMR spectra, see Table 1; EIMS m/z (%): 447 $[M + H]^+$ (3), 255 (4), 225 (100); EIMS m/z (%) : 445 $[M - H]^-$ (4), 223 (100); HRESIMS m/z 445.1887 $[M - H]^-$ ($C_{24}H_{29}O_8$ $[M - H]^-$, calculated 445.1862).

2.4. Antiprotozoal assays

All experiments were performed in triplicate, using 3 wells per condition. DMSO did not show toxicity at the maximum concentration used (0.1%).

2.4.1. Antileishmanial activity

The antileishmanial activity of the isolated compounds was tested *in vitro* against *Leishmania donovani* (WHO designation: MHOM/ET/1967/L82), according to a method previously described [23]. This method is based on a dying agent specific of died parasites, thus allowing the