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Minor secondary metabolites from the bark of *Entandrophragma congoënse* (Meliaceae)☆

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ABSTRACT

Two new tirucallane-type triterpenoids were isolated from the bark of *Entandrophragma congoënse* (Meliaceae) along with five known compounds gladoral A, bipendensin, 4-hydroxymethyl-3,5-dimethyldihydrofuran-2(3*H*)-one, scopoletin and 5,7-dimethoxy-6-hydroxycoumarin. Their structures were elucidated by means of spectroscopic analyses including 1D and 2D-NMR spectroscopy, high resolution mass spectrometric data as well as the comparison of data with those reported in the literature. The tested compounds (**1**–**4**) displayed moderated antiplasmodial activity against erythrocytic stages of chloroquine-resistant *Plasmodium falciparum* strain NF54 and low cytotoxicity on L6 cell lines. All the isolated compounds are reported for the first time from the genus *Entandrophragma*.

1. Introduction

Entandrophragma congoënse (De Wild) A. chev., is a towering tree belonging to Meliaceae family, which grows up to 25 m high in the tropical rainforests in Africa and South America [1]. The species of *Entandrophragma* have been used as timbers and herbal medicines by traditional healers in the Cameroonian folk medicine for the treatment of malaria [2], rheumatism [3] and gastric ulcer [4]. Previous chemical studies on some members of this genus reported limonoids [5], protolimonoids [6], highly oxygenated acyclic triterpenes [3,7], steroids [8] and other terpenes derivatives [9] with interesting biological properties such as: antiplasmodial, anti-inflammatory, antiulcer and antifeedant activities [2,3,10,11]. In the continuation of our effort in the search for antiplasmodial and cytotoxic metabolites from plants [12], we have investigated the minor constituents of the bark of *E. congoënse* (De Wild.) A. Chev (Meliaceae). Herein, we describe the isolation and structure elucidation of two new triterpenoids, namely congoensins A–B (**1–2**) as well as their antiplasmodial and cytotoxicity activities. The trivial names were given according to the plant species.

2. Experimental

2.1. General procedures

Melting points were determined on a Gallenkamp melting point apparatus (Loughborough, U.K.) and are uncorrected. Optical rotations were measured on a Perkin-Elmer polarimeter, model 241. To monitor analytical HPLC elution, a photodiode array detector ($\lambda = 205$ nm) was used in the wavelength range of 200–800 nm. Xcalibur software (Thermo Fisher Scientific,

☆ Dedicated with best wishes to Prof. Dr. Hartmut Laatsch on the occasion of his 69th birthday.

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Bremen, Germany) was used for data acquisition and for manually browsing the acquired data. IR measurements were obtained on a Perkin-Elmer (Model 1600) FTIR spectrometer. The NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-500 NMR spectrometer. Chemical shifts (δ) were quoted in parts per million (ppm) from internal standard tetramethylsilane (TMS). The $^3J_{C,\ H}$ couplings were measured by means of pulsed field gradient HMBC spectra recorded by varying the *J*-refocusing time between $t = 0.04$ and 0.14 s. The high-resolution mass spectra were obtained with an LTQ Orbitrap Spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a HESI-II source. Flash column chromatography was performed using silica-gel 60 (Merck, 0.040–0.063 mm). Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex; Amersham Biosciences Ltd., purchased from Sigma-Aldrich Chemie, Steinheim, Germany). Preparative reversed-phase HPLC was carried out with a Gilson system consisting of 322 pump with a UV detector 152 ($\lambda = 205$ nm) using a Nucleodur Gravity column from Macherey–Nagel (Düren, Germany) (250 × 16 mm, 5 μm particle size). Separation was achieved by using a H_2O (A)-MeOH (B) gradient program as follows (flow rate 6 mL min^{-1}): 50% A linear gradient for 2 min, follows by variation from 50% B to 0% A for 4 min, after 100% B isocratic for 9 min. Afterwards, the system returned to its initial condition (50% A) within 2 min, and was equilibrated for 3 min.

2.2. Plant material

The bark of *E. congoënse* was collected in June 2012 at Nkomokui, a locality near Yaoundé (Cameroon) and the botanical identification was made by Mr. Victor Nana. A voucher specimen was deposited at the National Herbarium of Cameroon under the number 43234 HNC.

2.3. Extraction and isolation

Dried and powdered bark of *E. congoënse* (~5.0 kg) was macerated two times with mixture of CH_2Cl_2/MeOH (1/1, *v*/*v*) for 48 h and then 8 h. Evaporation under reduced pressure afforded a crude extract (386 g). A part of the crude extract (350 g) was subjected to a silica gel flash column chromatography using stepwise gradient of *n*-hexane/EtOAc followed by a gradient of EtOAc/MeOH, to afford six fractions: A (65 g, pure *n*-hexane); B (112 g, *n*-hexane/EtOAc 50%); C (23 g, pure EtOAc); D (21 g, EtOAc/MeOH 30%), E (32 g, EtOAc/MeOH 50%) and F (15 g, pure MeOH). Fraction A was found to contain mainly fatty acids. Part of fraction B (110 g) was submitted to a silica gel column chromatography eluting with a gradient of *n*-hexane/EtOAc (0 to 75%) at atmospheric pressure to afford four sub-fractions B_1–B_4. The third sub-fraction B_3 (54.7 g) eluted with *n*-hexane/EtOAc (45%) was applied to Sephadex LH-20 eluting with MeOH to afford four series B_{31}–B_{34}. Compound **2** (1.3 mg) was obtained from series B_{31}, while series B_{32} (26.6 g) was subjected again to a silica gel column chromatography using cyclohexane/EtOAc (10–30%) to afford compound **4** (5.6 mg, Cyclohexane/EtOAc 25%). Furthermore, series B_{33} (12.0 g) and B_{34} (9.2 g) were combined and further purified on a silica gel column chromatography using the gradient of cyclohexane/EtOAC (15–40%) to afford compounds **6** (2.7 mg, cyclohexane/EtOAc 30%) and **7** (2.4 mg, cyclohexane/EtOAc 20%). Fractions C and D were combined and subjected to a silica gel column chromatography using a stepwise of CH_2Cl_2–MeOH gradient to give seven sub-fractions labeled CD1–7. Sub-fractions CD3 (11.8 g) and CD4 (10.2 g) were further purified on a silica gel column chromatography using the gradient of cyclohexane/ethyl acetate (15–95%) to afford two series of fractions: f1–48 from CD3 and h1–32 from CD4 which were combined based on their TLC profiles. Fractions f23–36 (7.5 g, cyclohexane-ethyl acetate 40%) were rechromatographed on a silica gel column using a mixture of cyclohexane-ethyl acetate with increasing polarity (from 10 to 50%) to give 40 fractions of ca. 150 mL, which were combined on the basis of their TLC and LC-MS profile to three series (I–III). Series II (2.7 g, cyclohexane/ethyl acetate 35%) were separately submitted for further purification to a reverse phase semi-preparative HPLC (see General procedures) to give compound **1** (5.3 mg, t_R 6.72 min). Fractions h12–27 (4.0 g, cyclohexane-ethyl acetate 30%) were further subjected to reverse phase semi-preparative HPLC as described above, to afford compounds **5** (4.8 mg, t_R 3.02 min) and **3** (5.0 mg, t_R 5.31 min).

Congoensin A (**1**): White powder (MeOH); $[\alpha]^{20}_D + 20.7°$ (*c* 1.3, $CHCl_3$); mp 136–138 °C; IR (KBr): ν_{max} 3398, 2950, 2877, 1706, 1462, 1383, 1150, 1024, 754 cm^{-1}; UV (MeOH) λ_{max} (PDA) 224, 280 nm; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) are shown in Table 1; HRESIMS *m*/*z* 482.3273 $[M + H]^+$ (calcd. for $C_{30}H_{44}O_4N$, 482.3265).

Congoensin B (**2**): White powder (MeOH); $[\alpha]^{20}_D + 27.6°$ (*c* 0.6, $CHCl_3$), mp 212–214 °C; IR (KBr) ν_{max} 3420, 2870, 2826, 2710, 1722, 1682, 1644, 1460, 1346, 1180, 1025 cm^{-1}; UV (MeOH) λ_{max} (PDA) 224 nm; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) are shown in Table 1; HRESIMS *m*/*z* 471.3476 $[M + H]^+$ (calcd. for $C_{30}H_{47}O_4$, 471.3469).

2.4. Biological activities

2.4.1. Antiplasmodial assay

In vitro activity against erythrocytic stages of chloroquine-sensitive of *Plasmodium falciparum* strain NF54 was assayed using a ^{3}H-hypoxanthine incorporation assay [13–15], the chloroquine- and pyrimethamine-resistant NF54 strain that originated from Thailand [14] and the standard drug chloroquine (Sigma-Aldrich). Compounds were dissolved in DMSO at 10 μg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL), Albumax® and washed human red cells A^+ at 2.5% hematocrit (0.3% parasitemia). Serial drug dilutions of 11 three-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 °C; 4% CO_2, 3% O_2, and 93% N_2. After 48 h 50 μL of [^{3}H] hypoxanthine (= 0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells were transferred onto a glass fiber filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression.