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**ABSTRACT**

A new xanthone, mboudiexanthone (1), together with five known compounds, euxanthone (2), isogarcinol (3), garcinol (4), betulinic acid (5) and zeorin (6) were isolated from the leaves of *Garcinia nobilis* Engl. The structures were determined by 1D and 2D NMR techniques and X-ray diffraction for 6. The *in vitro* antiproliferative properties of isolated compounds were evaluated against the human breast cancer cell line MCF-7. All compounds showed an antiproliferative activity with an IC<sub>50</sub> value down to ~11 μM for isogarcinol.

**1. Introduction**

Plants of the genus *Garcinia* (family *Clusiaceae*), widely distributed in tropical Africa, Asia, New Caledonia and Polynesia, have yielded an abundance of biologically active and structurally intriguing natural products (Ampofo and Waterman 1986). Apart from...
its use as a preservative and food flavour, *Garcinia* extracts have been used in traditional medicine for treatment of ailments. A decoction of fruit rind is given as a purgative in the treatment of intestinal parasites, bilious digestive conditions, dysentery, rheumatism and in the treatment of tumours (Raina et al. 2016). Extracts are also used as a cardio-tonic to treat angina (CSIR 1956). In veterinary practice, extracts of *Garcinia* are used as a rinse for diseases of the mouth while the fruit is used in rickets and enlargement of spleen, and for healing of bone fractures (Khare 2007). *Garcinia* species are known to contain a wide variety of oxygenated and prenylated xanthones, as well as polyisoprenylated benzophenones such as guttiferones (Nguyen et al. 2005). Xanthones show a wide range of biological and pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, anticholinesterase, cytotoxic activities, anti-allergy, antiallergy, antiparasitic and antihelminth activities to help in human health and also weight loss and appetite-reducing properties (Chin et al. 2008; Louh et al. 2008). Guttiferones have been reported as anti-HIV, trypanocidal and cytotoxic agents (Gustafson et al. 1992; Komguem et al. 2005; Merza et al. 2006). Our previous studies of the chemistry of *Garcinia nobilis* led to the isolation of xanthones derivatives including the isolation from the leaves of d l-hydroxy-2,5-dimethoxyxanthone with cytotoxic activity against human cervix carcinoma cell line KB-3-1 (Fouotsa et al. 2014). We also isolated caroxanthone from the stem bark of *G. nobilis* which exhibited α-glucosidase and antibacterial activities but did not showed any α-chymotrypsin inhibitory activity (Fouotsa et al. 2012; 2013). As part of our ongoing research program on the identification of bioactive constituents from plants in the genus *Garcinia*, we have investigated the methanol extract of the leaves of *G. nobilis*. These have been subjected to petro- leum ether liquid–liquid extraction, flash and column chromatography techniques that led to the separation of six compounds. In this article, we describe the isolation and characterisation of one new xanthone derivative (1) together with five known compounds (2–6). These compounds have been tested for their antiproliferative activity on breast cancer cell line, MCF-7.

2. Results and discussion

Two hundred and fifty grams of the methanolic extract of the leaves of *G. nobilis* were partitioned with petroleum ether and ethyl acetate (EtOAc). The petroleum ether extract (78.8 g) was then subjected to successive flash and column chromatography techniques over silica gel and Sephadex to obtain a new xanthone derivative named mboudiexanthone (1), along with six known molecules identified as euxanthone (2), isogarcinol (3), garcinol (4), betulinic acid (5) and zeorin (6) (Figure 1).

Compound 1 was isolated as a yellow powder. Its molecular formula C_{18}H_{15}O_{5} was determined by HR-ESI-MS. A positive ferric chloride test revealed its phenolic nature.

The UV spectrum showed absorption bands at \( \lambda_{\text{max}} \) 352, 264, 241 and 201 nm, indicating a xanthone derivative. The IR spectrum showed absorptions at 3730, 3370, 2922, 1739 and 1622 cm\(^{-1}\) suggesting a carbonyl group in the xanthone skeleton (Meli et al. 2005). The \(^{1}\)H NMR spectrum of xanthone 1 showed signals at \( \delta \) 3.39 (2H, d, \( J \) = 7.3 Hz, H-1’), 5.32 (1H, t, \( J \) = 7.3 Hz, H-2’), 1.74 (3H, s, H-4’) and 1.75 (3H, s, H-5’) suggesting the presence of a 3-methyl-2-butenyl moiety, which was connected to C-2.
(δC 124.2) on the basis of HMBC correlations (Supplementary information). The protons H-1′ showed HMBC correlations with C-1 (δC 158.9), C-2 (δC 124.2) and C-3 (δC 137.8). The analysis of an aromatic AB system at δ 7.52 (1H, d, J = 8.5 Hz, H-3) and 6.90 (1H, d, J = 8.5 Hz, H-4) on one hand, and δ 7.30 (1H, d, J = 8.9 Hz, H-6) and 6.71 (1H, d, J = 8.6 Hz, H-7) on the other hand, suggested the presence of two different ortho-protons in the skeleton. H-3 attached to C-3 showed HMBC correlations with C-1 (δC 158.9), C-1′ (δC 27.2) and C-4a (δC 154.0), H-4 attached to C-4 showed long range correlations with C-2 (δC 124.2), C-9a (δC 107.8), and C-4a (δC 154.0). Likewise, H-6 correlated with C-10a (δC 143.2), C-5 (δC 135.9) and C-8 (δC 154.0), while H-7 correlated with C-8a (δC 107.3), C-8 (δC 154.0) and C-5 (δC 135.9). The signals at δC 186.4 (C-9), δH 12.16 (1H, s, 1-OH) and 11.17 (1H, s,8-OH) indicated the presence of chelated carbonyl and hydroxyl groups in product 1. The HMBC spectrum highlighted a correlation between proton 1-OH (δH 12.16) and carbons C-9a (δC 107.8), C-1 (δC 158.9), and C-2 (δC 124.2). The signal at δH 11.17 (8-OH) showed cross peaks with the signals at δC 154.0 (C-8), 109.9 (C-7) and 107.3 (C-8a). From these spectral data, compound 1 was
confirmed as a trioxygenated xanthone which was named mboudiexanthone (1,5,8-tri-
hydroxy-2-(3-methyl-2-butenyl)xanthone) (Figure 1).

The in vitro antiproliferative properties of isolated compounds were screened
against the human breast cancer cell line MCF-7. Dose-response curves as well as IC₅₀
values are given in Supplementary information. All molecules showed an antiproliferative
activity with a growth inhibition higher than 50% at 20 μM — 50 μM, except for compound 6
that reached 111.45 μM. Isogarcinol (3) was the most active with an IC₅₀ value of 10.76 μM. Garcinol (4) also showed a significant activity with an IC₅₀ value of 13.77 μM. Both compounds 3 and 4 are polyisoprenylated benzophenones and previous studies revealed that substrates belonging to the benzophenone family exhibit an antiproliferative activity against several tumour cell lines in different experimental models (Wu et al. 2014). A significant activity was also observed with betulinic acid (5) (IC₅₀ values of 19.52 μM). Betulinic acid has been reported as one of the most active agents against cancer development in the group of pentacyclic triterpenoids (Paduch and Kandefer-Szerszen 2014). However, zeorin 6, another pentacyclic triterpenoid, was the least active compounds tested in this work, showing an IC₅₀ value of 111.45 μM. Mboudiexanthone (1) and euxanthone (2), two xanthonoid derivatives exhibited an antiproliferative activity with IC₅₀ values of 35.26 and 32.91 μM, respectively. These findings are in agreement with previous studies on the anticancer properties of xanthonoids, considering that several traditionally used medicinal plants contain xanthones as active constituents (Anantachoke et al. 2012). Structure–activity relationship analysis of xanthones derivatives such as mangostin indicates that the maintenance of the isopentene group at C-8 is essential for the cytotoxic activity (Chi et al. 2018). This may justify the moderate activity observed with the isolated new compound mboudiexanthone. The overall antiproliferative properties of compounds isolated in this work are consistent with the literature which reports that xanthonoids, triterpenoids and benzophenones are generally responsible for the pharmacological activity of Garcinia species (Hemshekhar et al. 2011).

3. Experimental

3.1. General procedures

IR spectra were recorded on a JASCO A-302 IR spectrophotometer (JASCO, Gross-
Umstadt, Germany). The ¹H, ¹³C and 2D NMR spectra were recorded on a Bruker
AMX-500 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) using CDCl₃ as
solvent. Homonuclear ¹H-¹H connectivities were determined by using the COSY 458
experiment. One-bond ¹H-¹H connectivities were determined by HMQC. Two and
three-bond ¹H-¹³C connectivities were determined by HMBC experiments. Proton
coupling constants (J) were measured in Hz. The HR-ESI-MS were recorded on a JEOL
HX 110 mass spectrometer. Column chromatography was carried out on silica gel 60
(70–230 and 240–300 mesh, E. Merck), and with Sephadex LH-20 (GE Healthcare Bio-
Sciences AB, Uppsala, Sweden). Preparative thin layer chromatography (PTLC) was
done on F254 PTLC plates (E. Merck, Darmstadt, Germany). Precoated silica gel TLC
was used to check the purity of compounds, and ceric sulphate spray reagent was used to visualise compounds on TLC.

3.2. Plant material

*Garcinia nobilis* Engl. was collected from Okola, Central Province, Cameroon in April 2010, and identified by Mr. Victor Nana of the Cameroon National Herbarium (Yaoundé) where a voucher specimen (50779/HNC/Cam/Mt Zamangoué) was deposited.

3.3. Extraction and isolation

The air-dried and ground leaves of *G. nobilis* (1.3 kg) were extracted three times with MeOH (10 L) at room temperature. The resulting extract was concentrated under reduce pressure to obtain a crude extract (250.0 g). The obtained extract was partitioned with petroleum ether (35.0 g) and ethyl acetate (17.0 g). 33.0 g of petroleum ether fraction were submitted to vacuum liquid chromatography (VLC; 25–40 µm, 8 cm × 60 cm, 300 g), eluting with n-hexane/ethyl acetate (EtOAc) of increasing polarity (10:0 1 L; 8:2 1 L, 6:4 750 mL, 4:6 500 mL, 0:10 500 mL), which were collected into 300 mL fractions and subsequently combined based on their thin layer chromatography (TLC) profile into four fractions A–D. Fraction B (3.0 g) was subjected to column chromatography on silica gel (25–40 µm, 4.0 × 60 cm) and eluted with petroleum ether/EtOAc by increasing polarity, 25 mL of each fraction were collected and subsequently combined on the basis of similar TLC into four fractions B1–B4. Fraction B1 was further purified using silica gel and column chromatography (25–40 µm; 4.0 × 60 cm) eluting with mixture of petroleum ether/EtOAc (2:8) and washing with methanol after condensation to afford betulinic acid (5, 10 mg) and zeorin (6, 5 mg). Isogarcinol (3, 32 mg) and garcinol (4, 7.5 mg) were obtained from fraction B2 by using preparative thin layer chromatography (PTLC) followed by further purification on silica gel chromatography (25–40 µm; 3.0 × 15 cm) eluting with petroleum ether/acetone solvent system by increasing the polarity.

B3 (2 g) was further purified by silica gel in the same column chromatography using petroleum ether/CH₂Cl₂/MeOH by increasing polarity and Sephadex LH-20 to afford 1,5,8-trihydroxy-2-prenylxanthone (1, 11 mg), Euxanthone (2, 17 mg). due to the TLC profile, B4 was kept.

**Mboudiexanthone (1)**: Yellow powder; C₁₈H₁₅O₅; UV (MeOH) λ<sub>max</sub> (nm) 352, 264, 241; 201. IR(KBr) ν<sub>max</sub>(cm⁻¹) 3730, 3370, 2922, 1739 and 1622. ¹H NMR (CDCl₃, 500 MHz) δ: 7.52(d; J = 8.5 Hz; 1H; H-3), 6.90(d; J = 8.5 Hz; 1H; H-4), 7.30(d; J = 8.5 Hz; 1H; H-5), 1.76(H-4), 1.74(H-5), 12.16(s; 1H; 1-OH), 11.17(s; 1H; 8-OH) and ¹³C NMR (CDCl₃, 125 MHz) δ: 158.9(C-1), 124.2(C-2), 137.8(C-3), 106.3(C-4), 135.9(C-5), 123.9(C-6), 109.9(C-7), 154.1(C-8), 186.4(C-9), 154.0(C-4a), 107.3(C-8a), 107.8(C-9a), 143.2(C-10a), 27.2(C-1′), 121.5 (C-2′), 133.9 (C-3′), 26.0 (C-4′), 26.0 (C-5′). HR-ESI-MS: m/z 311,0932 [M − 1] (calcd. for C₁₈H₁₅O₅/−1, 311,0925).
Euxanthone (2): Yellow needles; EI-MS: m/z 228 M⁺, C₁₃H₈O₄; mp 231–232 °C. UV λ_max (nm):(log ε): 387, 288, 259, 235, 202. IR (KBr)υ_max (cm⁻¹): 3300, 1640, 1600, 1580, 1480. ¹H NMR and ¹³C NMR are consistent with published data (Wenkui et al. 1998).

Isogarcinol (3): Brown crystal, ESI-MS: m/z 603.37 [M+H]⁺, C₃₈H₅₀O₆; mp 251 °C; UV λ_max (nm):(log ε): 317 (3.82), 277 (4.14), 233 (4.07); IR (KBr)υ_max (cm⁻¹): 3300, 1640, 1600, 1580, 1480. ¹H NMR and ¹³C NMR are consistent with published data (Jaideep et al. 2013).

Garcinol (4): Yellow crystal; ESI-MS: m/z 603.37 [M+H]⁺, C₃₈H₅₀O₆; mp 132 °C; UV λ_max (nm):(log ε): 279 (4.18), 232 (4.04); IR (KBr)υ_max (cm⁻¹): 3300, 2920, 1720, 1640, 1590, 1440, 1370, 1290, 1190. ¹H NMR and ¹³C NMR are consistent with published data (Jaideep et al. 2013).

Betulinic acid (5): Colourless amorphous powder; MS m/z 456 M⁺ m.p 297 °C; UV: 206 nm; IR (KBr)υ_max (cm⁻¹): 3473, 3063, 2953, 2887, 2712, 1682, 1643, 1457, 1375, 1221, 1194, 1106, 1035, 980, 876, 871, 789. ¹H NMR and ¹³C NMR are consistent with published data (Eder et al. 2008).

Zeorin (6): MS m/z 444 M⁺, C₃₀H₅₂O₂; m.p. 237–240 °C. IR (KBr)υ_max (cm⁻¹): 3385, 2980, 1465, 1380, 1260, 1250, 1210, 1160, 1140, 1044, 1020, 980, 965, 875, 830, 760, 730, 710, 690. ¹H NMR and ¹³C NMR are consistent with published data (Konig and Wright 1999).

3.4. X-ray crystallographic study of 6
Crystal data for 6. (C₃₀H₅₂O₂) x 0.5 CH₂Cl₂. M = 444, hexagonal, space group P6₁22 (no. 178), a = 16.76179(13) Å, c = 68.9708(6) Å, V = 16781.7(3) Å³, Z = 24, T = 100.0(1) K, μ(CuKα) = 0.927 mm⁻¹, Dcalc = 1.107 g/cm³, 283,999 reflections measured (6.088° ≤ 2Θ ≤ 151.65°), 11,582 unique (Rint = 0.0926, Rsigma = 0.0230) which were used in all calculations. The final R₁ was 0.0562 for 10773 reflections with l > 2σ(l) and wR₂ was 0.1373 for all data. CCDC 1970901 contains the supplementary crystallographic data for this article (Supplementary information). These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/conts/retrieving.html.

3.5. Antiproliferative activity assay
3.5.1. Cell culture and treatment
The breast carcinoma cell line MCF-7 was cultured in DMEM (Dulbecco’s Modified Eagle’s Medium) supplemented with 10% FCS and 1% antibiotics comprising 100 IU/mL penicillin and 100 μL/mL streptomycin. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

3.5.2. WST-1 assay for cell proliferation inhibition
Cell proliferation was evaluated using reagent WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium] (Roche Diagnostics, Germany) as described by Delie et al. (2013). Cells at a density of 10⁴ per well were seeded in 96-well plates and incubated overnight. The next day, they were exposed to different
concentrations (5–100 µM or 20–200 µM) in triplicate of compounds solubilised in DMSO and incubated for 48 h. Then the medium in each well was aspirated, WST-1 solution was diluted 1:10 with fresh medium, added to each well and the plates were incubated at 37 °C for 45 min. The absorbance was recorded at 450 nm/690 nm using Synergy Multi-Mode Microplate Reader (BioTek). Results were expressed as percentage of inhibition of cell proliferation relatively to the control without treatment. Concentration-response analysis was performed to determine the compound concentrations required to inhibit the growth of cancer cells by 50% (IC50) using GraphPad Prism 8.00 software.

4. Conclusion

The present work indicated that *G. nobilis* is a good source of bioactive xanthones and benzophenones. All molecules showed an antiproliferative activity with a growth inhibition higher than 50% at 20–50 µM, except for compound 2 that reached 200 µM. Their cytotoxicity provided baseline information for their possible use for the control of cancer diseases.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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