Prenyled Xanthone Derivatives with Antiplasmodial Activity from 
*Allanblackia monticola* Staner L.C.

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Further study of the methanolic extract of the stem bark of *Allanblackia monticola* Staner L.C. resulted in the isolation of a new prenylated xanthenedione, designated allanxanthone C, together with the five known xanthones, garciniafuran, tovophyllin A, rubraxanthone, ncoroewain and mangostin and one saponin, stigmastrol-3-O-β-D-glucopyranoside. The structure of the new compound was established by detailed spectroscopic analysis to be 1,2-dihydro-3,6,8-trihydroxy-1,1,7-tri(3-methylbut-2-enyl)xanthen-2,9-dione (3-hydroxyapetalinone C). The methanol extract and pure compounds were tested on two strains of *Plasmodium falciparum*, F32 (chloroquine sensitive) and FeM29 (chloroquine resistant). The IC₅₀ values obtained ranged from 0.6 to 8.9 μg/ml. Their cytotoxicity was estimated on human melanoma cells (A375) and the cytotoxicity/antiplasmodial ratio was found to be between 15.45 and 30.46. The antimicrobial activities against a range of microorganisms of the crude extract and some of these compounds are also reported.

**Key words** *Allanblackia monticola*; Clusiaceae; allanxanthone C; xanthone; antiplasmodial; cytotoxicity; antimicrobial activity

There has been a recent increase in the research efforts on secondary metabolites of the genus *Allanblackia*.¹⁻³ Our interest was stimulated by the fact that, many of the secondary metabolites from this genus such as xanthones, biflavonoids, benzophenones and pentacyclic triterpenes exhibited a wide range of biological and pharmacological activities, including cytotoxic, anti-inflammatory, antimicrobial, antifungal and HIV inhibitory activities.²⁻⁵ *Allanblackia monticola* Staner L.C., which belongs to the plant family Clusiaceae, is a large forest tree found throughout the west and south province of Cameroon, where it is used as medicinal plant to treat several diseases including, respiratory infections, diarrhoea and toothache.⁶⁻⁷

In a previous paper, we have reported the isolation and structural elucidation of a novel polyisoprenylated xanthone, allanxanthone B, along with the known compounds: tovophyllin A, rubraxanthone, garciniafuran, lupeol and stigmasterol-3-O-β-D-glucopyranoside. The signals at δ 6.55 (1H, s, H-4) and 2.73 (2H, dd, J 7.2 Hz, H-2) could be assigned to a sp³ carbon atom (δ 56.9, C-1).¹⁴,¹⁵ Furthermore, the signals at δ 5.25 (1H, t, J = 7.2 Hz, H-2''), 3.35 (2H, d,
The fact that one of the methylene proton of the gem bis (3-methylbut-2-enyl) group resonate at the lower field of δ 3.43 instead of δ 2.73 was probably due to the anisotropie effect of the carbonyl group and the cross peaks in the Heteronuclear Multiple Bond Correlation (HMBC) spectrum (Fig. 1), between these methylene protons and carbons C-2 (δ 201.1) and C-9a (δ 116.6) suggesting that the gem bis(3-methylbut-2-enyl) is located at the C-1 position. The third prenyl group was located at the C-7 position, based on analysis of the NOESY spectrum which showed cross peak between the allylic proton at δ 3.35 and the chelated hydroxyl proton at δ 13.50 in addition to the correlations observed in the HMBC spectrum (Fig. 1) between protons H-2’ (δ 3.35) and carbons C-8 (δ 180.3), C-7 (δ 112.6), and C-6 (δ 162.3) and between the chelated hydroxyl proton at δ 13.50 and carbons C-8 (δ 180.3), C-7 (δ 112.6), and C-8a (δ 105.6).

From the above spectroscopic data, the structure of allanxanthone C (I) was established as 1,2-dihydro-3,6,8-trihydroxy-1,1,7-tri(3-methylbut-2-enyl)xanthene-2,9-dione. The crude extract and compounds 1, 2 and 3 were assayed for their antimalarial activities against two strains of Plasmodium falciparum: F32 (chloroquine-sensitive) and FeM29 (chloroquine resistant) and for their cytotoxicity against human melanoma cells (A375). All these compounds were found to be moderately active against the two strains of P. falciparum and also showed weak cytotoxicity against human melanoma A375 cells (Table 1). When the cytotoxicity-antiplasmodial ratio (CAR, Table 1) was calculated, it appeared that despite the moderate antiplasmodial activity observed with pure compounds, a higher specificity (×2) was obtained after purification.

The crude extracts and compounds 1, 2 and 3 were also tested for their antimicrobial potency against the Gram-positive bacteria Staphylococcus aureus (ATCC 6538), Gram-negative Vibrio anguillarum (ATCC 19264), and the pathogenic fungi Candida tropicalis (ATCC 66029). The results obtained show that, crude extract and all these compounds were inactive against C. tropicalis and V. anguillarum. Against S. aureus, compounds 1 and 2 were also inactive whereas compound 3 and crude extract displayed moderate activity with a diameter of inhibition zone of 16 mm and 12 mm respectively. From the above results, we can conclude that compound 3 and crude extract are twice less active against S. aureus than the reference compound, oxacillin for which the diameter of inhibition is 30 mm.

### Experimental

**General Experimental Procedures** Melting points were determined on a Buchi apparatus and were uncorrected. UV spectra were obtained on a Shimadzu-265 Spectrometer. IR spectra were recorded on a Perkin-Elmer 727B spectrometer in KBr disks. NMR spectra were recorded on a Bruker instrument equipped with a 5 mm 1H and 13C probe operating at 300 and 75 MHz respectively with TMS as internal standard. 1H assignments were made based on two dimensional Correlated Spectroscopy COSY and NOESY (mixing time 800 ms experiments while 13C assignments were made based on HSQC and HMBC experiments. Silica gel 230—400 Mesh (Merck) were used for flash and column chromatography, while precoated aluminium silica gel 60 F254 sheets were used for TLC with a mixture of cyclohexane and ethyl acetate as eluents; spots were visualised under UV lamps (254 nm) or (365 nm) or by MeOH–H2SO4 reagent.

**Plant Material** The stem bark of A. monticola was collected in January 2004, at Bangante in the West Province of Cameroon. The identification was confirmed by Dr. L. Zapfack, Botanic Department, University of Yaounde I. A voucher specimen documenting the collection is deposited at the National Herbarium of Cameroon.

**Extraction and Isolation** Air-dried and powdered stem bark of A. monticola (3.1 kg) was extracted at room temperature with methanol (10 l) and concentrated to dryness to afford a viscous residue (190 g). This residue was then fractionated by flash column chromatography using silica gel (70—230 mesh) eluted with a mixture of cyclohexane—EtOAc (7.5 : 2.5), (1 : 1) (2.5 l of each solvent), EtOAc (3 l) and EtOAc–MeOH (7.5 : 2.5) (2 l of each solvent), EtOAc–MeOH (7.5 : 2.5) (1 : 1) (2.5 l of each solvent) to give four main fractions labelled A (16 g), B (17 g), C (50 g) and D (95 g). Fraction A was column chromatographed over silica gel (70—230 mesh) eluted with a mixture of cyclohexane and ethyl acetate as eluents; spots were visualised under UV lamps (254 nm) and (365 nm) or by MeOH–H2SO4 reagent.

<table>
<thead>
<tr>
<th>Compound</th>
<th>FcM29-Cameroon (Chloroquine resistant)</th>
<th>F32 (Chloroquine sensitive)</th>
<th>A375 (Human melanoma cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
<td>24 h</td>
</tr>
<tr>
<td>MeOH extract</td>
<td>3.1±0.08</td>
<td>2.13±0.1</td>
<td>3.3±0.3</td>
</tr>
<tr>
<td>1</td>
<td>2.6±0.9</td>
<td>0.6±0.02</td>
<td>3.2±0.0</td>
</tr>
<tr>
<td>2</td>
<td>8.9±3.1</td>
<td>—</td>
<td>2.8±0.9</td>
</tr>
<tr>
<td>3</td>
<td>2.6±0.8</td>
<td>1.72±0.0</td>
<td>2.2±0.05</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>0.213b</td>
<td>0.215b</td>
<td>0.035b</td>
</tr>
</tbody>
</table>

* a) Cytotoxicity/antiplasmodial activity ratio. b) This was routinely tested in the laboratory every month.
tions 4—8), and norcowanin (2) as yellow powder (80 mg) (fractions 11—13) whereas fractions (31—54) affords pure garciniafan (6) as yellow needles (11 mg). From series B, eluted with a mixture of cyclohexane–EtOAc (7.5:2.5), after repeated column chromatography, allanxanthone C (1) (200 mg) was obtained as yellow oil. Series C eluted with a mixture of cyclohexane–EtOAc (1:1) (2), was essentially constituted of stigmasterol-3-O-β-D-glucopyranoside (4) (1700 mg), whereas series D contained very polar compounds that were difficult to separate.

**Assay for Antiplasmodial Activity** The antiplasmodial activity of the plant methanol extract and pure compounds was evaluated using the radioautographic micromethod described by Desjardins et al., with the modifications reported by Benoit et al. The extract and pure compounds were analyzed three times in triplicate in 96-well culture plates (TPP, Switzerland) with cultures mostly at ring stages (synchronization interval, 16 h) at 0.5—1% para-sitemia (hematocrit, 1%). Parasite cultures were incubated with extract and each pure compound for two time intervals, 24 and 72 h. Two strains of *P. falciparum* were employed in this experiment and parasite growth was estimated by [3H]-hypoxanthine incorporation. The control parasite cultures free from extract and any compound were referred to as 100% growth. IC₅₀ values were determined graphically in concentration versus percent inhibition curves.

**Assay for Cytotoxicity** The cytotoxicity of the extract and pure compounds was estimated against human melanoma A375 cells. Cells were cultured in the same conditions as *P. falciparum*. For the determination of extract and pure compounds toxicity, cells were distributed in 96-well plates at 2·10⁴ cells per well in 100 µl, then 100 µl of culture medium containing extract or pure compounds at various concentrations were added. Cell growth was estimated by [3H]-hypoxanthine incorporation after 24 h incubation exactly as for the *P. falciparum* contact period. The [3H]-hypoxanthine incorporation in the presence of the extract or pure compounds compared with that of control cultures without extracts.

**Assay for Antimicrobial Activity** The qualitative antimicrobial assay employed was a classical disc diffusion technique. The culture medium used for the bacteria was Mueller Hinton agar (DIFCO). Whereas Sabouraud agar was used for growing the fungi. Paper discs were impregnated with 20 µl of DMSO solution containing each sample (1 mg/ml) and allowed to evaporate at room temperature. Oxacillin (20 µl of a 1 mg/ml solution) was used as standard for positive control. The plates with micro-organisms were incubated for 24 h at 37°C for *S. aureus* and for 48 h at 27°C for *F. anguillarum* and *C. tropicalis*. The diameters of the inhibition zone around each disc were measured and recorded at the end of the incubating period.

Allanxanthone C (1): 1,2-Dihydro-3,6,8-trihydroxy-1,1,7-tri(3-methylbut-2-enyl)xanthan-2,9-dione (3-hydroxyapetalone C): Yellow oil; UV λmax (log ε) (nm): 242 (3.80), 280 (3.60), 305 (3.70), 417 (3.51). IR (KBr) cm⁻¹: 3350, 1674, 1578, 1474, 1427, 1269, 1160, 1136; +ESI-TOF-MS m/z 665.5455 [M + H⁺] +, 456.5580 ([M - C₆H₄O₁]²⁺ required for 465.5580); msms m/z (rel. int.): 409 (25) [M⁻ - C₆H₄], 397 (55) [M⁻ - C₇H₄], 341 (100) [M⁻ - C₇H₈ - C₆H₄], 285 (65), 257 (95), 229 (78), 231 (15), 69 (7.5). 1H-NMR (300 MHz, acetone-δ₆) δ 13.50 (1H, s, H-OH), 9.50 (1H, br s, OH-6), 9.30 (1H, br s, OH-3), 6.55 (1H, s, H-4), 6.45 (1H, s, H-5), 5.25 (1H, t, J = 7.2 Hz, H-2'), 4.74 (2H, t, J = 7.6 Hz, H-2'), 3.43 (2H, dd, J = 7.6, 13.6 Hz, H-1'), 3.35 (2H, d, J = 7.2 Hz, H-1').

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**References**
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24) Benoit F., Valentin A., Peliisser Y., Diafouka F., Marion C., Dakuyo Z., Meddah for the biological assays.