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

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Further study of the methanol 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, norcowanin and mangostin and one saponin, stigmasterol-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 FcM29 (chloroquine resistant). The IC_{50} 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.

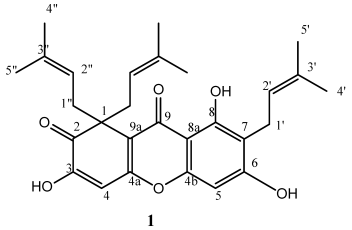
Key words *Allanblackia monticola*; Clusiaceae; allanxanthone C; xanthone; antiplasmodial; cytotoxicity; antimicrobial activity

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.[2,4,5] *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]

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 from the CH_2Cl_2–MeOH (1 : 1) extract of the stem bark of this plant.[7] In the present paper, we report the isolation and characterization of seven compounds isolated from the MeOH extract of this plant, including a new triprenylated xanthenedione, designated allanxanthone C (**1**), together with the known compounds norcowanin (**2**),[8] mangostin (**3**),[9] tovophyllin A (**5**),[10] garciniafuran (**6**),[11] rubraxanthone (**7**)[12] and stigmasterol-3-*O*-β-D-glucopyranoside (**4**).[13] The structure of known compounds were identified from their spectral data and confirmed by comparison with those published in the literature. We also report on the antiplasmodial, cytotoxic and antimicrobial activities of the methanol extract and some isolated compounds.

Extensive column chromatography of a methanol extract of the stem bark of *A. monticola* led to the isolation allanxanthone C (**1**).

Allanxanthone C (**1**) was obtained as a sticky yellow oil. It reacts positively to ferric chloride test, suggesting the presence of a phenolic groups. Its molecular formula, $C_{28}H_{32}O_6$, was deduced from the high resolution Electro Spray Ionisation-Time of Flight (ESI-TOF) mass spectrometry which showed the pseudomolecular ion $[M+H]^+$ at *m/z* 465.5545 (Calcd for 465.5580). The IR spectrum of **1** confirmed the presence of phenolic group (3350 cm^{-1}) and suggested the presence of conjugated carbonyl group (1674 cm^{-1}) and xanthone carbonyl group (1643 cm^{-1}).[14] The ^{1}H-NMR and Heteronuclear Single Quantum Coherence (HSQC) spectra of allanxanthone C (**1**) showed the presence of a hydrogen-bonded hydroxyl at δ 13.50 (1H, s, 8-OH) and two singlet protons at δ 6.55 (1H, s, H-4) and δ 6.45 (1H, s, H-5), δ 109.5 (d, C-4) and 93.5 (d, C-5). The presence of a set of signals at δ 4.74 (2H, t, J=7.6 Hz, H-2″), 3.43 (2H, dd, J=7.60, 13.6 Hz, H-1″), 2.73 (2H, dd, J=7.6, 13.6 Hz, H-1″) and 1.47 (12H, s) was typical of a *gem* bis(3-methylbut-2-enyl) groups linked to a sp^3 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,



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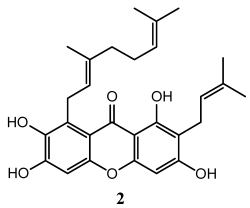

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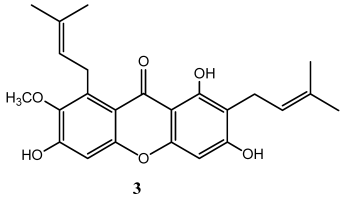

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J=7.2 Hz, H-1′), 1.80 (3H, s, H-4′) and 1.65 (3H, s, H-5′), δ 131.7 (C-3′), 123.2 (C-2′), 22.1 (C-1′), 25.9 (C-4′) and 18.0 (C-5′) revealed the presence of the third 3-methylbut-2-enyl group. In addition, the Attach Proton Test (APT) ^{13}C spectrum indicated the presence of two carbonyls at δ 180.3 (C-9) and 201.1 (C-2) and nine other quaternary carbons.

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 anisotropic 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 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 (**1**) 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 antiplasmodial activities against two strains of *Plasmodium falciparum*: F32 (chloroquine-sensitive) and FcM29 (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.

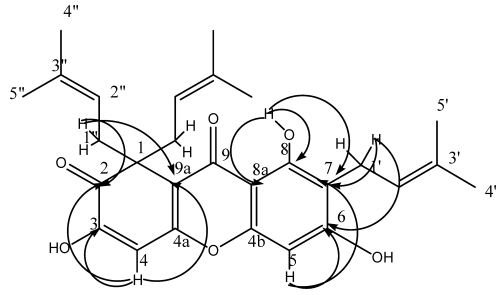

Fig. 1. Significant HMBC Correlation for Allanxanthone C (**1**)

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 ^{13}C 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 ^{13}C assignments were made based on HSQC and HMBC experiments. Silica gel 230—400 Mesh (Merck) and silica gel 70—230 Mesh (Merck) were used for flash and column chromatography, while precoated aluminium silica gel 60 F_{254} sheets were used for TLC with a mixture of cyclohexane and ethyl acetate as eluents; spots were visualised under UV lamps (254 nm) and (365 nm) or by MeOH–H_2SO_4 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) 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 (230—400 mesh), eluted with a mixture of cyclohexane–EtOAc (9 : 1) (2.5 l) to afford tovophyllin A (**5**) as yellow powder (15 mg) (fractions 5—8) and mangostin (**3**) (fractions 12—23) as yellow crystals (1200 mg). Fractions (30—51) (2.4 g) were rechromatographed, eluted with cyclohexane–EtOAc (8.5 : 1.5) (2.2 l) as eluent to afford rubraxanthone (**7**) as yellow powder (14 mg) (frac-

Table 1. *In Vitro* Antiplasmodial Activities and Cytotoxicities of MeOH Extract and Pure Compounds **1**, **2**, **3** on Various *P. falciparum* Strains and on Human Melanoma A375 Cells

	IC_{50} (μg/ml)±S.D.					
	FcM29-Cameroon (Chloroquine resistant)		F32 (Chloroquine sensitive)		A375 (Human melanoma cells)	
	24 h	72 h	24 h	72 h	24 h	CAR[a]
MeOH extract	3.1±0.08	2.13±0.1	3.3±0.3	3.3±0.2	51.0±2.5	15.45
1	2.6±0.9	0.6±0.02	3.2±0.0	3.2±0.3	83.8±7.1	26.19
2	8.9±3.1	—	2.8±0.9	2.89±0.4	79.8±4.0	13.5
3	2.6±0.8	1.72±0.0	2.2±0.05	3.15±0.02	79.2±6.0	30.46
Chloroquine	0.213[b]	0.215[b]	0.035[b]	0.035[b]	>2000	>100

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 garciniafuran (**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 l), 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 radioactive micromethod described by Desjardins *et al.*,[16] with the modifications reported by Benoit *et al.*[17] 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% parasitemia (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 used for this experiment and parasite growth was estimated by [^{3}H]-hypoxanthine incorporation. The control parasite cultures free from extract and any compound were referred to as 100% growth. IC_{50} 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 $2 \cdot 10^4$ cells per well in 100 μl, then 100 μl of culture medium containing extract or pure compounds at various concentration were added. Cell growth was estimated by [^{3}H]-hypoxanthine incorporation after 24 h incubation exactly as for the *P. falciparum* contact period. The [^{3}H]-hypoxanthine incorporation in the presence of the extract or pure compounds were compared with that of control cultures without extracts.[18]

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).[19] Whereas Sabauraud 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 evaporated 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 *V. 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)xanthen-2,9-dione (3-hydroxyapetalinone C): Yellow oil; UV λ_{max}^{EtOH} (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* 465.5545 $[M+H]^+$ ($C_{28}H_{33}O_6$, required for 465.5580); msms *m/z* (rel. int.): 409 (25) $[M^+-C_4H_8]$, 397 (35) $[M^+-C_5H_9]$, 341 (100) $[M^+-C_5H_9-C_4H_8]$, 285 (65), 257 (95), 229 (78), 231 (15), 69 (7.5). ^{1}H-NMR (300 MHz, acetone-d_6) δ 13.50 (1H, s, OH-1), 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′), 2.73 (2H, 2H, dd, J=7.6, 13.6 Hz, H-1″), 1.80 (3H, s, H-4′), 1.65 (3H, s, H-5′), 1.47 (12H, s, H4″, H-5″); ^{13}C-NMR (75 MHz, acetone-d_6), δ 201.1 (C-2), 180.3 (C-9), 162.3 (C-6), 160.7 (C-8), 160.1 (C-3), 154.2 (C-4a, C-4b), 135.2 (C-3″), 131.7 (C-3′), 123.2 (C-2′), 119.2 (C-2″), 56.9 (C-1), 38.4 (C-1″), 25.9 (C-4′), 25.7 (C-4″, C-5″), 22.1 (C-1′), 18.0 (C-5′).

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