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Allanxanthone B, a polyisoprenylated xanthone from the stem bark of *Allanblackia monticola* Staner L.C

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Abstract

In addition to five known compounds including three xanthones, tovophyllin A, rubraxanthone and garciniafuran, one pentacyclic triterpene, lupeol and one phytosterol, stigmasterol, a polyisoprenylated xanthone named allanxanthone B was isolated from the stem bark of *Allanblackia monticola*. The structure of the new compound was assigned as 2-geranyl-1,3,6-trihydroxy-2′,2′-dimethyl[5′,6′:7,8]xanthone by means of spectroscopic analysis. The antimicrobial activities of some of these compounds against a range of micro-organisms are also reported.
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Keywords: *Allanblackia monticola*; Guttiferae; Allanxanthone B; Xanthones; Lupeol; Stigmasterol; Antimicrobial activities

1. Introduction

Allanblackia monticola Staner L. C., which belongs to the plant family Guttiferae is widely distributed in West Province of Cameroon, where it is used for the treatment of certain human ailments such as respiratory infections, diarrhoea and toothache (Raponda-Walker and Sillans, 1961). Further phytochemical studies of plants belonging to the genus *Allanblackia* have revealed the presence of xanthones, benzophenones, biflavonoids, phytosterols and saponins (Locksley and Murray, 1971; Blunt et al., 1999; Nkengfack et al., 2002). Some of these compounds exhibit a wide range of biological and pharmacological activities such as cytototoxic, anti-inflammatory, antimicrobial and antifungal (Nagem and Peres, 1997; Nagem et al., 2000); as well as HIV inhibitory activity (Blunt et al., 1999). As part of our continuous search for biologically active compounds from *Allanblackia* species, we now report the phytochemical analysis of the stem bark of *A. monticola* as well as the in vitro antimicrobial activity of some of the isolated compounds.

2. Results and discussion

Extensive column chromatography of a methylene chloride–methanol (1:1) extract of the stem bark of *A. monticola* led to the isolation of a new polyisoprenylated xanthone, allanxanthone B (**1**), along with three known xanthones, tovophyllin A (**3**) (Graham et al., 1993); rubraxanthone (**2**) (Ampofo and Waterman, 1986); garciniafuran (**6**) (Nilar and Leslie, 2002); one pentacyclic triterpene, lupeol (**5**) (Wenkert et al., 1978) and one phytosterol, stigmasterol (**4**) (Diakow et al., 1978).

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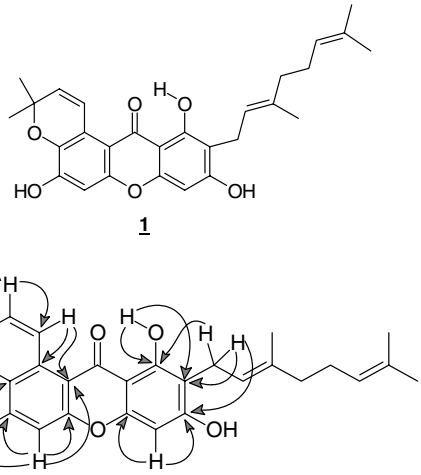
Allanxanthone B (**1**), $C_{28}H_{30}O_6$, was obtained as yellow powder, m.p. 158–160 °C. The compound gave a dark green color with methanolic ferric chloride, indicating that it was phenolic. The UV spectrum of (1) was typical of a 1,3,6,7-oxygenated xanthone and showed bathochromic shift with $AlCl_3$ and NaOAc reagents indicative of the presence of free hydroxyl groups at C-1 and at C-3 or C-6 (Lockley et al., 1966). The ^1H and ^{13}C NMR spectra (see experimental and Table 1) showed the presence of a chelated hydroxyl group [δ_H 13.70 (1H, *s*, 1-OH)], a chelated carbonyl [δ_C 181.5 (*s*, C-9)], two isolated aromatic protons [δ_H 6.40 (1H, *s*, H-4) and 6.80 (1H, *s*, H-5); δ_C 102.0 (*d*, C-5) and 93.0. (*d*, C-4), a dimethylchromene ring [δ_H 1.30 (6H, *s*, H_3-5, H_3-6) 5.90 and 8.15 (each 1H, *d*, $J = 10$ Hz); δ_C 30.1 (*q*, C-5, C-6′); 79.5 (*s*, C-2′); 131.8 (*d*, C-3′); 122.0 (*d*, C-4′) and a geranyl moiety [δ_H 1.60, 1.64, 1.78 (3H each, *s*, 3× CH_3); 2.06 (4H, *m*, H-4″, H-5″); 3.45 (2H, *d*, $J = 7$ Hz, H-1″); 5.10 (14, *brt*, $J = 7$ Hz, H-6″); 5,25 (1H, *brt*, $J = 7$ Hz, H-2″) (Asai et al., 1995; Pornpipat na Pattalung et al., 1994). The absence of the signal (δ_H 7.75–7.80) due to the aromatic proton (H-8) located at a *peri* position to the carbonyl group suggested that the methyl chromene was fused in an angular manner at C-8 through an oxygen atom at C-7. This was confirmed, on one hand, by the chemical shift of one *cis*-olefinic proton of the chromene ring which appeared in lower field (δ_H 8.15) caused by the anisotropic effect of the carbonyl group and, on the other hand, by the HMBC (Fig. 1) spectrum in which the same olefinic proton showed cross-peaks with C-8 (δ_C 109.1) and C-8a (δ_C 119.2). The cross-peaks between a chelated hydroxyl group at δ_H 13.70 and allylic proton at δ_H3,45 in the NOESY spectrum (Fig. 2) proved that the geranyl moiety was located at the C-2-position while the remaining proton was at C-4 position. The *ortho*-position of the oxygenated carbons of ring B was supported by the value of their ^{13}C NMR chemical shifts (Nilar and Leslie, 2002). From the above spectroscopic data, structure of allanxanthone B was unambiguously assigned as, {2-geranyl-1,3,6-trihydroxy-2′,2′-dimethylpyrano [5′,6′: 7,8]-xanthone}. Further confirmation of the structure of allanxanthone B came from the comparison of its ^1H NMR data with those reported in the literature for toxyloxanthone B (Chen and Chen, 1985) and garcinone B (Ishiguro et al., 1995) isolated, respectively, from *Hypericum sampsonii* and *Hypericum patulum* and which have the same substitution pattern as compound **1**.

Crude extracts and compounds (**1**, **2**, **3** and **6**) were tested for their antimicrobial potency against Gram-pos-

Table 1
^1H (300.135 MHz) and ^{13}C (75.469 MHZ) assignments for allanxanthone B (**1**) in CD_3 $COCD_3$

Attribution	^{13}C	Multiplicity	^1H [*m*, J (Hz)]	HMBC
1	161.5	*s*	–	–
2	111.0	*s*	–	–
3	163.0	*s*	–	–
4	93.0	*d*	6.40 (*s*)	4a, 3, 2, 9a
4a	156.2	*s*	–	–
5a	153.5	*s*	–	–
5	102.1	*d*	6.80 (*s*)	4b, 8a, 6
6	158.2	*s*	–	–
7	144.1	*s*	–	–
8	109.1	*s*	–	–
8a	119.2	*s*	–	–
9	181.5	*s*	–	–
9a	103.0	*s*	–	–
2′	79.5	*s*	–	–
3′	131.8	*d*	5.90 (*d*, 10.33)	2′, 4′, 6′, 5′
4′	122.0	*d*	8.15 (*d*, 10.33)	3′, 8, 8a
5′	30.1	*q*	1.30 (*s*)	2′, 3′, 6′
6′	30.1	*q*	1.30 (*s*)	2′, 3′, 5′
1″	22.0	*t*	3.45 (*d*, 7.20)	2, 1, 3
2″	123.1	*d*	5.25 (*t*, 7.20)	1″, 2, 3″, 4″, 10″
3″	135.2	*s*	–	–
4″	39.7	*t*	1.66 (*m*)	3″, 5″
5″	26.5	*t*	1.64 (*m*)	4″, 5″, 3″, 7″
6″	124.1	*d*	5.10 (*t*)	–
7″	131.3	*s*	–	–
8″	25.6	*q*	1.53 (*s*)	–
9″	17.7	*q*	1.43 (*s*)	–
10″	16.4	*q*	1.66 (*s*)	–
1-OH	–	–	13.4 (*s*)	1, 2, 9a
3-OH	–	–	1.68 (*brs*)	3, 2, 4
6-OH	–	–	2.68 (*brs*)	6, 5



1

Fig. 1. Significant HMBC correlations of compound **1**.

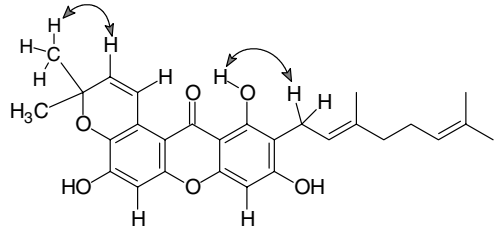


Fig. 2. Selected NOESY correlations of compound **1**.

Table 2
Antibacterial activity of crude extract and compounds **1**, **2**, **3** and **6**

Micro-organisms	Inhibition zone (mm)					
	Crude extract	**1**	**2**	**3**	**6**	Oxacillin
V. anguillaium	–	–	–	–	–	
S. aureus	15	–	12	–	–	30
C. tropicalis	–	–	–	–	–	

–, not active against the tested micro-organism.

itive (*Staphylococcus aureus*, *Vibrio anguillarium* and *Candida tropicalis*) bacteria in an agar well diffusion assay. As shown in Table 2, crude extract and the four xanthones were found to be inactive against *V. angillarium* and *C. tropicalis*. While against *S. aureus*, crude extracts and compound **2** displayed moderate activity, compounds (**1**, **3** and **6**) were found to be inactive.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Buchi apparatus and are uncorrected. UV spectra were obtained on a Shimadzu-265 spectrophotometer. NMR spectra were run on a Bruker instrument equipped with a 5mm ^{1}H and ^{13}C probe operating at 300.135 and 75.469 MHz, respectively, with TMS as internal standard. ^{1}H assignments were made using 2D-COSY and NOESY (mixing time 800 ms experiments while ^{13}C assignments were made using 2D - HSQC and HMBC experiments. Silica gel, 230–400 Mesh (Merk) and silica gel 70–230 Mesh (Merck) were used for flash and column chromatography, respectively, while precoated aluminium sheets silica gel 60 F_{254} (Merck) were used for TLC with a mixture of cyclohexane-ethyl acetate as eluents; spot were visualised by UV (254 nm)and (365 nm) or by MeOH–H_2SO_4.

3.2. Plant material

The stem bark of *A. monticola* was collected in July, 2003 at Bagangte in West Province of Cameroon. This sample was identified by Dr. L. ZAPFACK, of Botanic Department, University of Yaoundé I, where a Voucher specimen in on deposit.

3.3. Extraction and isolation

Air dried, powdered stem bark of *A. monticola* (3 kg) was extracted at room temperature with a mixture of CH_2Cl_2–MeOH (1:1) and concentrated to dryness to afford a viscous residue (250 g). This residue was then fractionated by flash column chromatography using silica gel (230–400 mesh) eluted with the mixture of cyclohexane–EtOA on time (7.5:2.5), cyclohexane–EtOAc (1:1); EtOAc and EtOAc–MeOH (7.5:2.5) to give four main fractions labelled A, B, C and D, respectively. Only fraction A and B showed a significant in vitro antimicrobial activity against a Gram-positive bacteria, *S. aureus* (diameter of inhibition = 13 and 9 mm, respectively).

Fraction A was then column chromatographed over *Si-gel* packed in cyclohexane; gradient elution was effected with cyclohexane–EtOAc mixture. A total of 90 fraction of ca. 300 ml each were collected and combined on the basis of TLC. The pure compounds were obtained either by direct crystallisation or after further purification by column chromatographies. Crystallisation of combined fraction 6–7 eluted with cyclohexane - EtOAc (19:1), gave lupeol (**5**) (10 g); the combined fractions (18–23) eluted with cyclohexane–EtOAc (18:2) gave tovophyllin A (**3**) (15 mg); the combined fraction (24–46) eluted with cyclohexane–EtOAc (17:3) afforded stigmasterol (**4**) (1.2 g) and garciniafuran (**6**) (1.5 mg).

Fraction B, treated in the same manner as A, yielded allanxanthone B(**1**) (14 mg) and rubraxanthone (**2**) (300 mg).

3.4. Antimicrobial assay

The crude extract and purified compounds from the stem bark of *A. monticola* were tested against micro-organisms, *S. aureus* (ATCC 6538), *C. tropicalis* (ATCC 66029) and *V. anguillarum* (ATCC 19264). The qualitative antimicrobial assay employed, was a classical discs diffusion procedure using Mueller Hinton agar (DIFCO). Paper discs were impregnated with 20 μL of DMSO solution of 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 positive control. The plates with micro-organisms was incubated at 37 °C for 24 h for *S. aureus* and at 27 °C during 48 h for *V. anguillarum* and *C. tropicalis*. The diameter of inhibition zone around each disc were measured and recorded at the end of the incubating period.

Allanxanthone B (***1***). Yellow powder, m.p. 158–160 °C, +ESI-TOF-MS *m/z* 464,2135 ($C_{28}H_{32}06$ require *m/z* 464,2190) msms *m/z* (rel. int.): 407(68); 285 (84); 257 (95); 229 (28). UV λ^{Et0H} max nm: 242, 253, 310, 348; (+NaOH): 265, 296, 357; (+$AlCl_3$): 260, 341, 390; (+NaOAc): 290, 354. For ^{1}H and ^{13}C NMR, see Table 1.

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