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A prenylated xanthone from *Allanblackia floribunda*

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Abstract

A new prenylated xanthone, named allanxanthone A, was isolated from the stem bark of *Allanblackia floribunda* in addition to known compounds, 1,5-dihydoxyxanthone, 1,5,6-trihydroxy-3,7-dimethoxyxanthone, stigmasterol and stigmasteryl-3-*O*-β-D-glucopyranoside. The structure of the new compound was assigned as 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-4-(1,1-dimethylprop-2-enyl) xanthone, by means of spectroscopic analysis. The ^{13}C NMR spectral data of 1,5-dihydroxyxanthone is reported here for the first time as well as the in vitro cytotoxic activity of xanthone metabolites against the KB cell line.

Keywords: *Allanblackia floribunda*; Guttiferae; Stem bark; Prenyl xanthone; Allanxanthone A; 1,5-Dihydroxyxanthone; 1,5,6-Trihydroxy-3,7-dimethoxyxanthone; Stigmasterol; Stigmasteryl-3-*O*-β-D-glucopyranoside; Cytotoxic activity

1. Introduction

Allanblackia floribunda Oliver, which belongs to the plant family Guttiferae (subfamily: Clusiodeae) is widely distributed in the coasts of West Africa. Extracts of leaves, stem bark and root bark of this plant, alone or combined with other plants, are widely used in many African countries including Cameroon, Gabon and Congo to treat certain human ailments such as upper respiratory tract infections, dysentery, diarrhoea, and toothache (Raponda-Waker and Sillans, 1961). Previous phytochemical studies of the hearthwood of *Allanblackia floribunda* (Locksley and Murray, 1971) and root bark of *Allanblackia stuhlmannii* (Blunt et al., 1999) reported the isolation of benzophenones (hydrocotin, guttiferone F), xanthones (1,3,5-trihydroxyxanthone and 4,5-dihydro-1,6,7-trihydroxy-4′,4′,5-trimethoxyfurano-[2′,3:3,4] xanthone) and biflavonoids (morelloflavone and volkensiflavone). Some of these exhibit a wide range of biological and pharmacological activities, e.g. cytotoxic, antiinflammatory, 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 continuing search for biologically active compounds from Cameroonian plants, we have examined the stem bark of *Allanblackia floribunda* Oliver and now report the isolation, structural elucidation and activity of a new polyoxygenated xanthone along with four known compounds.

2. Results and discussion

The dried and ground stem bark of *Allanblackia floribunda* was extracted at room temperature with a mixture of CH_2Cl_2–MeOH (1:1) and the extract concentrated to dryness to give a sticky residue. Bioassay-directed fractionation of this residue by silica gel column chromatography monitored by cytotoxicity towards the KB cell line, afforded a series of active fractions from which were isolated a novel xanthone, allanxanthone A (**1**) and four known compounds (**2**)–(**5**) which were identified by comparison of their spectral data with the literature values as 1,5-dihydroxyxanthone (**2**) (Jackson et al., 1967, 1968), 1,5,6-trihydroxy-3,7-dimethoxyxanthone (**3**) (Biswas et al., 1977), stigmasterol (**4**) (Diakow et al., 1978) and stigmasteryl-3-*O*-β-D-glucopyranoside (**5**) (Berry et al., 1962; Claeys et al., 1992; Jares et al., 1992), respectively. Although,

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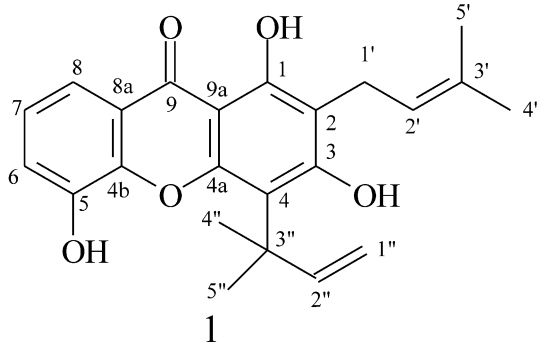
1,5-dihydroxyxanthone (**2**) is a known compound which has been previously reported from other genera belonging to the family Guttiferae such as *Calophyllum* (Jackson et al., 1967), *Garcinia* (Jackson et al., 1968), *Mammea* and *Mesua* (Gunasekera et al., 1975), it is the first time that it has been isolated from *Allanblackia* species. Its ^{13}C NMR spectral data, which have been assigned on the basis of Jmod, HSQC and HMBC experiments, are reported here for the first time (see Experimental).



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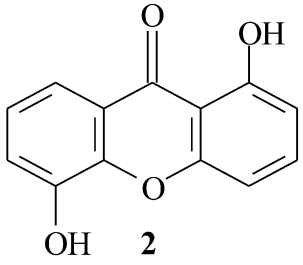

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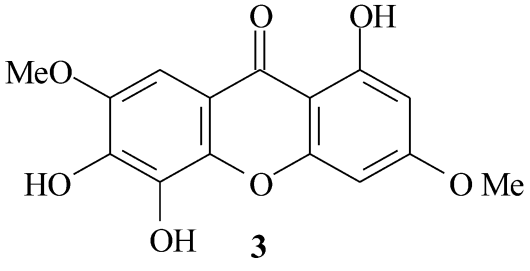

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Allanxanthone A (**1**) was obtained as yellow crystals, mp 178–180 °C, and reacted positively to $FeCl_3$ reagent. Its molecular formula, $C_{23}H_{24}O_5$, was established from HREI–mass spectrometry (*m*/*z* 380.1622; calc. for $C_{23}H_{24}O_5$: 380.1624) and corresponds to 12 °C of unsaturation. The broad band decoupled ^{13}C NMR spectrum of compound (**1**) showed 22 carbon signals which were sorted by Jmod and HSQC techniques as four methyls, two methylenes, five methines and 12 quaternary carbons including a carbon (δ 182.2) of a carbonyl group, five oxygenated sp^2 carbons, five sp^2 and one sp^3 carbons. The IR spectrum of (**1**) exhibited strong vibration bands due to free hydroxyl groups (3460 cm^{-1}), chelated hydroxyl group (3265 cm^{-1}) and a conjugated carbonyl group (1642 cm^{-1}). The UV absorption bands (205, 282 and 340 nm) indicated 1 to be an hydroxyl xanthone derivative (Jackson et al., 1966; Locksley et al., 1966). In the ^1H NMR spectrum, a chelated hydroxyl group at δ 13.65 and two D_2O exchangeable broad two-proton singlets at δ 8.50, due to two free phenolic hydroxyl groups, were observed. The ^1H NMR spectrum of (**1**) also showed the presence of an ABX spin system at δ 7.68 (1H, *dd*, $J=8.1$ and 1.7 Hz), 7.34 (1H, *dd*, $J=8.1$ and 1.7 Hz) and 7.21 (1H, *t*, $J=8.1$ Hz) assignable to a 1,2,3-trisubstituted benzene ring. The presence of a γ,γ-dimethylallyl group was inferred from ^1H and ^{13}C NMR spectra which displayed one one-proton triplet at δ_H 5.25 (1H, *t*, $J=6.8$ Hz) /δ_C 123.1, one two-protons doublet at δ 3.35 (2H, *d*, $J=6.8$ Hz)/22.3 and two sharp three-protons singlets at δ 1.79/26.2 and 1.65/17.9 and δ_C 132.2. Furthermore, the set of signals consisting of one one-proton double doublet at δ 6.60 (1H, dd, $J=16.0$ and 9.3 Hz), two one-proton double doublets at δ 5.45 (1H, *dd*, $J=16.0$ and 1.1 Hz), 5.30 (1H, *dd*, $J=9.3$ and 1.1 Hz) and one six-protons singlet at δ 1.80 established the presence of a 1,1-dimethylallyl substituent. In the HMBC spectrum (Fig. 1), one of the ABX system protons, which appeared at δ 7.68, gave cross peaks with the carbonyl carbon (δ 182.2), one tertiary CH aromatic carbon δ 120.7) and one aromatic carbon with a *O*-function (δ 147.2 or 147.4). These results proved that, one of the aromatic protons (δ 7.68) was located at a *peri*-position (C-8) to the carbonyl group (Cardona et al., 1986; Goh et al., 1992) and that the C-5 position was substituted by OH group. Hence, the aromatic protons which showed a double doublet signal at δ 7.34 and a triplet signal at δ 7.21 were attributed to H-6 and H-7, respectively.

The above results were fully supported, on one hand, by COSY spectrum and on the other hand, by the EI–mass spectrum which showed an important ion peak at

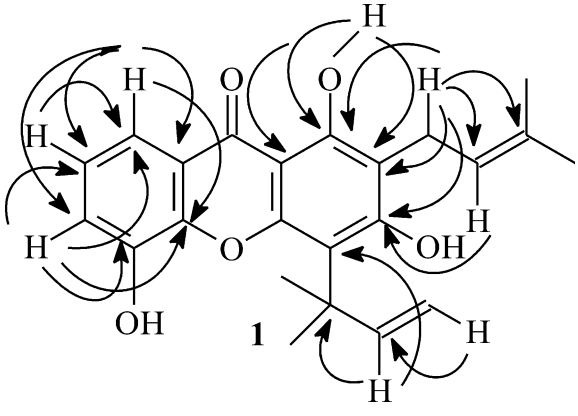

Fig. 1. Significant HMBC correlations for compound **1**.

m/z 269 $[M-111]^+$ corresponding to the loss of two C_4 units, C_4H_7 and C_4H_8, from the molecular ion, characteristic of the presence of two *gem*-dimethylallyl groups. The EI–mass spectrum also showed two important ion fragments at *m*/*z* 189 and 136 resulting from retro Diels-Alder fragmentation of ion peak at *m*/*z* 325 $[M\text{-}C_4H_7]^+$ and which confirmed that, the ring A (*m*/*z* 189) contains one hydroxyl group and two *gem*-dimethylallyl moieties while the ring B (*m*/*z* 136) bears only one hydroxyl group. Therefore, it remained to be established unambiguously the position of the two *gem*-dimethylallyl groups on ring A. This was deduced from the long-range heteronuclear correlation experiment (HMBC) as follows. In the HMBC spectrum, the chelated OH proton (δ 13.65) caused cross peaks with two aromatic carbons at δ 104.0 and 112.1, indicating that the C-2 position was not substituted by a group possessing *O*-function. Furthermore, the C-2 carbon (δ 112.1) was correlated to the allylic protons (δ 3.35) of the 3,3-dimethylallyl group which in turn also showed cross peaks with two oxygenated aromatic carbons at δ160.0 (C-1) and 162.1 (C-3). This finding clearly indicated that, the γ,γ-dimethylallyl group was located at the C-2 and the OH group at C-3 position. Thus, the α,α-dimethylallyl moiety was assigned to C-4. On the basis of the above results, the structure of allaxanthone A (**1**) was determined to be 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-4-(3-methylprop-2-enyl)xanthone, which was supported by other long-range correlations in the HMBC spectrum as shown in Fig. 1.

The three xanthone metabolites (**1**)–(**3**) isolated here were screened for in vitro cytotoxicity against the KB cancer cell line (derived from a nasopharyngeal carcinoma of human origin) as described in Experimental. They were all found to be moderately cytotoxic with EC_{50} values of 1.5 (**1**), 3.3 (**2**) and 2.5 (**3**) μg ml^{-1}, respectively.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Kofler hot plate and are uncorrected. IR spectra (KBr) were recorded on a Perkin–Elmer spectrophotometer. UV spectra were obtained on a Shimadzu-265 spectrophotometer. NMR spectra were run on a Bruker spectrometer equipped with a 5 mm ^1H and ^{13}C probe operating at 300.1 and 75.5 MHz, respectively, with TMS as internal standard.

3.2. Plant material

The stem bark of *Allanblackia floribunda* Oliver, was collected in September 1999 at Kribi in South province of Cameroon. The sample was identified by Dr L. Zapfack, of Botanic Department, University of Yaounde I, where a voucher specimen is on deposit.

3.3. Extraction and isolation

Air-dried, powdered stem bark of *Allanblackia floribunda* (5.0 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 (300.0 g). This residue was then subjected to flash column chromatography over silica gel (230–400 mesh) eluted with *n*-hexanes–EtOAc (1:1), EtOAc and EtOAc–MeOH (7:3) to give three main fractions labelled A, B and C, respectively. Only the pure EtOAc fraction (fraction B, 50.0 g) showed a significant in vitro cytotoxic activity against the KB cell line (EC_{50}, 20 μg ml^{-1}). This fraction was then subjected to column chromatography over Si gel (70–230 mesh) eluted with a gradient of increasing polarity with n-hexanes–EtOAc resulting in the collection of 50 fractions (F_1–F_{50}) of ca. 250 ml per fraction which were combined on the basis of TLC analysis. Fractions F_{10}–F_{15} (8.0 g), eluted with the mixture of *n*-hexane–EtOAc (17:3), were rechromatographed over Si gel using the mixture of *n*-hexane-EtOAc (9:1) as eluent, to afford compound (**4**) (300.0 mg). Fractions F_{20}–F_{30}, eluted with a mixture of *n*-hexane–EtOAc (3:2), were concentrated to dryness and the residue obtained (6 g) was subjected to column chromatography over Si gel, eluted with a mixture of *n*-hexane–EtOAc (13:7), to give compounds (**3**) (250.0 mg) and (**2**) (185.0 mg). Fractions F_{35}–F_{45}, eluted with a mixture of *n*-hexane–EtOAc (1:4), were also concentrated to dryness. The brown residue obtained (5.67 g), was further subjected to repeated CC over Si gel eluted with a mixture of *n*-hexane–EtOAc to afford, compounds (**1**) (180.0 mg) and (**5**) (225.0 mg), respectively.

3.4. Allanxanthone A (*1*)

Yellow crystals, mp 178–180 °C; UV λ_{max} (MeOH) nm (log ε): 205 (3.75), 282 (4.41), 340 (3.01); IR ν_{max} (KBr) cm^{-1}: 3460, 3265, 1642, 1604, 1464, 1280, 1110; ^1H NMR (300.1 MHz, acetone-d_6): δ 1.65 (3H, *s*, H-5′), 1.79 (3H, *s*, H-4′), 1.80 (6H, *s*, H-4″, H-5″), 3.35 (2H, *d*, $J=6.8$ Hz, H-1′), 5.25 (1H, *t*, $J=6.8$ Hz, H-2′), 5.30 (1H, *dd*, $J=9.3$ and 1.1 Hz, H-1″, *cis*), 5.45 (1H, *dd*, $J=16.0$ and 1.1 Hz, H-1″, *trans*), 6.60 (1H, *dd*, $J=16.0$ and 9.3 Hz, H-2″), 7.21 (1H, *t*, $J=8.1$ Hz, H-7), 7.34 (1H, *dd*, $J=8.1$ and 1.7 Hz, H-6), 7.68 (1H, *dd*, $J=8.1$ and 1.7 Hz, H-8), 8.50 (2H, *br*, OH-3 and OH-5, exch. with D_2O), 13.65 (1H, *s*, 1-OH, exch. with D_2O); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 161.0 (*s*, C-1), 112.1 (*s*, C-2), 159.0 (*s*, C-3), 111.9 (*s*, C-4), 144.2 (*s*, C-4a), 147.3 (*s*, C-4b), 152.0 (*s*, C-5), 120.7 (*d*, C-6), 124.6 (*d*, C-7), 116.0 (*d*, C-8), 122.0 (*s*, C-8a), 182.0 (*s*, C-9), 108.0 (*s*, C-9a), 22.3 (*t*, C-1′), 123.1 (*d*, C-2′), 134.1 (*s*, C-3′), 25.8 (*q*,

C-4′), 17.9 (*q*, C-5′), 112.4 (*t*, C-1″), 151.2 (*s*, C-2″), 42.0 (*s*, C-3″), 28.5 (*q*, C-4″ and C-5″); ^{1}H–^{13}C HMBC correlations (300.1 MHz, acetone-d_6): see Fig. 1; HREIMS $[M]^+$ *m/z* 380.1622 (alc. for $C_{23}H_{24}O_5$, 380.1624); EIMS *m/z* (rel. int.) 380 (87), 365 (70, M^+-Me), 337 (74, $M^+-C_3H_7$), 365 (92, $M^+-C_4H_7$), 310 (67, $M^+-[Me_+C_4H_7]$), 309 (100, $M^+-[Me+C_4H_8]$), 297 (47), 295 (56), 294 (60), 281 (62), 283 (51), 269 (78, $M^+-[C_4H_8+C_4H_7]$), 265 (16), 241 (10), 213 (10), 189 (31), 164 (10), 154 (11), 137 (21), 136 (35), 115 (18), 91 (15), 56 (18), 55 (20), 43 (75).

3.5. Assay for cytotoxic activity

Cytotoxicity of the crude extracts and the purified compounds against human epidermoid carcinoma (KB) of the nasopharynx cancer cell line were evaluated using the protocol described in the literature by Likhitwitayawuid et al. (1993).

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