Afzeliixanthones A and B, Two New Prenylated Xanthones from *Garcinia afzelii* ENGL. (Guttiferae)

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Two new prenylated xanthones, afzeliixanthones A (1) and B (2), together with three known xanthones (3—5) and two phytosterols, β-sitosterol and stigmasterol, were isolated from the CH_2Cl_2/MeOH (1 : 1) extract of the stem bark of *Garcinia afzelii* ENGL. collected in the South West Province of Cameroon. Structures were mainly established using one and two-dimensional NMR and mass spectroscopies. The antioxidant activities of the crude extracts as well as the new compounds (1) and (2) were evaluated.

Key words *Garcinia afzelii*; Guttiferae; xanthone

Plants of the Guttiferae family are used worldwide in traditional medicine for the treatment of diseases and are well known to be rich sources of xanthones, biflavonoids and benzophenones.[1—3] In particular, for trees belonging to thegenus *Garcinia*, it has been found that approximately half of the species studied contain xanthones which are of chemotaxonomic interest.[3—7]

This has led us to study *G. afzelii* ENGL., a small tree widely distributed in Cameroon and Ghana, whose leaves and flowers are used for their antibacterial properties. Chewing sticks are used for dental hygiene by most people in the southern region of Ghana.[8,9]

In this paper we report the isolation and structure elucidation of two new prenylated xanthones (**1**) and (**2**), along with three known xanthones 1,5-dihydroxyxanthone (**3**),[10] 1,7-dihydroxyxanthone (**4**),[11] 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)xanthone (**5**)[12] and two phytosterols, β-sitosterol and stigmasterol,[13] and their antioxidant activities.

Results and Discussion

Extensive silica gel column chromatography of the CH_2Cl_2/MeOH (1 : 1) extract of the stem bark of *G. afzelii* using solvents of increasing polarity gave *n*-hexane, ethyl acetate (EtOAc) and methanol (MeOH) soluble fractions. The separation of these fractions with silica (Si) gel open-column chromatography, and repeated preparative TLC chromatography led to the isolation of **1**—**5**, together with a mixture of β-sitosterol and stigmasterol.

Afzeliixanthone A (**1**) was isolated as a yellow oil. It gave a dark green colour with methanolic ferric chloride, indicating that it was phenolic compound. Its molecular formula was determined to be $C_{19}H_{18}O_6$ by HR-EI-MS (*m*/*z* 342.1058, M^+; Calcd for $C_{19}H_{18}O_6$, 342.1103). The IR spectrum showed absorption bands ν_{max} at 3400 (hydroxyls groups) and 1672 cm^{-1} (conjugated ketone functional group) and the UV spectrum showed maxima λ_{max} MeOH ($\log \varepsilon$) at 221 (2.01), 253 (2.69) and 332 nm (1.29), typical of a hydroxylated xanthone.[14]

The ^{1}H- (Table 1) and ^{13}C-NMR spectra (Table 1) aided by distorsionless enhancement by polarization transfer (DEPT) and heteronuclear single quantum correlation (HSQC) experiments disclosed the presence of 19 carbons that were assignable to one chelated carbonyl, 10 quaternary carbons, four methine, one methylene and three methyl carbons. The analysis of this data indicated the presence of a C-8-OH proton intramoleculary H-bonded to the C-9 carbonyl oxygen atom of a xanthone nucleus [δ_H 13.50 (s, 1H, 8-OH), δ_C 162.2 (s, C-8)], a pair of *meta*-coupled aromatic protons [δ_H 6.32 (d, J=2.3 Hz, 1H, H-7), δ_C 101.4 (d, C-7); δ_H 6.20 (d, J=2.3 Hz, 1H, H-5), δ_C 97.6 (d, C-5)] on the ring A, an aromatic proton [δ_H 6.85 (s, 1H, H-3), δ_C 100.5 (d, C-3)] as well as the presence of one prenyl group [δ_H 3.60 (d, J=6.3 Hz, 2H, 2H-1′), δ_C 24.9 (t, C-1′); δ_H 5.20 (t, J=6.2 Hz, 1H, H-2′), δ_C 124.1 (d, C-2′); δ_H 1.68 (s, 3H, 3H-4′), δ_C 25.5 (q, C-4′); δ_H 1.76 (s, 3H, 3H-5′), δ_C 18.3, (q, C-5′)], and a methoxyl group [δ_H 3.80 (s, 3H, 1-OCH_3), δ_C 62.5]. The substitution pattern of ring A, was determined using the heteronuclear multiple bond connectivity (HMBC) correlations (Table 1) between the proton resonance at δ_H 13.50 and the two carbons C-7 (δ_C 101.4) and C-8a (δ_C 108.2); and, in addition, by the correlation between the H-7 and C-8 (δ_C 162.2), C-8a (δ_C 108.2), C-6 (δ_C 164.4) and C-5 (δ_C 97.6) resonances; and between the H-5 and C-10a (δ_C 158.7), C-6 (δ_C 164.4) and C-7 (δ_C 101.4) resonances. The position of the prenyl group was deduced to be at C-2 (δ_C 110.2) on the ring B by the HMBC correlations (Table 1) between the H-1′ and C-1 (δ_C 155.1) and C-3 (δ_C 100.5) resonances; and the H-3 and C-4 (δ_C 141.4), C-4a (δ_C 145.2), C-2 (δ_C 110.2), C-1 (δ_C 155.1) and C-1′ (δ_C 24.9) resonances and by the nuclear Overhausser effect spectroscopy (NOESY) correlations (Fig. 1) between the three proton resonance of the methoxyl group at the C-1 position and the two H-1′ protons of the prenyl group. Finally the two remaining hydroxyl groups whose proton resonances occurred at δ_H 9.99 [(s, 1H, 4-O-H), δ_C 141.4, (s, C-4)] and at δ_H 9.75 [(s, 1H, 6-O-H), δ_C 164.4, (s, C-6)] were placed at C-4 and C-6, respectively, by

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) Assignments for Compounds (**1**) and (**2**) in Aceton-d_6

No.	Compound (**1**)			Compound (**2**)		
	^{1}H	^{13}C	HMBC	^{1}H	^{13}C	HMBC
1	—	155.1, s	—	—	159.5, s	
2	—	110.2, s	—	—	110.3, s	
3	6.85 (1H, s)	100.5, d	C-1, 2, 4, 4a, 1′	6.20 (1H, s)	100.5, d	C-1, 4a, 1′
4	—	141.4, s	—	—	156.0, s	
4a	—	145.2, s	—	—	150.7, s	
5	6.20 (1H, d, 2.3)	97.6, d	C-7, 8a	6.88 (1H, d, 8.1)	119.7, d	C-7, 8a, 6
6	—	164.4, s	—	7.27 (1H, dd, 2.0, 8.3)	128.4, d	C-10a, 8, 7
7	6.32 (1H, d, 2.3)	101.4, d	C-5, 6, 8a, 8	—	146.2, s	
8	—	162.2, s		7.50 (1H, d, 2.0)	114.1, d	C-9, 10a, 6
8a	—	108.2, s	—	—	117.2, s	
9	—	181.5, s	—	—	179.6, s	
9a	—	112.2, s	—	—	112.3, s	
10a	—	158.7, s	—	—	145.3, s	
1′	3.60 (2H, d, 6.3)	24.9, t	C-1, 3, 2′, 3′	6.50 (1H, d, 10.0)	115.1, d	C-1, 3, 3′, 2
2′	5.20 (1H, t, 6.2)	124.1, d	C-1′, 2, 4′, 5′	5.65 (1H, d, 10.0)	126.0, d	C-4′, 5′, 2
3′	—	130.1, s	—	—	74.0, s	
4′	1.68 (3H, s)	25.5, s	C-2′, 3′, 5′	1.50 (3H, s)	14.3, s	C-2′, 5′
5′	1.76 (3H, s)	18.3, s	C-2′, 3′ 4′	1.50 (3H, s)	14.3, s	C-4′, 2′
1-OMe	3.80 (3H, s)	62.5, s	C-1	3.90 (3H, s)	63.2, s	C-1
7-OMe	—	—	—	3.75 (3H, s)	56.2 s	C-7
4-OH	9.99 (1H, s)	—	C-3, 4a	9.99 (1H, s)	—	C-3, 4a
6-OH	9.75 (1H, s)	—	C-5, 7	—	—	—
8-OH	13.50 (1H, s)	—	C-7, 8a	—	—	—

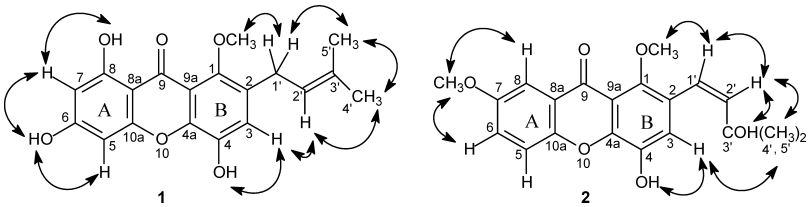

Fig. 1. Selected NOESY Correlations of Compounds **1** and **2**

the following NOESY correlations (Fig. 1), H-7 (δ_H 6.32)/8-OH; H-7 (δ_H 6.32)/6-OH; H-5 (δ_H 6.20)/6-OH and H-3 (δ_H 6.20)/4-O-H, and the remaining two oxygen bearing quaternary carbons were assigned to the C-4a and C-10a positions (Table 1). Analysis of the data suggested the structure of **1** to be 4,6,8-trihydroxy-2-(3-methylbut-2-enyl)-1-methoxyxanthone which was confirmed by comparison of the NMR data with those of the related compounds, cudraxanthones E and F.[15)]

Afzeliixanthone B (**2**), obtained as yellow oil, had a molecular formula $C_{20}H_{20}O_6$ based on the results of HR-EI-MS (*m/z* 356.1242, M^+; Calcd for $C_{20}H_{20}O_6$, 356.1259). The IR spectrum showed absorption bands for hydroxyl and conjugated ketone (1664 cm^{-1}) functional groups. The UV spectrum of **2** exhibited bands very similar to those of **1**. The ^{1}H- and ^{13}C-NMR spectra (Table 1) aided by DEPT and HSQC experiments disclosed the presence of 20 carbons that were arranged similarly to those of **1**, except for the replacement of the *meta*-coupled aromatic protons with three aromatic protons of the ABX-type [δ_H 7.50 (d, J=2.0 Hz, 1H, H-8), δ_C 114.1 (d, C-8); δ_H 7.27 (dd, J=2.0, 8.3 Hz,1H, H-6), δ_C 128.4 (d, C-6); δ_H 6.88 (d, J=8.1 Hz, 1H, H-5), δ_C 119.7 (d, C-5)] and the replacement of the prenyl group with a (3-hydroxy-3-methylbut-1(*Z*)-enyl) group [δ_H 6.50 (d, J=10.0 Hz, 1H, H-1′), δ_C 115.1 (d, C-1′); δ_H 5.65 (d, J=10.0 Hz, 1H, H-2′), δ_C 126.0 (d, C-2′); δ_H 1.50 (s, 6H, 3H-4′, 3H-5′), δ_C 14.3 (q, C-4′, C-5′)]. The *Z*-configuration of the double bond was shown by the coupling constant of 10.0 Hz between H-1′ and H-2′.[16)] The location of the (3-hydroxy-3-methylbut-1(*Z*)-enyl) group was deduced to be at C-2 by the correlations seen in the NOESY spectrum (Fig. 1) between the protons of the methoxyl group at C-1 and the doublet of one proton H-1′ (δ_H 6.50), and by the HMBC correlations (Table 1) between the H-1′ and C-1 (δ_C 159.5), C-2 (δ_C 110.3) and

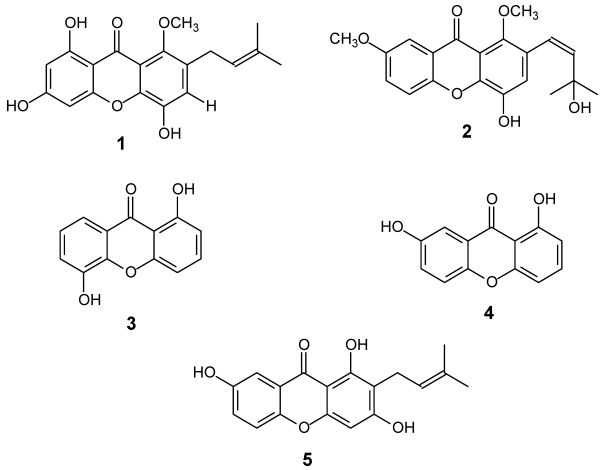

Fig. 2. Structures of Compounds **1**—**5**

Table 2. Free Radical-Scavenging Activity of CH_2Cl_2/MeOH (1 : 1) Extract and Compounds **1** and **2**

	Item tested			Reference	
	CH_2Cl_2/MeOH (1 : 1) extract	**1**	**2**	BHA[a)]	α-Tocopherol
IC_{50} (μg/100 ml)	20.5	17.7	14.0	13.5	13.8

a) BHA: 2,6-di-*tert*-butyl-4-hydroxy-anisol. DPPH: α,α-diphenyl-β-picrylhydrazyl.

C-3 (δ_C 100.5) resonances. Finally one additional methoxyl group [δ_H 3.75 (s, 3H, 7-O-CH_3), δ_C 56.2 (q)] was located at C-7 on ring B in good agreement with the HMBC long range correlations (Table 1) ($^3J_{CH}$) between H-8 and C-9 (δ_C 179.6) and C-6 (δ_C 128.4) resonances; H-6 and C-10a (δ_C 145.3) and C-8 (δ_C 114.1) resonances and finally between the H-5 and C-4a (δ_C 150.7) and C-7 (δ_C 146.2) resonances.

Therefore **2** was identified as 4-hydroxy-2-([*Z*]-3-hydroxy-3-methyl-but-1(enyl)-1,7-dimethoxyxanthone.

The anti-oxidant activity of the CH_2Cl_2/MeOH (1/1) extract of the stem bark of *G. afzelii* and compounds **1** and **2** were investigated using the DPPH free radical scavenging method on TLC. The CH_2Cl_2/MeOH (1/1) extract was found to exhibit significant anti-oxidant effects, based on the scavenging of the stable DPPH free radical (Table 2). The anti-oxidative properties of compounds **1** and **2** were also assessed. The tested compounds showed high antioxidant capacity in good agreement with the fact that phenolic compounds are known to be antioxidants with excellent hydrogen or electron donor capacity.[17)] As with other *Garcinia* species, such as *G. cambodgia* and *G. virgata*, *G. afzelii* can be exploited and used in cosmetic preparations since the crude extract from *G. afzelii* is a free radical scavenger.

Experimental

General Procedure Melting points were determined on a Mettler FP 61 apparatus and are uncorrected. UV spectra were obtained on a Spectroline type spectrophotometer. NMR spectra were run on a Varian Unity-Inova Spectrometer instrument equipped with a 5 mm ^{1}H and ^{13}C probe operating at 400 and 100 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 column chromatography and column chromatography respectively, while precoated aluminium sheets silica gel 60 F_{254} (Merck) were used for TLC with a mixture of cyclohexane–EtOAc as eluents ; spot were visualised by UV (254 nm) and (365 nm) or by MeOH–H_2SO_4.

Plant Material The stem bark of *Garcinia afzelii* Engl. was collected in November 2003 at Bolo Mebeka (Kumba) in South West Province of Cameroon. The authentication was achieved by Mr Nole of University of Yaounde I, by comparison with the specimen at National Herbarium of Yaounde (Cameroon) and where a Voucher specimen is on deposit.

Extraction and Isolation Air dried powdered stem bark of *G. afzelii* (5.0 kg) was extracted with a mixture of CH_2Cl_2/MeOH (1/1) at room temperature for 48 h. The extract was filtered and concentrated under reduced pressure to yield a brown viscous extract (62.0 g). This extract was subjected to flash column chromatography over silica gel (70—230 mesh, Merck) and eluted using *n*-hexane/EtOAc/MeOH mixtures of increasing polarity. A total of 80 sub-fractions (*ca.* 300 ml each) were collected and combined on the basis of TLC analysis leading to five main fractions A (12.0 g) , B (7.0 g), C (5.0 g), D (2.0 g), and E (15.0 g), respectively, sub-fractions 1—15 (eluting with *n*-hexane : EtOAc = 3 : 1), sub-fractions 16—30 (eluted with *n*-hexane : EtOAc = 1 : 1), sub-fractions 31—45 (eluted with *n*-hexane : EtOAc = 1 : 4), sub-fractions 46—60 (eluted with EtOAc : MeOH = 19 : 1), and sub-fractions 61—80 (eluted with MeOH). The main fraction A was re-chromatographed on silica gel column with *n*-hexane : EtOAc gradient. A total of 20 fractions of *ca.* 100 ml each were collected and combined on the basis of TLC. Fractions 1—10 eluted with hexane : EtOAc = 9 : 1 yielding 1,5-dihydroxyxanthone (**3**) (3.5 mg). Fractions 11—20 eluted with hexane : EtOAc = 17 : 3 yielding β-sistosterol (7.0 mg) and stigmasterol (11.0 mg). The main fraction B was chromatographed on a silica gel column with a *n*-hexane : EtOAc gradient. A total of 25 fractions of *ca.* 100 ml each were collected and combined on the basis of TLC. Fractions 1—10 eluted with *n*-hexane : EtOAc = 17 : 3 yielding 1,7-dihydroxyxanthone (**4**) (3.0 mg) and fractions 11—25 eluted with *n*-hexane : EtOAc = 7 : 3 yielding 1,3,7-trihydroxy-2-prenylxanthone (**5**) (5.0 mg).

The main fraction C was re-chromatographed over silica gel using a *n*-hexane : EtOAc gradient. A total of 30 fractions of *ca.* 100 ml each were collected and combined on the basis of TLC. Fractions 1—10 eluted with *n*-hexane : EtOAc = 3 : 1 yielding 4,6,8-trihydroxy-2-(3-methylbut-2-enyl)-1-methoxyxanthone (**1**) (3.0 mg) and fractions 11—20 eluted with *n*-hexane : EtOAc = 1 : 1 yielding 4-hydroxy-2-([*Z*]-3-hydroxy-3-methylbut-1-enyl)-1,7-dimethoxyxanthone (**2**) (3.5 mg).

Finally, main fractions D and E were found to contain mainly very polar compounds.

Afzeliixanthone A (**1**): Yellow oil, UV λ_{max} (MeOH) nm (log ε): 204 (4.24), 235 (4.21), 245 (4.18), 290 (3.98), 322 (3.82), 402 (3.51) nm. IR cm^{-1}: 3400, 2920, 1672, 1600, 1580. ^{1}H- and ^{13}C-NMR see Table 1. HR-EI-MS *m*/*z* 342.1058 (Calcd for $C_{19}H_{18}O_6$: 342.1103).

Afzeliixanthone B (**2**): Yellow oil, UV λ_{max} (MeOH) nm (log ε) 204 (4.24), 235 (4.21), 249 (4.20), 292 (3.99), 322 (3.82), 405 (3.51). IR cm^{-1}: 3400, 2920, 1664, 1600. ^{1}H- and ^{13}C-NMR see Table 1; HR-EI-MS *m*/*z* 356.1242 (Calcd for $C_{20}H_{20}O_6$, 356.1259).

Determination of the Radical Scavenging Activity The reaction mixture containing 5 μl of test sample (1 mM in DMSO) and 95 μl of DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma, 300 μM) in ethanol was placed in a 96-well micro titer plate (Molecular Devices, U.S.A.) and incubated at 37 °C for 30 min. The absorbance was measured at 515 nm. Percent radical scavenging activity was determined by comparison with a DMSO containing control (Table 4). IC_{50} values represent concentration of compounds required to scavenge 50% of DPPH radicals. BHA (3-*t*-butyl-4-hydroxyanisole) was used as a positive control. All the chemicals used were of analytical grade (Sigma, U.S.A.).

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