C-Glucoside xanthone from the stem bark extract of *Bersama engleriana*

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ABSTRACT

Background: The genus *Bersama* belongs to the Melianthaceae family and comprises of four species (*B. swinnyi*, *B. yangambiensis*, *B. abyssinica*, and *B. engleriana*) all of which are very high trees; the latter two detected species are found in Cameroon. Previous phytochemical investigation on *B. yangambiensis*, *B. swinnyi*, and *B. abyssinica* led to the isolation of triterpenes, saponins, flavonoids, and xanthones. **Method:** The stem bark of *B. engleriana* were collected in the village, Baham near Bafoussam city, Cameroon in August 2003 and identifi ed by Dr. Onana National Herbaruim, Yaoundé, Cameroon. The air dried and powdered stem bark of *B. engleriana* (1 kg) was extracted at room temperature with CH2Cl2–MeOH (1:1) 5 L for 48 hours. The mixture of the solvent was removed by evaporation to yield 200 g of crude extract. The latter was then dissolved in CH2Cl2 to give the CH2Cl2 soluble fraction of 5 g and a remaining gum of 195 g. Part of the remaining gum (22 g) was dissolved in water and extracted four times with butanol to give 12 g of red oil; which was then separated by paper chromatography, with butanol-acetic acid-water (4:1:5), to give 3 g of orange gum; purification was carried out on HPLC with MeOH (100%) to yield 2 g of mangiferin (1) as red oil. The CH2Cl2 soluble extract was eluted on silica gel n-hexane-CH2Cl2 gradient ratio and Sephadex LH-20 (n-hexane -CH2Cl2 -MeOH, (7:4:0.5) to afford compounds swinniol (2), Δ4-stigmaster-3β-ol (3), 4-methylstigmaster-5,23-dien-3β-ol(4). **Results:** Herein, we carried out a phytochemical study of the stem bark of *B. engleriana*, and we report herein the isolation and structural elucidation of mangiferin, in addition to three triterpenes, previously reported from other species of the genus.[3,5] The assignment of the signals of mangiferin was determined using 1H, 13C-NMR, and 2D-NMR spectral data (HMQC, COSY, HMBC). The terpenoids were identifi ed by comparison of their 1H and 13C-NMR spectra with the literature data. Fractionation of the CH2Cl2–MeOH (1:1) extract of the stem bark of *B. engleriana* Guike gave mangiferin (1), in addition to three previously reported triterpenes, swinniol (2), Δ4-stigmaster-3β-ol (3), and 4-methylstigmaster-5,23-dien-3-β-ol (4). **Conclusions:** A chemical investigation of the CH2Cl2-MeOH extract of the stem bark of *Bersama engleriana* afforded a xanthone C-glucoside (mangiferin) and fi rst isolation of three terpenoids from this species: swinniol (2), Δ4-stigmaster-3β-ol (3), and 4-methylstigmaster-5,23-dien-3-β-ol (4). The complete 1H and 13C chemical shift assignments of mangiferin were determined using 1D and 2D NMR spectroscopic data (COSY, HMQC, HMBC, DEPT). The structures of the terpenoids were determined from their 1H and 13C NMR data and compared with the literature data.

Key words: *Bersama engleriana*, melianthaceae, terpenoids, xanthone, mangiferin

INTRODUCTION

The genus *Bersama* belongs to the Melianthaceae family and comprises of four species (*B. swinnyi*, *B. yangambiensis*, *B. abyssinica,* and *B. engleriana*) all of which are very high trees; the latter two detected species are found in Cameroon.[1] *B. abissinica* is used in the form of a decoction for stomach ache, while *B. engleriana* is used to treat syphilis, injuries, and fever. The pharmacological activity of *B. yangambiensis* was studied,[2] the antitumor action of *B. abyssinica* is known[3] and the anti-HIV activity of the Ethiopian species has been studied.[4] Previous phytochemical investigation on *B. yangambiensis*, *B. swinnyi,* and *B. abyssinica* led to the isolation of triterpenes,[3,5] saponins,[6] flavonoids,[3] and xanthones.[3,7] In continuation of our investigation of the Cameroonian medicinal plants, we carried out a phytochemical study

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of the stem bark of *B. engleriana*, and we report herein the isolation and structural elucidation of mangiferin, in addition to three triterpenes, previously reported from other species of the genus.[3,5] The assignment of the signals of mangiferin was determined using ^{1}H, ^{13}C-NMR, and 2D-NMR spectral data (HMQC, COSY, HMBC). The terpenoids were identified by comparison of their ^{1}H and ^{13}C-NMR spectra with the literature data.

MATERIALS AND METHODS

General

^{1}H-NMR (500 MHz, $CDCl_3$ and DMSO), ^{13}C-NMR (125 MHz, $CDCl_3$ and DMSO), and the 2D spectra (^{1}H-^{1}H COSY, HMQC, HMBC) were recorded on the JEOL EAC 600 MHz spectrometer, with Trimethylsilane (TMS) as an internal standard. The IR spectrum (KBr) was taken on a HORIBA FT-720 spectrometer. Optical rotation was determined with the help of a HORIBA SEPA-300 spectropolarimeter. Electron impact-mass spectrometry (EI-MS) analyses were recorded on a JEOL SX102A mass spectrometer. Column chromatography was carried out on silica gel 60 (Merck; 230 – 400 mesh) and Sephadex LH-20 (Pharmacia Co. Tokyo, Japan). TLC was performed on silica gel 60 F_{254} plated (0.25 mm, Merck Co.), Column chromatography (CC) was carried out on Kieslgel 60 (Merck; 230–400 mesh) and spots were detected under UV light and colored by spraying with 10% H_2SO_4 solution followed by heating.

Plant material

The stem bark of *B. engleriana* were collected in the village Baham near Bafoussam city, Cameroon in August 2003 and identified by Dr. Onana National Herbaruim, Yaoundé, Cameroon where a voucher specimen (No 3454/SRFK) was also deposited.

Plant identification was performed by Dr. Onana, Cameroon National Herbaruim, Yaoundé, where a voucher specimen (No 3441/SRFK) was deposited.

Extraction and Isolation

2-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (**mangiferin**, **1**) — The air dried and powdered stem bark of *B. engleriana* (1 kg) was extracted at room temperature with CH_2Cl_2–MeOH (1:1) 5 L for 48 hours. The mixture of the solvent was removed by evaporation to yield 200 g of crude extract. The latter was then dissolved in CH_2Cl_2 to give the CH_2Cl_2 soluble fraction of 5 g and a remaining gum of 195 g. Part of the remaining gum (22 g) was dissolved in water and extracted four times with butanol to give 12 g of red oil; which was then separated by paper chromatography, with butanol-acetic acid-water (4:1:5), to give 3 g of orange gum; purification was carried out on HPLC with MeOH (100%) to yield 2 g of mangiferin as red oil; (^{1}H-NMR, 500 MHz, and ^{13}C-NMR, 125 MHz, DMSO) Table 1.

Table 1: ^{1}H, ^{13}C-NMR data of 1 (mangiferin)

Position	^{13}C	^{1}H, m, *J* (Hz)	HMBC
1	161.7	13.80 (1-OH)	
2	107.5	-	
3	163.8	-	
4	93.3	6.40, s	C-3, C-4a, C-4b
4a	156.2	-	
4b	101.2	-	
5	102.4	6.86, s	C-8a, C-8b, C-7
6	150.9	-	
7	143.9	-	
8	107.8	7.41, s	CO, C-8a, C-8b, C-7
8a	111.4	-	
8b	154.6	-	
CO	179.0	-	
1´	73.1	4.60, d, 8.3	C-1, C-3, C-2, C-6´
2´	70.3	4.03, t, 9.5	C-1´, C-3´
3´	79.0	3.16, m,	C-5´, C-4´
4´	70.6	3.16, m	C-5´
5´	81.5	3.16, m	C-4´, C-6´
6´	61.4	3.40, dd, 11.0, 2.1 3.60, dd, 11.0, 4.6	C-5´

Table 2: ^{1}H and ^{13}C-NMR data of compounds 2 – 4

Position	2		3		4	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	37.9	1.21	36.2	1.77	36.4	1.73
2	25.4	1.44	25.8	1.82	23.6	1.78
3	78.3	3.50	77.1	3.93	77.7	3.89
4	42.1	-	122.4	4.40	40.7	2.42
5	48.7	1.60	145.7	-	147.3	-
6	19.1	1.01	20.4	1.98	126.1	4.67
7	35.1	1.35	33.8	1.19	34.5	1.16
8	41.3	-	49.3	1.85	49.6	1.75
9	50.2	1.50	52.5	1.54	53.1	1.50
10	37.1	-	39.6	-	37.9	-
11	20.4	1.28	23.5	1.39	22.4	1.31
12	24.5	1.22	25.7	1.47	24.3	1.42
13	37.0	1.52	44.6	-	45.2	-
14	43.6	-	47.9	1.67	47.6	1.53
15	29.9	1.43	30.4	1.52	32.3	1.61
16	29.7	1.66	28.5	1.77	29.7	1.68
17	58.3	-	40.7	1.67	38.3	1.91
18	47.6	1.79	15.3	0.70	16.1	0.77
19	49.5	2.70	15.9	0.94	19.1	0.97
20	150.1	1.51	49.4	1.40	47.3	1.44
21	30.6	1.72	19.2	0.76	17.6	0.71
22	33.0	1.39	32.2	1.81	32.6	2.30
23	70.6	3.60/3.70	32.5	1.60	126.5	4.22
24	14.0	0.79	48.8	1.10	149.2	-
25	16.9	0.82	32.3	1.90	34.1	1.97
26	16.5	0.95	20.8	0.82	21.3	0.89
27	15.1	0.92	31.7	0.97	31.8	0.91
28	204.1	9.50	19.7	0.75	20.3	0.74
29	108.9	4.56/4.66	21.4	0.87	22.1	0.85
30	19.4	1.67	-	-	19.3	1.16

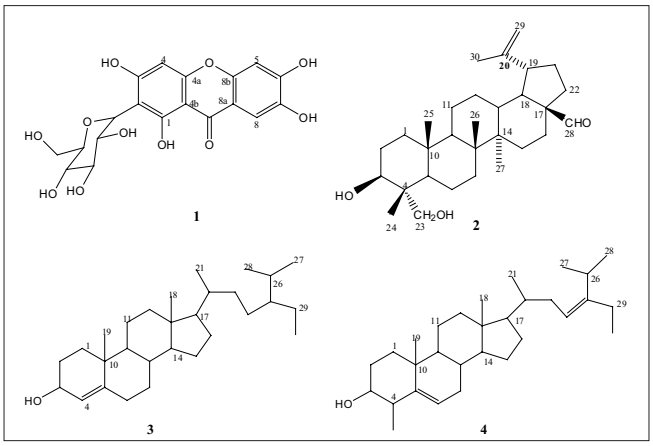

Figure 1: Structures isolated from stem bark extract of *Bersama engleriana*

Terpenoids – The CH_2Cl_2 soluble extract was eluted on silica gel *n*-hexane-CH_2Cl_2 gradient ratio and Sephadex LH-20 (*n*-hexane -CH_2Cl_2 -MeOH, (7:4:0.5) to afford compounds (2-4).

23-Hydroxy betulinaldehyde (swinniol, 2)
White powder, 12 mg; (^{1}H-NMR, 500 MHz, and ^{13}C-NMR, 125 MHz, $CDCl_3$) Table 2.

Δ^4-stigmaster-3β-ol (3)
White powder, 20 mg; (^{1}H-NMR, 500 MHz, and ^{13}C-NMR, 125 MHz, $CDCl_3$) Table 2.

4-methylstigmaster-5,23-dien-3β-ol (4)
White powder, 24 mg; (^{1}H-NMR, 500 MHz, and ^{13}C-NMR, 125 MHz, $CDCl_3$) Table 2.

RESULTS AND DISCUSSION

Fractionation of the CH_2Cl_2–MeOH (1:1) extract of the stem bark of *B. engleriana* Guike gave mangiferin (**1**), in addition to three previously reported triterpenes (**2**-**4**) [Figure 1]. The ^{1}H-NMR of compound **1** revealed the presence of three downfield aromatic singlet signals at δ 6.40 (1H, H-4), 6.86 (1H, H-5), and 7.41 (1H, H-8). Furthermore, the characteristic chemical shifts of the sugar moiety were observed at δ 4.60 (1H, d, J = 8.3 Hz, H-1´), 4.03 (1H, t, J = 9.5 Hz, H-2´), 3.16 (3H, H-3´, H-4´, H-5´), 3.40 (1H, dd, J = 11.0, 2.1 Hz, H-6´a), and 3.60 (1H, dd, J = 11.0, 4.6 Hz, H-6´b). The ^{13}C-NMR revealed 19 carbons atoms in the molecule. The aglycone, had 13 carbons, including 10 quaternary aromatic carbons at δ 161.7 (C-1), 107.5 (C-2), 163.8 (C-3), 156.2 (C-4a), 101.2 (C-4b), 150.9 (C-6), 143.9 (C-7), 111.4 (C-8a), 154.6 (C-8b), and 179.0 (CO), and three methines at δ 93.3 (C-4), 102.4 (C-5), and 107.8 (C-8). The anomeric carbon at δ 73.1 (C-1´), suggested the C-glucoside moiety (8-9). In addition the other shifts of the sugar carbons appeared at δ 70.3 (C-2´), 79.0 (C-3´), 70.6 (C-4´), 81.5 (C-5´), and 61.4 (C-6´). The location of the OH at C-1 was based on the downfield singlet proton at δ 13.80, hydrogen bonding with the carbonyl. The location of the protons at C-4, C-5, and C-8 was based on their singlet form in ^{1}H-NMR, the COSY and HMBC. The other positions were given according to the HMBC correlations [Table 1]. From the above-mentioned comprehensive NMR data, compound **1** was identified to be mangiferin.[3,7] The ^{1}H-NMR of **2** showed five methyl signals at δ 0.79, 0.82, 0.95, 0.92, and 1.67; accounting for H-24, H-25, H-26, H-27, and H-30, and three protons geminal to the hydroxyl groups at δ 3.50, 3.60, and 3.70 (H-3, H-23a, and H-23b). An aldehyde proton was found at δ 9.50 (H-28), two unsaturated methylenes protons were found at δ 4.56 and 4.66 (H-29a and H-29b). There were a large number of cyclic methylene groups with a chemical shift range (1.00 – 2.50). The ^{13}C-NMR data were in agreement with this information and revealed four carbons in the low field at δ 78.3, 70.6, 108.9, and 204.1, accounting for C-3, C-23, C-29, and C-28, respectively. The 3-OH was assigned a *β*-configuration from the large value of C-3 (78.3), which could be around 75 ppm in the α-orientation.[5] The *β*-C-23 stereochemistry was based on the chemical shift of H-23 (3.50/3.60), as this value could be 3.70/4.20 for the *β*-C-24 configuration.[5] The configuration of C-25, C-26, and C-28 was assigned to be *β*-oriented from the large multiplets H-5, H-9, and H-18. The stereochemistry of C-20 and C-27 was deduced from H-13 and H-19, which are sharp singlets. Based on this result and comparison with the data of a similar compound,[10] compound **2** was identified as 23-Hydroxy betulinaldehyde, (swinniol), previously isolated from *Bersama swinnyi*.[5] The stucture of compounds **3** and **4** could be easily determined from the comparison of their ^{1}H and ^{13}C-NMR with literature values.[3,11]

Chemotaxonomic Significance

Mangiferin is common in the plant kingdom and has been reported from the genus *Hymenophyllum*,[9] *Arrabidaea*[12] and *Davallia*.[8] Its isolation from *B. engleriana* is of great interest, as the previous investigation of *B. abyssinica*, a Kenyan species[3] and *B. yangambiensis*, a D.R. Congo species[7] reveals the presence of this compound. Thus, this compound may represent a chemotaxonomic marker of this genus, supporting the various uses of these species in traditional medicine.[2]

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