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Glucuronide triterpene saponins from *Bersama engleriana*

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Abstract

Five 3-*O*-glucuronide triterpene saponins (**1**–**5**) were isolated from the stem bark of *Bersama engleriana* Gurke along with two known saponins, polyscias saponin C and aralia saponin 15, and one major C-glycoside xanthone, mangiferin. The structures of the saponins were established mainly by means of spectroscopic methods (one- and two-dimensional NMR spectroscopy as well as FAB-, HRESI-mass spectrometry) as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl]-betulinic acid (**1**), 3-*O*-[β-D-glucopyranosyl-(1 → 2)-[β-D-galactopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl]-oleanolic acid (**2**), 3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-oleanolic acid (**3**), 3-*O*-[β-D-galactopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-oleanolic acid (**4**), and 3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-oleanolic acid (**5**).

Keywords: *Bersama engleriana*; Melianthaceae; Triterpene saponins; Glucuronic acid; Betulinic acid; Oleanolic acid

1. Introduction

Bersama engleriana Gurke (Melianthaceae) is a tree that occurs in forests and forest margins of tropical and subtropical Africa. The chemical constituents and biological activity of this plant have not been previously investigated but phytochemical studies on related species revealed the presence of bufadienolides (Kupchan et al., 1971; Vanhaelen et al., 1972), triterpenoids (Monkhe et al., 1998; Bowen et al., 1985), glucuronide triterpene saponins (Vanhaelen, 1972a) and a C-glycoside xanthone (Bowen et al., 1985; Vanhaelen, 1972b). *Bersama* species are generally used for their antitumor (Kupchan et al., 1971; Bowen et al., 1985), spamolytic (Makonnen and Hagos, 1993), cardiotonic (Lock, 1962; Vanhaelen et al., 1972), antibacterial (Taniguchi and Kubo, 1993) and antiviral (Asres et al., 2001) activities.

As part of our continuing search for new bioactive triterpene saponins from Cameroonian medicinal plants (Naheed et al., 2002; Tapondjou et al., 2002, 2003; Mitaine Offer et al., 2004) we have investigated the stem bark of *B. engleriana* collected in the Western highlands of Cameroon. The present paper reports the isolation and the structural elucidation of five new triterpene saponins (**1**–**5**) along with two known saponins, polyscias saponin C and aralia saponin 15, and one known C-glycoside xanthone, mangiferin.

2. Results and discussion

The dried and powdered stem bark of *B. engleriana* was successively extracted with MeOH/CH_2Cl_2 (1:1) and MeOH at room temperature. The combined extracts were

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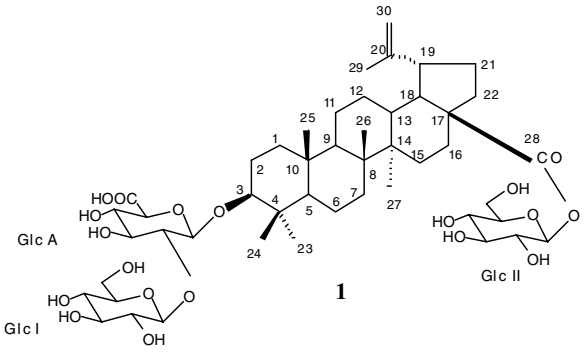

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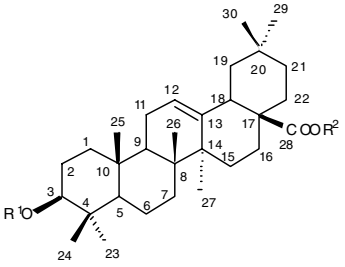

	R^1	R^2
2	Glc(1→2) [Gal-(1→3)]- GlcA-	H
3	Glc-(1→3)-GlcA-	Xyl-(1→6)-Glc-
4	Gal-(1→3)-GlcA-	Glc-(1→4)-Glc-
5	Glc-(1→3)-Gal-(1→3)-GlcA-	Xyl-(1→6)-Glc-

concentrated to dryness to afford a crude extract which was suspended in water and partitioned against EtOAc and *n*-BuoH. The *n*-BuOH layer yielded after evaporation of the solvent a crude saponin mixture which was fractionated by column chromatography over Sephadex LH-20 in MeOH to eliminate complex mixture of polyphenolic compounds. The residual saponin mixture was fractionated by column chromatography over silica gel and further purifications were achieved by repeated medium-pressure liquid chromatography (MPLC) over silica gel to afford seven saponins including five new ones (**1**–**5**). Structure elucidation was based on high resolution mass spectra, and 1D and 2D NMR measurements. In addition to saponins, one major C-glycoside xanthone (1,3,6,7-tetrahydroxy-2-C-β-D-glucopyranosylxanthone, mangiferin) was isolated and identified by comparison of its spectroscopic data with those reported in the literature and by co-TLC with an authentic sample (Lacaille-Dubois et al., 1996). It is important to notice that mangiferin seems to be a chemotaxonomic marker of the *Bersama* genus of Melianthaceae as it has also been previously isolated in large amount from *B. abyssinica* (Bowen et al., 1985) and *B. yangambiensis* (Vanhaelen, 1972b).

Acid hydrolysis of the saponin mixture gave a mixture of betulinic acid and oleanolic acid identified by co-TLC comparison with authentic samples and comparison of ^{13}C NMR data with literature data (Janeczko et al., 1990; Mimaki et al., 2004). The sugars were identified as glucose, glucuronic acid, galactose and xylose by co-TLC comparison with authentic samples. The common D-configuration for glucose, galactose and xylose was determinated by GC analysis of their chiral derivatives in the acidic hydrolysate (Haddad et al., 2003; Elbandy et al., 2003), and for glucuronic acid, by the measurement of optical rotation after separation of the sugar by prep. TLC of the crude sugar residue.

Compound **1** was isolated as a white amorphous powder. Its negative FABMS exhibited a quasi-molecular ion peak at *m/z* 955 $[M-H]^-$, indicating a molecular weight of 956. The molecular formula was established as $C_{48}H_{76}O_{19}$ by the positive-ion mode HRESIMS showing a pseudo-molecular ion peak at *m/z* 979.4897 $[M+Na]^+$ (calcd for 979.4879 $C_{48}H_{76}O_{19}Na$). Further fragment ion peaks were observed in the FABMS spectrum at *m/z* 793 $[M-H\text{-}162]^-$, 631 $[M-H\text{-}162\text{-}162]^-$ and 455 $[M-H\text{-}162\text{-}162\text{-}176]^-$ corresponding to the successive loss of two hexosyl and one hexosyluronic acid moiety. This result suggested that saponin **1** contained three sugar units, one of them being an hexosyluronic acid. The extensive studies of the 1D and 2D NMR spectra (^1H NMR, ^{13}C NMR, COSY, TOCSY, NOESY, HSQC and HMBC) led to the identification of the aglycone part of **1** as betulinic acid (Janeczko et al., 1990). In the ^{13}C NMR spectrum the chemical shifts of C-3 (δ 89.6) and C-28 (δ 175.2) of betulinic acid (Table 1) indicated that **1** is a bidesmosidic glycoside. The three anomeric protons detected at δ_H 6.17, 5.24 and 4.73 in the ^1H NMR spectrum gave correlations with anomeric carbons at δ 94.7, 104.1 and 104.2 respectively in the HSQC experiment. Complete assignments of each glycosidic proton system were achieved by analysis of COSY, TOCSY and NOESY experiments starting from the readily identifiable anomeric H-atoms. The units with anomeric protons at δ 5.24 (*d*, $J = 7.4$ Hz), 6.17 (*d*, $J = 8.0$ Hz), and 4.73 (*d*, $J = 6.6$ Hz) corresponded respectively to a terminal β-D-glucopyranose (Glc I), a β-D-glucopyranosyl ester (Glc II) and a β-D-glucuronopyranosyl acid (GlcA). The deshielded value of C-2 (δ 80.4) of GlcA suggested the point of linkage of the terminal glucosyl moiety. The sequencing of the glycosidic chains in saponin **1** was confirmed by analysis of HMBC and NOESY experiments. The HMBC spectrum showed correlations between H-1 (δ 6.17) of Glc II and C-28 (δ 175.2) of betulinic acid, between H-1 (δ 4.73) of GlcA and C-3 (δ 89.6) of betulinic acid, and between H-1 (δ 5.24) of the terminal Glc I and C-2 (δ 80.4) of GlcA. These connectivities were also confirmed by correlations observed in the NOESY spectrum between H-3 (δ 3.22) of betulinic acid and H-1 (δ 4.73) of GlcA, between H-1 (δ 5.24) of Glc I and H-2 (δ 4.22) of GlcA. Thus, compound **1** was elucidated as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl]-betulinic acid.

For all the remaining compounds **2**–**5**, the aglycone part was identified as oleanolic acid from the extensive studies

Table 1
^{13}C NMR data (150 MHz) of the aglycones of compounds **1-5** in pyridine-d_5 (δ ppm)

C	1	2	3	4	5
1	38.9	38.6	38.1	38.0	38.1
2	25.6	27.8	27.8	27.8	27.9
3	89.6	90.0	89.0	90.2	89.0
4	41.5	39.2	39.1	39.3	39.3
5	55.3	55.3	55.3	55.2	55.3
6	17.9	18.1	18.0	18.0	18.0
7	34.1	32.0	32.7	31.9	31.9
8	40.6	39.3	39.2	39.0	38.9
9	50.2	47.5	47.5	47.5	47.4
10	39.2	36.4	36.4	36.3	36.4
11	20.6	23.5	23.1	23.0	22.8
12	23.3	122.2	122.1	122.4	122.4
13	37.9	144.2	144.2	143.6	143.6
14	42.2	41.2	41.4	41.6	41.6
15	30.4	26.0	25.9	26.3	25.7
16	31.7	23.3	23.1	23.2	23.2
17	56.6	46.3	46.0	46.6	46.6
18	47.0	41.7	41.4	41.2	41.2
19	49.0	46.1	46.3	45.8	45.8
20	150.5	30.5	30.4	30.2	30.2
21	30.3	33.8	33.7	33.4	33.4
22	36.7	32.4	32.7	32.5	32.6
23	27.4	27.5	27.4	27.3	27.7
24	14.5	16.2	16.1	16.1	16.5
25	15.8	14.9	15.0	14.9	15.0
26	16.0	17.0	16.9	16.9	16.9
27	15.9	25.8	25.8	25.6	25.6
28	175.2	180.0	176.5	176.5	176.5
29	109.6	23.4	23.2	23.2	23.2
30	19.0	32.9	32.8	32.7	32.7

of 1 D and 2D NMR spectroscopic data (Mimaki et al., 2004). They differ from each other through the oligosaccharidic chains containing from two to three sugar units located at C-3 and/or C-28 of the aglycone.

Compound **2** was isolated as a white amorphous powder. Its HRESIMS (positive-ion mode) exhibited a pseudo-molecular ion peak at *m/z* 979.4856 $[M+Na]^+$ (calcd for $C_{48}H_{76}O_{19}Na$, 979.4879), ascribable to a molecular formula $C_{48}H_{76}O_{19}$. Its FAB-mass spectrum (negative-ion mode) showed a quasi-molecular ion peak at *m/z* 955 $[M-H]^-$ indicating a molecular weight of 956. Further fragment ion peaks in the FAB-mass spectrum were observed at *m/z* 793 $[M-H\text{-}162]^-$, 631 $[M-H\text{-}162\text{-}162]^-$, and 455 $[M-H\text{-}162\text{-}162\text{-}176]^-$ corresponding to the successive loss of two hexosyl and one hexosyluronic acid moiety exactly as in compound **1**, thus indicating that compound **2** as **1** also contains three sugar units. In the ^{13}C NMR spectra the chemical shifts of C-3 and C-28 of the aglycone appearing respectively at δ 90.0 and 180.0 (Table 1) indicated a sugar unit composed of three monosaccharides was located at C-3 position of oleanolic acid. The three anomeric protons were observed on the ^1H NMR spectrum at δ 4.77 (*d*, $J = 7.6$ Hz), 5.25 (*d*, $J = 7.3$ Hz) and 5.54 (*d*, $J = 7.4$ Hz) and correlated in the HSQC spectrum with three anomeric C-atoms at δ 104.5, 104.1 and 102.8 respectively (Table 2). Complete assignments of each glycosidic ring from 2D NMR spectra led to the identification of the monosaccharide units as one terminal β-D-galactopyranosyl (Gal), one terminal β-D-glucopyranosyl (Glc II) and one 2,3-disubstituted β-D-glucuronopyranosyl acid (GlcA) moieties (Table 2). The structure of the sugar chain at C-3 was defined by NOESY correlations between H-3 (δ 3.15) of oleanolic acid and H-1 (δ 4.77) of GlcA, H-2 (δ 4.36) of GlcA and H-1 (δ 5.54) of the terminal Glc I, and between H-3 (δ 4.34) of GlcA and H-1 (δ 5.25) of the terminal Gal. A cross peak correlation was also depicted in the HMBC spectrum between H-1 (δ 5.25) of Gal and C-3 (δ 86.0) of GlcA. Thus, compound **2** was elucidated as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-[β-D-galactopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl]-oleanolic acid.

Compound **3** was obtained as a white amorphous powder. Its positive-ion mode HRESIMS showed a pseudo-molecular ion peak at *m/z* 1111.5345 $[M+Na]^+$ (calcd for $C_{53}H_{84}O_{23}Na$ 1111.5301) consistent with a molecular formula of $C_{53}H_{84}O_{23}$. The negative-ion mode FAB-mass spectrum exhibited an intense quasi-molecular ion peak at *m/z* 1087 $[M-H]^-$ indicating a molecular mass of 1088, 132 amu higher than **2**, and in agreement with the molecular formula $C_{53}H_{84}O_{23}$. Subsequent ion peaks were observed at *m/z* 955 $[M-H\text{-}132]^-$, 793 $[M-H\text{-}132\text{-}162]^-$, 631 $[M-H\text{-}132\text{-}162\text{-}162]^-$ and 455 $[M-H\text{-}132\text{-}162\text{-}162\text{-}176]^-$ corresponding to the successive loss of one pentosyl, two hexosyl and one hexosyluronic acid moiety. The presence of four sugar units was confirmed from the observation in the ^1H NMR spectrum of signals for four anomeric protons at δ 4.58 (*d*, $J = 7.3$ Hz), 4.84 (*d*, $J = 7.5$ Hz), 5.08 (*d*, $J = 7.6$ Hz) and 6.08 (*d*, $J = 8.0$ Hz) giving correlations in the HSQC spectrum with four anomeric C-atoms at δ 104.1, 105.6, 104.6 and 95.1, respectively (Table 2). The ^1H and ^{13}C NMR data (Table 2) assigned from TOCSY, HSQC and HMBC experiments allowed the identification of the four sugar units as a 3-substituted β-D-glucuronopyranosyl acid (GlcA), a terminal β-D-glucopyranosyl (Glc I), a terminal β-D-xylopyranosyl (Xyl) and a β-D-glucopyranosyl ester (Glc II) moieties. The ^{13}C NMR spectrum indicated that the sugars were present in two saccharide chains, one attached to C-3 (δ 89.0) and another to C-28 (δ 176.5) of oleanolic acid. Sequencing of the two glycosidic chains was achieved by analysis of HMBC and NOESY experiments. Cross peak correlations were depicted in the HMBC spectrum between H-1 (δ 4.84) of GlcA and C-3 (δ 89.0) of oleanolic acid, and between H-1 (δ 5.08) of Glc I and C-3 (δ 83.5) of GlcA. This was further confirmed by NOESY correlations observed between H-1 (δ 4.84) of GlcA and H-3 (δ 3.24) of oleanolic acid, and between H-3 (δ 4.12) of GlcA and H-1 (δ 5.08) of Glc I. Other HMBC correlations were observed between H-1 (δ 4.58) of Xyl and C-6 (δ 69.5) of Glc II, and the cross peak correlating H-1 (δ 6.08) of Glc II and C-28 (δ 176.5) implying an ester linkage between the disaccharidic chain and oleanolic acid. Thus, compound **3** was elucidated as 3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-oleanolic acid.

Table 2
^{1}H and ^{13}C NMR data of sugar moieties of compound **1-5** in pyridine-d_5 (δ in ppm and J in Hz)[a]

		1		**2**		**3**		**4**		**5**	
		δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
At C-3 GlcA	1	104.2	4.73, *d* (6.6)	104.5	4.77, *d* (7.5)	105.6	4.84, *d* (7.5)	104.1	4.68, *d* (7.6)	105.3	4.74, *d* (7.5)
	2	80.4	4.22	77.8	4.36	74.0	4.00	74.0	3.96	74.5	3.88
	3	77.3	4.14	86.0	4.34	83.5	4.12	85.4	4.28	85.8	4.22
	4	71.1	3.94	71.6	4.22	71.2	4.46	70.2	4.08	70.6	3.90
	5	77.6	3.78	77.7	3.86	77.5	3.85	77.3	4.02	77.0	3.76
	6	n.d.		170.0		173.2		n.d.		n.d.	
Glc I	1	104.1	5.24, *d* (7.4)	102.8	5.54, *d* (7.4)	104.6	5.08, *d* (7.6)				
	2	75.7	3.90	75.6	3.90	74.3	3.92				
	3	77.0	4.08	77.8	4.16	77.0	4.10				
	4	72.7	4.14	71.8	3.87	70.7	3.94				
	5	76.9	4.00	77.7	3.80	78.4	3.92				
	6	61.4	4.18, 4.26	62.7	4.08, 4.42	61.7	4.04, 4.18				
Gal	1			104.1	5.25, *d* (7.4)			103.6	5.17, *d* (7.2)	104.4	5.20, *d* (7.7)
	2			72.3	4.32			72.0	4.22	71.0	4.44
	3			74.4	4.00			76.8	3.96	83.3	4.08
	4			69.6	4.26			69.4	4.18	69.0	4.50
	5			76.7	3.98			76.3	3.90	76.0	4.02
	6			61.7	4.12, 4.32			61.6	4.04, 4.26	61.5	4.06, 4.28
Glc IV	1									104.7	5.04, *d* (8.0)
	2									75.8	3.94
	3									77.4	4.07
	4									70.3	4.10
	5									77.6	3.80
	6									61.4	4.04, 4.16
At C-28 Glc II	1	94.7	6.17, *d* (8.0)			95.1	6.08, *d* (8.0)	95.0	6.02, *d* (7.4)	95.0	6.04, *d* (7.4)
	2	73.6	4.04			73.4	4.05	73.0	4.02	73.1	4.04
	3	77.6	4.20			77.5	4.18	77.5	4.15	77.6	4.14
	4	70.3	4.15			70.1	4.04	77.5	4.10	71.1	4.12
	5	78.5	3.92			77.7	3.98	78.2	3.86	78.3	3.88
	6	62.1	4.14, 4.40			69.5	3.80, 4.64	61.4	4.14, 4.28	69.4	3.78, 4.60
Xyl	1					104.1	4.58, *d* (7.3)			104.2	4.56, *d* (7.6)
	2					74.3	3.85			74.2	3.80
	3					76.2	4.38			76.6	4.00
	4					70.2	4.12			70.1	4.02
	5					66.3	3.50, 4.15			66.2	3.45, 4.13
Glc III	1							102.3	5.46, *d* (7.8)		
	2							75.3	3.80		
	3							77.3	4.30		
	4							71.5	4.10		
	5							77.5	3.76		
	6							62.5	3.96, 4.36		

[a] Overlapped ^{1}H NMR signals are reported without designated multiplicity, n.d.: not determined.

Compound **4** appeared as a white amorphous powder. Its positive-ion mode HRESIMS exhibited an intense quasi-molecular ion peak at m/z 1141.5383 $[M+Na]^+$ (calcd for $C_{54}H_{86}O_{24}Na$ 1141.5407) consistent with a molecular formula $C_{54}H_{86}O_{24}$. Its FAB-mass spectrum (negative ion mode) exhibited an intense pseudo-molecular ion peak at m/z 1117 $[M-H]^-$ indicating a molecular mass of 1118, which was compatible with the molecular formula $C_{54}H_{86}O_{24}$. Subsequent ion peaks were observed in the FAB-mass spectrum at m/z 955 $[M-H\text{-}162]^-$, 793 $[M-H\text{-}162\text{-}162]^-$ and 455 $[M-H\text{-}162\text{-}162\text{-}162\text{-}176]^-$ attributed to the loss of three hexosyl and one hexosyluronic acid moieties. The ^{1}H, ^{13}C NMR and HSQC spectra showed four anomeric signals of sugars at δ_H 6.02/δ_C 95.0, δ_H 5.46/δ_C 102.3, δ_H 5.17/ δ_C 103.6 and δ_H 4.68/δ_C 104.1. The extensive analysis of 1D and 2D NMR spectra revealed the presence of a terminal β-D-glucopyranosyl (Glc III), a terminal β-D-galactopyranosyl (Gal), a 4-substituted β-D-glucopyranosyl ester (Glc II), and a 3-substituted β-D-glucuronopyranosyl acid moieties (GlcA) (Tables 1 and 2). The deshielded values observed for C-3 (δ_C 85.4, δ_H 4.28) of GlcA and the cross peak correlation depicted in the NOESY spectrum between H-3 (δ 4.28) of GlcA and H-1 (δ 5.17) of Gal indicated that GlcA is substituted at the C-3 position by Gal. By the same manner the deshielded values observed for C-4 (δ_C 77.5, δ_H 4.10) of

Glc II and the cross peak correlation observed in the NOESY spectrum between H-4 (δ_H 4.10) of Glc II and H-1 (δ_H 5.46) of Glc III enabled the location of the terminal glucopyranosyl at C-4 of Glc II. Thus, the structure of compound **4** is established as 3-*O*-[β-D-galactopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-oleanolic acid.

Compound **5** was obtained as a white amorphous powder. Its HRESIMS (positive-ion mode) showed a pseudomolecular ion peak at *m/z* 1273.5892 $[M+Na]^+$, (calcd for $C_{59}H_{94}O_{28}Na$ 1273.5829), consistent with a molecular formula of $C_{59}H_{94}O_{28}$. The FAB-mass spectrum (negative ion mode) exhibited an intense quasi-molecular ion peak at *m/z* 1249 $[M-H]^-$, indicating a molecular mass of 1250, 132 amu higher than that of **4**, which was compatible with the molecular formula $C_{59}H_{94}O_{28}$. Subsequent ion peaks were observed in the FAB-mass spectrum at *m/z* 1117 $[M-H-132]^-$, 955 $[M-H-132-162]^-$, 793 $[M-H-132-162-162]^-$, 631 $[M-H-132-162-162-162]^-$ and 455 $[M-H-132-162-162-162-176]^-$ which revealed the successive loss of one pentosyl, three hexosyl, and one hexosyluronic acid moiety. Compound **5** contains five sugar units identified from 2D NMR spectra as a β-D-glucopyranosyl ester (Glc II), a terminal β-D-xylopyranosyl (Xyl), a terminal β-D-glucopyranosyl (Glc IV), a 3-substituted β-D-galactopyranosyl (Gal), and a 3-substituted β-D-glucuronopyranosyl acid (GlcA) moieties (Table 2). The HMBC and NOESY experiments were used for sequencing the sugar chains. The HMBC spectra showed correlations between H-3 of the genin (δ 3.28) and C-1 (δ 105.3) of GlcA as for compounds **2** and **3**, and between H-1 (δ 5.04) of the terminal Glc IV and C-3 (δ 83.3) of Gal. Cross peak correlations were depicted in the NOESY spectrum between H-3 (δ 4.22) of GlcA and H-1 (δ 5.20) of Gal, and between H-3 (δ 4.08) of Gal and H-1 (δ 5.04) of the terminal Glc IV, thus establishing the sugar chain at C-3 position of the genin as 3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]. A cross peak correlation was also depicted in the HMBC spectrum between H-1 (δ 4.56) of the terminal Xyl and C-6 (δ 69.4) of the Glc II ester. The structure of compound **5** is then established as 3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-oleanolic acid.

On the basis of their spectral data compound **6** was identified as polyscias saponin C (3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl]-oleanolic acid) previously isolated from *Polyscias scutellaria* (Paphassarang et al., 1989) while compound **7** was identified as aralia saponin 15 (3-*O*-[β-D-galactopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl]-oleanolic acid) previously isolated from *Calendula arvensis* (Pizza et al., 1987).

Referring to the review article on 2,3-bidesmosidic glucuronide saponins of oleanolic acid (Tan et al., 1999) and the up to date literature on triterpenoid saponins, compounds **1**–**5** were established as new glucuronide saponin derivatives.

3. Experimental

3.1. General

Optical rotations were measured with a AA-10R automatic polarimeter. 1D and 2D NMR spectra (^1H and ^{13}C NMR, ^1H-^1H COSY, TOCSY, NOESY, HMBC and HSQC) were performed using a UNITY-600 spectrometer at an operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4L-X computer system (600 MHz for ^1H and 150 MHz for ^{13}C NMR spectra). Conventional pulse sequences were used for COSY, HMBC and HMQC. All chemical shifts (δ) are given in ppm units with reference to tetramethylsilane (TMS) as internal standard and the coupling constants (*J*) are in Hz. The samples were solubilized in pyridine-d_5. Fast-atom bombardment (FAB) mass spectra (negative-ion mode) were obtained on a Jeol-SX-102 mass spectrometer, using glycerol as matrix. HRESIMS was carried out on a Q-TOF 1 micromass spectrometer. GC analysis was carried out on a Thermoquest gas chromatograph using a DB-1701 capillary column (30 m × 0.25 mm i.d) with He as carrier gas. TLC and HPTLC were carried out on precoated silica gel 60 F_{254} (Merck); solvent systems used were for monosaccharides and saponins: $CHCl_3$/MeOH/AcOH/H_2O (60:32:12:8); for aglycones: $CHCl_3$/MeOH (9:1). Spray reagents: for saponins and aglycones, Komarowsky reagent, a 5:1 solution of 2% 4-hydroxybenzaldehyde in MeOH and 50% H_2SO_4 soln.; for sugars, diphenylamine/phosphoric acid reagent. Isolations were carried out using column chromatography (CC) [silica gel 60 (Merck, 70–200 μm) and Sephadex LH-20], Medium-pressure liquid chromatography (MPLC) (silica gel 60, Merck, 15–40 μm), Gilson pump M 305, Büchi column (460 × 25 mm and 460 × 15 mm).

3.2. Plant material

Stem bark of *B. engleriana* Gurke was collected in the village Fongo Tongo, near the Dschang city located in the Menoua division of the Western highlands of Cameroon in September 2002. The specimen of the plant (voucher no. 24725/HNC) was deposited in the National Herbarium of Cameroon, Yaoundé where the identification was confirmed by Dr B. Satabie, Botanist.

3.3. Extraction and isolation

Dried and finely powdered stem bark of the plant (3.5 kg) was macerated for 24 h with the solvent mixture MeOH/CH_2Cl_2 (1:1) (6 l). After removal of the solvent, the residual powder was re-extracted with MeOH (3 l) for 12 h. Evaporation of the solvent from combined extracts in vacuo gave a dark residue (165 g), which was suspended in water (1000 ml) and partitioned against EtOAc (4 × 250 ml) and H_2O-sat. *n*-BuOH (4 × 200 ml). The *n*-BuOH soluble portion was evaporated to dryness to afford a brown gum (85 g). The TLC of the above extract sprayed with the

Komarowsky reagent revealed its high content of complex polyphenolic compounds in addition to saponins. In order to eliminate these polyphenolic compounds from saponin mixture, the extract was submitted to CC (Sephadex LH-20) eluted with MeOH. During the evaporation of MeOH from the polyphenolic mixture soluble fractions, a yellow compound precipitating in MeOH was obtained and thereafter purified by recrystallisation in acetone to yield pure mangiferin (6.28 g). The overall saponin mixtures obtained (15.23 g) were fractionated by CC (silica gel 60, 70–200 μm) with the eluent $CHCl_3$/MeOH/H_2O (64:40:8) to afford six sub-fractions; I (665 mg), II (750 mg), III (1.4 g), IV (1.8 g), V (1.6 g), and VI (2.2 g). After co-TLC of the different subfractions, similar ones were combined for purification. Subfractions V–VI (3.8 g) were again submitted to CC (Sephadex LH-20, MeOH) to remove the residual complex polyphenolic mixture and thereafter purified by repeated MPLC (Silica gel 60, 15–40 μm) with $CHCl_3$/MeOH/H_2O (64:40:8) to yield **6** (15 mg), **7** (12 mg). Sub-fractions III–IV (3.2 g) were purified by repeated MPLC (Silica gel 60, 15–40 μm) with $CHCl_3$/MeOH/H_2O (65:35:10, lower phase) to yield **3** (9 mg), **4** (13 mg) and **5** (8 mg). Sub-fraction II (750 mg) under the same conditions afforded **1** (7.5 mg) and **2** (8 mg).

3.4. 3-O-[β-D-Glucopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-28-O-[β-D-glucopyranosyl]-betulinic acid (1)

White amorphous powder; $[\alpha]_D^{22} + 26.6°$ (MeOH; *c* 0.15); ^{1}H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz): see Tables 1 and 2; HRESIMS *m/z*: 979.4897 $[M+Na]^+$ (calcd for $C_{48}H_{76}O_{19}Na$, 979.4879); FABMS (negative-ion mode) *m/z*: 955 $[M-H]^-$, 793 $[M-H-162]^-$, 631 $[M-H-162-162]^-$, and 455 $[M-H-162-162-176]^-$.

3.5. 3-O-[β-D-Glucopyranosyl-(1 → 2)-[β-D-galactopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl]-oleanolic acid (2)

White amorphous powder; $[\alpha]_D^{22} + 20.0°$ (MeOH; *c* 0.05); ^{1}H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz): see Tables 1 and 2; HRESIMS *m/z*: 979.4856 $[M+Na]^+$ (calcd for $C_{48}H_{76}O_{19}Na$, 979.4879); FABMS (negative-ion mode) *m/z*: 955 $[M-H]^-$, 793 $[M-H-162]^-$, 631 $[M-H-162-162]^-$, and 455 $[M-H-162-162-176]^-$.

3.6. 3-O-[β-D-Glucopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-oleanolic acid (3)

White amorphous powder; $[\alpha]_D^{22} + 20.0°$ (MeOH; *c* 0.15); ^{1}H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz): see Tables 1 and 2; HRESIMS *m/z*: 1111.5345 $[M+Na]^+$ (calcd for $C_{53}H_{84}O_{23}Na$, 1111.5301); FABMS (negative-ion mode) *m/z*: 1087 $[M-H]^-$, 955 $[M-H-132]^-$, 793 $[M-H-132-162]^-$, 631 $[M-H-132-162-162]^-$, and 455 $[M-H-132-162-162-176]^-$.

3.7. 3-O-[β-D-Galactopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-O-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-oleanolic acid (4)

White amorphous powder; $[\alpha]_D^{22} + 6.6°$ (MeOH; *c* 0.15); ^{1}H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz): see Tables 1 and 2; HRESIMS *m/z*:1141.5383 $[M+Na]^+$ (calcd for $C_{54}H_{86}O_{24}Na$ 1141.5407); FABMS (negative-ion mode) *m/z*: 1117 $[M-H]^-$, 955 $[M-H-162]^-$, 793 $[M-H-162-162]^-$, and 455 $[M-H-162-162-162-176]^-$.

3.8. 3-O-[β-D-Glucopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-28-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-oleanolic acid (5)

White amorphous powder; $[\alpha]_D^{22} + 60.0$ (MeOH, 0.15); ^{1}H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz): see Tables 1 and 2. HRESIMS *m/z*:1273.5892 $[M + Na]^+$ (calcd for $C_{59}H_{94}O_{28}Na$ 1273.5829); FABMS (negative-ion mode) *m/z*: 1249 $[M-H]^-$, 1117 $[M-H-132]^-$, 955 $[M-H-132-162]^-$, 793 $[M-H-132-162-162]^-$, 631 $[M-H-132-162-162-162]^-$, and 455 $[M-H-132-162-162-162-176]^-$.

3.9. Acid hydrolysis

The crude saponin mixture (0.5 g) in H_2O (4 ml) was refluxed with 15 ml of 2 N aq. CF_3COOH at 120 °C for 2 h. Thereafter the mixture was diluted in water (15 ml) and extracted with $CHCl_3$ (3 × 8 ml), which was washed with H_2O and evaporated to afford the aglycones. After repeated evaporations of the aq. layer by adding MeOH to remove the acid, the crude sugar residues was analysed by TLC (silica gel, $CHCl_3$–MeOH–AcOH–H_2O, 60:32:12:8) in comparison with standard sugars. R_f 0.29 (GlcA), 0.53 (Glc), 0.50 (Gal), 0.65 (Xyl). The absolute configuration of sugar residues was determined by GC analysis of their chiral derivatives as described in the previous papers (Haddad et al., 2003; Elbandy et al., 2003). D-Glucose, D-Galactose, D-Xylose were detected by co-injection of the silylated hydrolysate with standard silylated samples, giving simple peaks at 18.60, 19.58, 13.46, respectively. The absolute configuration of Glucuronic acid was determined to be D by the measurement of optical rotation after its separation by prep.TLC from the crude sugar mixture. $\alpha_D = +7.0°$ (c 0.21, H_2O, 2 h).

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