Carbohydrate Research 346 (2011) 2699–2704

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Cytotoxic and antioxidant triterpene saponins from *Butyrospermum parkii* (Sapotaceae)

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ARTICLE INFO

Article history:
Received 4 July 2011
Received in revised form 14 September 2011
Accepted 17 September 2011
Available online 24 September 2011

Keywords:
Butyrospermun parkii
Butyrospermun paradoxum
Sapotaceae
Saponin
Cytotoxic activity
Antioxidant activity

ABSTRACT

Three new triterpenoid saponins, elucidated as 3-*O*-β-D-glucopyranosyloleanolic acid 28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside (parkioside A, **1**), 3-*O*-[β-D-apifuranosyl-(1→3)-β-D-glucopyranosyl]oleanolic acid 28-*O*-[β-D-apifuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)β-D-xylopyranoside (parkioside B, **2**) and 3-*O*-β-D-glucuronopyranosyl-16α-hydroxyprotobassic acid 28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside (parkioside C, **3**), were isolated from the *n*-BuOH extract of the root bark of *Butyrospermum parkii*, along with the known 3-*O*-β-D-glucopyranosyloleanolic acid (androseptoside A). The structures of the isolated compounds were established on the basis of chemical and spectroscopic methods, mainly 1D and 2D NMR data and mass spectrometry. The new compounds were tested for both radical scavenging and cytotoxic activities. Compound **2** showed cytotoxic activity against A375 and T98G cell lines, with IC_{50} values of 2.74 and 2.93 μM, respectively. Furthermore, it showed an antioxidant activity comparable to that of Trolox or butylated hydroxytoluene (BHT), used as controls, against 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), oxygen and nitric oxide radicals.

1. Introduction

Butyrospermun parkii Kotschy (Sapotaceae), also known as *Butyrospermun paradoxum* (Don) Hepper, is a small tree that grows up to 14 m high. The seeds of this plant contain an edible fat that is used in traditional medicine to treat scabies, ulcers, wounds and nasal stiffness.[1,2] Previous work on the seeds of this plant collected in the northern part of Cameroon revealed the presence of fatty acids and triglycerides.[1] The antibacterial and antifungal properties of extracts from leaves, stem bark, root bark, fruit and seed kernels of *B. parkii* have also been evaluated,[2] and the characterization of some bioactive metabolites from the seeds of this plant were reported in 2009 by some of us.[3] Because plants belonging to the Sapotaceae family have been known as rich source of saponins,[4–7] we examined the *n*-BuOH extract from the root bark of *B. parkii* as part of our research work on bioactive saponins from Cameroonian medicinal plants.[8–13] In this paper we report the isolation, structure elucidation, antioxidant and cytotoxic activities of three new triterpene saponins, designated as parkioside A, B and C (**1**–**3**, Fig. 1), from the root bark of this plant. Together with the new compounds, the known 3-*O*-β-D-glucopyranosyloleanolic acid (androseptoside A) was also isolated.

2. Results and discussion

The dried and pulverized root barks of *B. parkii* (3 kg) were extracted three times (each for 24 h) with MeOH (95%) at room temperature. The filtrate obtained was concentrated under reduced pressure to yield a dark residue (276 g). This extract was suspended in water and successively partitioned against EtOAc and *n*-BuOH, yielding, after evaporation to dryness, 56 and 110 g of EtOAc and *n*-BuOH extracts, respectively. One part of the *n*-BuOH extract was subjected to Sephadex LH-20 column chromatography using MeOH as the eluant to separate saponins from polyphenolic compounds and sugars. The saponin part consisted by two main fractions (fraction A and B). Fraction A was repeatedly purified by column chromatography on silica gel to afford the known 3-*O*-β-D-glucopyranosyloleanolic acid (androseptoside A) by comparison with data reported in the literature.[14] Fraction B afforded three new saponins, parkioside A (**1**), B (**2**) and C (**3**) (Fig. 1), the structures of which were elucidated through the analysis of

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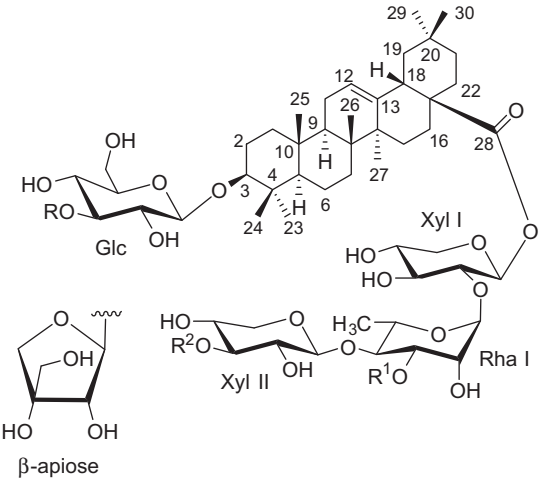
doi:10.1016/j.carres.2011.09.014



1: R = H, R^1 = H, R^2 = H
2: R = β-api (Api I), R^1 = α–L-rha (Rha II), R^2 = β-api (Api II)

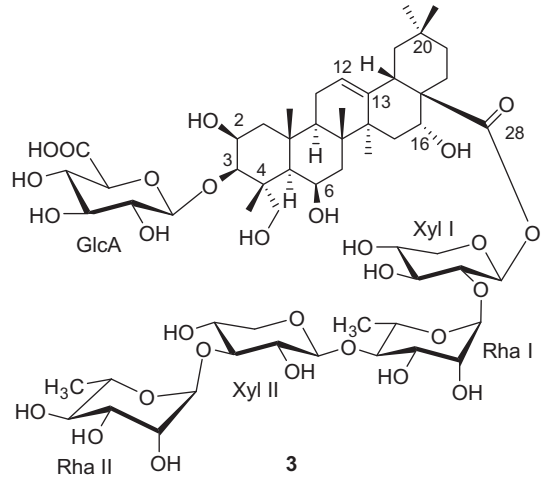


Figure 1. Structures of compounds **1**–**3**.

ESI-MS, tandem MS–MS and 1D and 2D NMR data, including ^{1}H, ^{13}C, COSY, TOCSY, ROESY, HMBC and HSQC.

Compound **1** was obtained as a white amorphous powder. Its ESI-MS (negative-ion mode) spectrum showed quasi-molecular ion-peaks at *m/z* = 1063.4 $[M+Cl]^-$ and *m/z* = 1027.4 $[M-H]^-$, indicating the molecular weight of 1028 amu, consistent with the molecular formula of $C_{52}H_{84}O_{20}$. Upon acid hydrolysis with 2 N TFA at 120 °C, compound **1** afforded an aglycone that was identified as oleanolic acid by co-TLC comparison with an authentic sample, and confirmed by the 2D NMR spectra of **1** (Table 1) compared with literature values.[15–17] The sugars obtained from the saponin hydrolysate were identified as glucose, xylose and rhamnose by TLC comparison with authentic samples, and confirmed by 1D-TOCSY, HSQC and HMBC data of **1**, as detailed below. The ^{1}H NMR spectrum of **1** displayed four sugar anomeric protons at δ 4.31 (d, *J* = 8.2 Hz), 4.44 (d, *J* = 7.8 Hz), 5.31 (br s) and 5.46 (d, *J* = 6.6 Hz), giving HSQC correlations with four anomeric carbons at δ 105.3, 105.9, 99.8 and 94.0, respectively (Table 2), confirming that compound **1** contains four sugar units. The ^{1}H NMR spectrum also revealed the presence of one rhamnose moiety, identified by the observation of a methyl doublet at δ 1.29 (*J* = 6.3 Hz) coupled with a glycosidic proton at 3.51 ppm. The analysis of ^{1}H–^{1}H COSY, 1D-TOCSY and HSQC data allowed the complete assignment of the glycosidic protons and carbons (Table 2), and the identification of one β-glucopyranosyl (Glc), one α-rhamnopyranosyl (Rha) and two β-xylopyranosyl (Xyl I and Xyl II) units. Sequencing and points of attachment in the glycosidic chains were established by analysis of HMBC and ROESY experiments. The glycosidic side chain at C-3 of the aglycone was identified as β-D-glucose, with its anomeric proton at δ 4.31 (d, *J* = 8.2 Hz) and its anomeric carbon at δ 105.3 (Table 2). The β-anomeric configuration of this sugar moiety was based on the observation of the large $^3J_{H\text{-}1,H\text{-}2}$ coupling constant. The location of this glucose unit at C-3 was confirmed by the HMBC correlation between the anomeric proton (δ 4.31) and the C-3 of the aglycone (δ 89.4). For the trisaccharide side chain at C-28 position of the aglycone, the HMBC spectrum showed correlation between H-1 of Xyl II (δ 4.44) and C-4 of Rha (δ 83.1), between H-1 of Rha (δ 5.31) and C-2 of Xyl I (δ 74.8), between H-1 of Xyl I (δ 5.46) and C-28 of the aglycone (δ 176.4). The tandem mass spectrometry (MS^n), which utilizes the collision-induced dissociation (CID) of target ions, has been used to confirm the sequences of the sugar chains. The MS^2 of *m/z* = 1027.4 $[M-H]^-$ gave fragments at *m/z* = 937.3 $[M-H-90]^-$, *m/z* = 895.4 $[M-H-132]^-$ corresponding to the cross-link cleavage of the terminal xylose unit[18,19] and the loss of the terminal xylose, respectively. Another important fragment ion was found at *m/z* = 617.2 $[M-H-2\times132-146]^-$, attributed to the loss of a triglycosidid chain made of two pentoses (xyloses) and a deoxyhexose, (rhamnose). The MS^3 of *m/z* = 617.4 gave a fragment at *m/z* = 411.0 $[617-162-44]^-$, corresponding to the loss of the CO_2 moiety at C-28 and a glucose unit. On the basis of the above analysis, the structure of **1** was thus elucidated as 3-*O*-β-D-glucopyranosyloleanolic acid 28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside (parkioside A).

Compound **2** was obtained as a white amorphous powder. Its ESIMS (negative-ion mode) showed quasi-molecular ion peaks at *m/z* = 1473.6 $[M+Cl]^-$ and *m/z* = 1437.7 $[M-H]^-$, indicating a molecular weight of 1438 amu consistent with a molecular formula of $C_{68}H_{110}O_{32}$. Comparison of the NMR data of **2** and **1** (Tables 1 and 2) revealed that the two compounds have the same aglycone (oleanolic acid) and different side chains at C-3 and C-28, compound **2** containing three more sugar units (two pentosyls and one deoxyhexosyl moieties). The supplementary signals were assigned to two β-D-apiofuranosyl moieties (Api I and Api II)[20,21] with their characteristic anomeric carbon atoms at δ 109.9 and 108.8 and anomeric protons at δ 5.21 (d, *J* = 2.8 Hz) and 5.16 (d, *J* = 3.6 Hz), respectively, and one terminal rhamnose moiety (Rha II) [anomeric carbon at δ 100.1, anomeric proton at δ 5.44 (br s), CH_3-6 at δ 1.22 (d, *J* = 6.6 Hz)]. The positions of these three supplementary sugar units were evidenced by the HMBC correlations and ^{13}C chemical shifts. The anomeric proton of Api I (δ 5.21) was found to be connected to C-3 (δ 85.0) of the glucopyranosyl moiety in the HMBC spectrum; this connection is confirmed by the chemical shift of the C-3 of the glucopyranosyl moiety. The second apifuranosyl unit was located at the C-3 of Xyl II due to the deshielded value observed for the signal of that carbon (δ 84.4) compared to the value observed for compound **1** (δ 76.8) (Table 2) and to the HMBC cross peak correlations between the anomeric proton at δ 5.16 (H-1 of Api II) and the carbon at δ 84.4, and between the proton at δ 3.39 (H-3 of Xyl II) and the anomeric carbon of Api II at δ 108.8. Finally, the terminal rhamnopyranosyl moiety was deduced to be located at the C-3 position of the inner rhamnosyl moiety (Rha I) because of its deshielded value observed (δ 83.7) compared to that observed in compound **1** (δ 70.9) and its HMBC correlation with the Rha II anomeric proton (δ 5.44). The MS^2 experiment of 1437.7 $[M-H]^-$ gave a fragment at *m/z* = 1291 $[M-H-146]^-$, corresponding to the loss of one terminal deoxyhexose. The MS^3 yielded fragments at *m/z* = 749 $[M-H-2\times146-3\times132]^-$, assigned to the loss of a pentaglycosidic ester-linked chain at C-28, and

Table 1
NMR spectroscopic data for the aglycone moieties of compounds **1**–**3** (400 MHz, CD_3OD)

Position	Parkioside A (**1**)		Parkioside B (**2**)		Parkioside C (**3**)	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	38.4	1.00, m	38.5	0.98, m	45.5	1.18, br d (12.5)
		1.60, m		1.57, m		2.04, dd (12.5, 4.5)
2	25.6	1.91, m	23.2	1.68, m	69.7	4.33, br d (4.5)
		1.66, m		1.91, m		
3	89.4	3.15, dd (11.2, 5.6)	89.6	3.15, dd (11.0, 5.2)	81.7	3.61, br d (3.8)
4	38.7		38.8		44.0	
5	55.7	0.78, m	55.7	0.78, m	47.4	1.33, br s
6	18.0	1.40, m	18.0	1.38, m	67.5	4.48, br s
		1.54, m		1.58, m		
7	32.7	1.40, m	32.6	1.41, m 1.47, m	40.0	1.57, br d (13.2)
		1.47, m				1.83, br d (13.2)
8	39.3		39.3		38.5	
9	47.6	1.56, dd (11.0, 4.6)	47.5	1.57, m	47.3	1.67, dd (11.8, 6.0)
10	36.4		36.5		35.8	
11	23.1	1.88, m	23.1,	1.89, m	23.2	2.05, m
12	122.3	5.26, br t (3.6)	122.3	5.26 br, t (3.4)	122.6	5.38 br, t (3.5)
13	143.4		143.4		142.6	
14	41.6		41.7		42.0	
15	27.8	1.19, m	27.9	1.19, m	35.1	1.46 br, d (14.7)
		1.58, m		1.56, m		1.73 br, d (14.7)
16	22.4	1.60, m	22.3	1.61, m	73.1	4.48 br, s
		2.04, m		2.05, m		
17	46.8		46.9		48.8	
18	41.4	2.83, dd (13.6, 4.2)	41.3	2.83, dd (13.4, 4.3)	40.9	2.99, dd (13.6, 4.0)
19	45.9	1.12, dd (13.8, 4.2)	45.9	1.13, dd (13.5, 4.8)	46.4	1.07, dd (13.2, 4.0)
		1.70 br t (13.8)		1.71 br, t (13.5)		2.29 br, t (13.2)
20	30.2		30.1		29.9	
21	33.5	1.22, m	33.6	1.21, m	35.0	1.16, m
		1.39, m		1.38, m		1.92, m
22	31.8	1.56, m	31.8	1.58, m	30.6	1.74, m
		1.72, m		1.70, m		1.91, m
23	27.2	1.06, s	27.3	1.06 s	64.1	3.43, d (10.0)
						3.75, d (10.0)
24	15.6	0.85, s	15.7	0.86 s	14.9	0.88, s
25	14.7	0.96, s	14.6	0.95 s	17.5	1.60, s
26	16.4	0.78, s	16.3	0.77 s	17.4	1.05, s
27	24.7	1.15, s	24.7	1.15 s	25.9	1.35, s
28	176.4		176.5		175.6	
29	32.0	0.90, s	32.0	0.90 s	31.9	0.95, s
30	22.6	0.92, s	22.6	0.92 s	23.5	0.88, s

m/z = 455 $[749-132-162]^-$, suggesting the supplementary loss of one diglycosidic ether-linked chain, composed by one pentose and one hexose moieties. This confirms that compound **2** contains seven sugar units: four pentosyls, two deoxyhexosyls and one hexosyl moiety. Thus, the structure of **2** was established as 3-*O*-[β-D-apifuranosyl-(1→3)-β-D-glucopyranosyl]-oleanolic acid 28-*O*-[β-D-apifuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]α-L-rhamnopyranosyl-(1→2)β-D-xylopyranoside (parkioside B).

Compound **3** was obtained as a white amorphous powder; its ESI-MS (negative-ion mode) spectrum gave a quasi-molecular ion peak at m/z = 1251.57 $[M-H]^-$, consistent with a molecular formula of $C_{58}H_{92}O_{29}$. The ^{1}H NMR spectrum (Tables 1 and 2) showed the characteristic signals of a triterpenoidic saponin, the aglycone of which was identified as 16α-hydroxyprotobassic acid (2β,3β,6β,16α,23-pentahydroxyolean-12-en-28-oic acid) by NMR data, which were in good agreement with those reported in the literature.[5,6] With respect to compound **1**, the ^{1}H NMR spectra of **3** showed the presence of an extra anomeric proton at δ 5.15 (br s), giving an HSQC correlation with the anomeric carbon at δ 101.3, and an additional methyl doublet at δ 1.25 (d, J = 6.2 Hz), indicating the presence of a second rhamnopyranosyl unit in compound **3**. Moreover, the presence of an additional carbonyl signal (δ 175.4) in the ^{13}C NMR spectrum of **3** and the loss of a hydroxymethylene group (δ 61.4 in compound **1**) suggested the presence in compound **3** of a glucuronic acid unit (GlcA) instead of a glucopyranosyl moiety. This was confirmed by the HMBC correlations observed between the protons at δ 3.43, δ 3.64 (H-4 and H-5 of GlcA) and the carbonyl at δ 175.4. The sugars composition was thus identified as one β-glucuronopyranosyl (GlcA), two α-rhamnopyranosyl (Rha I and Rha II) and two β-xylopyranosyl (Xyl I and Xyl II) units. The complete assignments of the glycosidic protons and carbons (Table 2) were possible through the analysis of ^{1}H-^{1}H COSY, TOCSY-1D and HSQC data. The glycosidic side chain at C-3 of the aglycone was identified as β-glucuronic acid, with its anomeric proton at δ 4.49 (d, J = 7.3 Hz) and its anomeric carbon at δ 103.1 (Table 2). The location of glucuronic acid at C-3 was confirmed by the HMBC correlation between the anomeric proton (δ 4.49) and C-3 (δ 81.7). The NMR data of the C-28 tetraglycosidic chain evidenced a close similarity with compound **1**, with the exception of an extra rhamnose unit (Rha II) present in **3** and the deshielded value observed for the C-3 carbon atom of Xyl II (δ 82.7) in **3** compared to the same carbon value in compound **1** (δ 76.8) (Table 2). Furthermore, the HMBC correlation observed between the anomeric proton of Rha II (δ 5.15) and the carbon at δ 82.7 (C-3 of Xyl II), indicated that Rha II is connected to C-3 of Xyl II, as further confirmed by the HMBC correlation between H-3 of Xyl II (δ 3.47) and the anomeric carbon of Rha II (δ 101.3). The MS^2 of m/z = 1251.4 $[M-H]^-$ gave fragments at m/z = 1105.3 $[M-H-146]^-$ and m/z = 695.2 $[M-H-2\times132-2\times146]^-$, attributed to the loss of a terminal deoxyhexose and a tetraglycosidic chain made of two pentoses and two deoxyhexose, respectively. The MS^3 of m/z = 695.2 gave an ion peak at m/z = 519 [695–176], indicating the loss of one glucuronic acid unit. Thus, compound **2** was elucidated as 3-*O*-β-D-glucuronopyr-

Table 2
NMR spectroscopic data for the sugar moieties of compounds **1**–**3** (400 MHz, CD_3OD)

Position	Parkioside A (**1**)		Parkioside B (**2**)		Parkioside C (**3**)	
	δ_C, type	δ_H(J in Hz)	δ_C, type	δ_H(J in Hz)	δ_C, type	δ_H(J in Hz)
C-3: 1	Glc: 105.3	4.31, d (8.2)	Glc: 105.3	4.32, d (7.9)	GlcA: 103.1	4.49, d (7.3)
2	74.3	3.17 br, t (8.2)	74.1	3.37 br, t (8.2)	73.5	3.34 br, t (8.5)
3	76.2	3.27 br, t (8.8)	85.0	3.40 br, t (8.6)	82.7	3.48 br, t (8.8)
4	70.2	3.32 br, t (8.3)	69.6	3.50 br, t (8.8)	72.2	3.43 br, t (9.0)
5	76.8	3.23, m	76.1	3.30, m	74.7	3.64, d (10.0)
6	61.4	3.65, dd (11.8, 5.3)	60.1	3.52, dd (11.6, 5.1)	175.4	
		3.83 br, d (11.8)		3.84 br, d (11.6)		
Api I 1			109.9	5.21, d (2.8)		
2			77.1	3.98, d (2.8)		
3			79.2			
4			73.4	3.78, d (9.6)		
				4.29, d (9.6)		
5			63.6	3.58 br, s		
C-28: Xyl I 1	94.0	5.46, d (6.6)	94.1	5.43 d (6.1)	94.0	5.42, d (6.4)
2	74.8	3.57, dd (9.1, 6.6)	75.0	3.59 dd (9.0, 6.1)	74.8	3.61, dd (9.0, 6.4)
3	75.4	3.56 br, t (9.0)	76.2	3.56 br t (9.0)	76.1	3.56 br, t (9.0)
4	69.4	3.51, m	68.6	3.51 m	69.6	3.53, m
5	65.2	3.29 dd (11.6,7.8)	65.4	3.29, dd (11.2, 7.7)	65.3	3.28, dd (11.4, 7.6)
		3.90 br d (11.6)		3.89 br, d (11.2)		3.91 br, d (11.4)
Rha I 1	99.8	5.31 br, s	100.3	5.35 br, s	99.9	5.36 br, s
2	70.4	3.92 br, s	70.4	3.94 br, s	70.9	3.94 br, s
3	70.9	3.85, dd (9.1, 3.4)	83.7	3.82, dd (9.1, 3.3)	71.0	3.86, dd (8.9, 3.1)
4	83.1	3.51 br, t (9.4)	77.1	3.51 br, t (9.2)	82.5	3.58 br, t (9.0)
5	67.4	3.75, m	67.3	3.75, m	67.4	3.79, m
6	16.8	1.29, d (6.3)	16.8	1.29, d (6.4)	16.9	1.32, d (6.2)
Xyl II 1	105.9	4.44, d (7.8)	106.1	4.47, d (7.6)	105.5	4.53, d (7.7)
2	74.7	3.19 br, t (8.3)	74.1	3.36 br, t (8.0)	74.9	3.40 br, t (8.2)
3	76.8	3.30 br, t (8.9)	84.4	3.39 br, t (8.5)	82.7	3.47, t (8.7)
4	69.6	3.45, m	68.5	3.51, m	68.4	3.56, m
5	65.8	3.17 br, t (11.1)	65.4	3.20 br, t (11.2)	65.7	3.23 br, t (11.2)
		3.83, dd (11.1, 5.2)		3.87, dd (11.2, 6.0)		3.90, dd (11.2, 6.1)
Rha II 1			100.1	5.44 br, s	101.3	5.15 br, s
2			71.0	3.91, dd (3.4, 1.8)	71.0	3.98, dd (3.2, 2.0)
3			70.5	3.71, (9.3, 3.4)	71.0	3.72, dd (9.5, 3.2)
4			73.9	3.38 br, t (9.3)	74.0	3.40 br, t (9.5)
5			68.0	4.21, m	68.6	4.01, m
6			16.6	1.22, d (6.4)	16.5	1.25, d (6.2)
Api II 1			108.8	5.16, d (3.6)		
2			76.9	3.83, d (3.6)		
3			78.7, C			
4			73.7	3.72, d (9.6)		
				4.13, d (9.6)		
5			65.4	3.51 br s		

anosyl-16α-hydroxyprotobassic acid 28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside (parkioside C).

It is worth noting that saponins having oleanolic acid derivatives, and more precisely 16α-hydroxyprotobassic acid, as aglycone, with similar sugar chains at C-3 and C-28 positions, as in compounds **1**, **2** and **3** have been frequently encountered in various plants belonging to Sapotaceae family.[5–7,22]

The radical scavenging activity of compounds **1**–**3** was screened against 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), nitric oxide (NO) and oxygen radicals. Compound **2** showed a dose dependent DPPH and ABTS radial scavenging comparable with Trolox used as control (Table 3). The activity of the new isolated saponins was also evaluated against oxygen radical scavengers as superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide (Fig. 2).

Table 3
DPPH, ABTS radical scavenging activity of compounds **1**–**3**

Compounds	DPPH IC_{50} μM	ABTS IC_{50} μM
Parkioside A (**1**)	>>1000	111.0(±4)
Parkioside B (**2**)	16.2(±2)	25(±2)
Parkioside C (**3**)	>>1000	70(±2)
Trolox	18.8(±1.5)	12.5(±0.3)

Compound **2** resulted to be the more active against superoxide radical, also with respect to Trolox and butylated hydroxytoluene (BHT) used as control and tested at the same concentration. Furthermore, it also showed lower activity with respect to compounds **1** and **3** towards the hydroxyl radical (Fig. 2). Finally, the

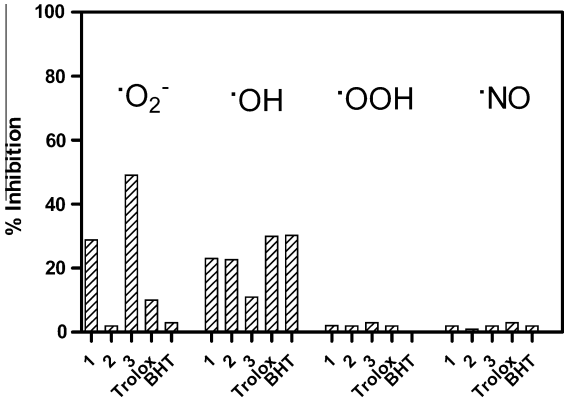


Figure 2. Radical scavenging activity of compounds **1**–**3** towards different radical species. For all compounds the radical scavenging activity was evaluated with the different assays as described in experimental and all the derivatives were used at a concentration of 20 μM.

Table 4
In vitro cytotoxic activity of compounds **1**–**3**

Compounds	Cell line (IC_{50} μM)[a] T98G	MDA-MB 231	A375	HCT116
Parkioside A (**1**)	23.68	35.97	27.32	55.44
95% CI	20.25–27.68	32.97–39.23	26.05–28.66	49.46–62.14
Parkioside B (**2**)	2.93	9.62	2.74	14.12
Parkioside C (**3**)	>100	>100	>100	>100
95% CI	2.61–3.29	7.65–12.10	2.42–3.11	12.36–16.13
Cisplatin	7.79	6.89	0.50	8.75
95% CI	6.83–8.52	5.65–7.39	0.37–0.68	8.03–9.54

[a] The IC_{50} value is the concentration of compound that affords a 50% reduction in cell growth (after 72 h of incubation). T98G = human glioblastoma multiforme cell line. MDA-MB 231 = human breast adenocarcinoma cell line. A375 = human malignant melanoma cell line. HCT116 = human colon carcinoma cell line. CI = confidence interval.

three new compounds did not show any scavenging activity towards hydrogen peroxide and nitric oxide.

The antiproliferative activity of compounds **1**–**3** was also evaluated against human breast adenocarcinoma (MDA-MB-231), malignant melanoma (A375), colon carcinoma (HCT116) and glioblastoma multiforme (T98G) cell lines. Compound **3** was not active in this assay. Compound **1** and **2** showed in vitro cytotoxic activity against all the cell lines tested (Table 4). Compound **2** was the most active, with IC_{50} values of 2.74 and 2.93 μM against A375 and T98G cell lines, respectively. It also showed a moderate activity against MDA-MB 231 and HCT116 cell lines (IC_{50} values of 9.62 and 14.12 μM, respectively). With respect to compound **2**, compound **1** was less active by about nine-fold against T98G and A375 cell lines and about four-fold against MDA-MB 231 and HCT116 cell lines. The higher cytotoxicity observed for compound **2** compared to compound **1** could be correlated with the presence of the apiose units, as already suggested for the triterpenoid saponins isolated from the aerial parts of *Conyza blinii*[23] and the pectic polysaccharide obtained from the cell wall of the marine phanerogam *Zostera marina*.[24] The inactivity of compound **3** in this assay could be attributed to its aglycone (16α-hydroxyprotobassic acid), which has been established to be not a structural determinant of cytotoxicity.[6]

3. Experimental

3.1. General methods

Optical rotations were measured on PerkinElmer 241 MC polarimeter. IR spectra were measured as a film on a KBr pellet using a FTIR-8400S Shimadzu spectrometer. ^1H and ^{13}C NMR, DEPT, COSY, HSQC, HMBC and ROESY experiments were performed on a Varian Mercury Plus Spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C). All chemical shifts (δ) are given in ppm units with reference to the residual solvent signals and the coupling constants (J) are in Hz. ESI mass spectra were carried out on an Agilent Technologies LC/MSD Trap SL (G2445D SL). ESIMS^n was performed in FIA (Flow Injection Analysis) using MeOH as solvent (5 mL/min, nebulizer pressure 15 psi), drying gas flow 4 L/min, drying gas temperature 325 °C. Column chromatography was performed using Silica Gel 60 Merck (63–200 μm and 32–63 μm). TLC was carried out on precoated Kieselgel 60 F_{254} (Merck) plates visualised by spraying with 50% H_2SO_4 and heating for 10 min at 110 °C.

3.2. Plant material

The root barks of *Butyrospermum parkii* (Sapotaceae) were collected in Bangwa village, near the city of Bangangte (West Region of Cameroon) in February 2009. Specimens documenting the collection were deposited at the Cameroon National Herbarium in Yaoundé (Ref.: No. 58927/HNC).

3.3. Extraction and isolation

The dried and pulverized root barks of *Butyrospermun parkii* (3 kg) were extracted three times (each for 24 h) with 9 L MeOH (95%) at room temperature. The filtrate obtained was concentrated under reduced pressure to yield a dark residue (276 g). This extract was suspended in water and successively partitioned against EtOAc and *n*-BuOH, yielding after evaporation to dryness 56 and 110 g of EtOAc and *n*-BuOH extracts, respectively. 100 g of the *n*-BuOH extract was subjected to Sephadex LH-20 column chromatography using MeOH as the eluent to separate the saponins from polyphenolic compounds and sugars. The saponins part was consisted by two main fractions: fraction A (16 g) and fraction B (17 g). Fraction A was repeatedly purified by column chromatography over silica gel (32–63 μm) with EtOAc–MeOH–H_2O (95:5:2) as the eluent and mainly afforded 3-*O*-β-D-glucopyranosyloleanolic acid (72 mg). Purification of fraction B on silica gel (32–63 μm) with EtOAc–MeOH–H_2O (85:15:5) and (80:20:10) led to the isolation of compounds **1** (85 mg), **2** (128 mg) and **3** (62 mg).

3.4. Identification

3.4.1. Parkioside A (1)

White amorphous powder; $[\alpha]_D^{25}$ −7.6 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3404, 2938, 1735, 1655, 1382, 1047 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (negative-ion mode), *m/z* = 1063.4 $[M+Cl]^-$ and *m/z* = 1027.4 $[M-H]^-$; ESIMS-MS MS^2 (1027.4) *m/z* = 937.3 $[M-H-90]^-$, *m/z* = 895.4 $[M-H-132]^-$ and *m/z* = 617.2 $[M-H-2\times132-146]^-$, MS^3 (617.4) m/z 411.0 [617–162–44].

3.4.2. Parkioside B (2)

White amorphous powder; $[\alpha]_D^{25}$ −51.8 (*c* 0.13, MeOH); IR (KBr) ν_{max} 3425, 2933, 1728, 1657,1386,1045 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (negative-ion mode) *m/z* = 1473.6 $[M+Cl]^-$ and 1437.7 $[M-H]^-$; ESIMS MS^2 (1437.7) *m/z* = 1291 $[M-H-146]^-$, MS^3 (1291) *m/z* = 749 $[M-H-2\times146-3\times132]^-$ and *m/z* = 455 [749−132−162].

3.4.3. Parkioside C (3)

White amorphous powder; $[\alpha]_D^{25}$ −39.9 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3417, 2930, 1740, 1637, 1383, 1047 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (negative-ion mode) *m/z* 1251.5 $[M-H]^-$; ESIMS MS^2 (1251.5) *m/z* = 1105.3 $[M-H-146]^-$ and *m/z* = 695.2 $[M-H-2\times132-2\times146]^-$, MS^3 (695.2) *m/z* = 519 [695−176].

3.5. Acid hydrolysis of compounds 1–3

A solution of each compound (3 mg) in water (1 mL) and 2 N aqueous CF_3COOH (10 mL) was heated at reflux at 100 °C on water bath for 2 h. The mixture was then diluted in water (10 mL) and extracted with EtOAc (3 × 3 mL). The combined EtOAc layers were washed with H_2O and evaporated to dryness to afford the aglycons. The aqueous residue was concentrated to dryness by adding repeatedly MeOH to remove acid and analysed by TLC (silica gel) in comparison with standard sugars by using a mixture of $CHCl_3$–MeOH–AcOH–H_2O (60:32:12:8) as the eluant. The absolute configuration of sugar residues was determined by GC analysis of their chiral derivatives.[25]

3.6. Radical scavenging activity tests

In order to measure antioxidant activity, the DPPH free radical scavenging assays were carried out according to the previously de-

scribed procedures.[26] ABTS radical cation decolorization assay, which relies on the decolorization of the blue-green ABTS cation radical caused by the antioxidant, was performed with a microplate assay, as previously described.[27] Scavenging activities of superoxide radicals and hydroxyl radicals were evaluated following the methods described by Kanga et al.[28] Scavenging of hydrogen peroxide was evaluated following the method described by Obied et al.[29] The scavenging activity of NO was evaluated as reported by Harput et al.[30]

3.7. MTT cytotoxicity assay

T98G cells (human glioblastoma multiforme cells) were cultured in Eagle's minimum essential medium (EMEM) with 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 μg/mL streptomycin and supplemented with 10% heat inactivated fetal bovine serum (HI-FBS) (PAA Laboratories GmbH, Austria, UE). MDA-MB 231 cells (human breast adenocarcinoma cells) and A375 cells (human malignant melanoma cells) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 2 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin and supplemented with 10% HI-FBS. HCT116 cells (human colon carcinoma cells) were cultured in RPMI1640 medium with 2 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin and supplemented with 10% HI-FBS. Cells were cultured in a humidified atmosphere at 37 °C in presence of 5% CO_2. The MTT cytotoxicity assay was performed as previously reported[12] with one modification, the concentrations of tested compounds ranged from 1 to 100 μM.

Acknowledgements

The authors are grateful to the IFS (International Foundation for Science, Stockholm, Sweden) through a grant to L. A. Tapondjou (RGA No. F/3976-2) and to the University of Camerino for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.09.014.

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