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

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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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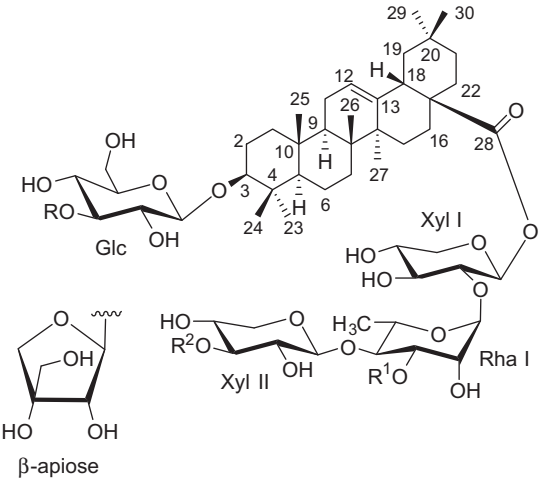

1: R = H, R^1 = H, R^2 = H
2: R = β-api (Api I), R^1 = α–L-rha (Rha II), R^2 = β-api (Api II)

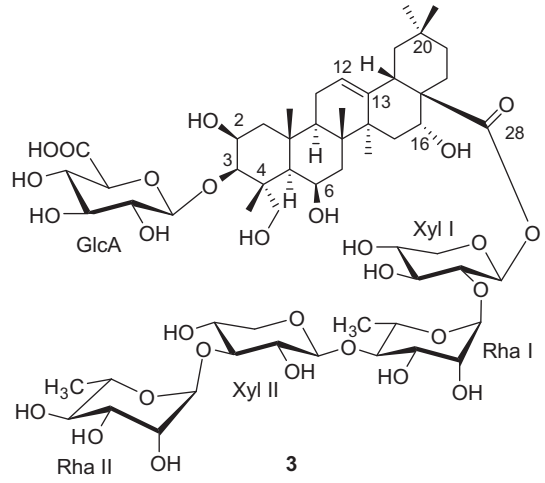

Figure 1. Structures of compounds **1**–**3**.

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