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Cycloartane-type glycosides from *Astragalus schottianus*

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ABSTRACT

Three new cycloartane-type triterpene glycosides were isolated from the roots of *Astragalus schottianus* Boiss. Their structures were established as 20(*R*),25-epoxy-3-*O*-β-D-xylopyranosyl-24-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxycycloartane (**1**), 20(*R*),25-epoxy-3-*O*-[β-D-glucopyranosyl(1 → 2)]-β-D-xylopyranosyl-24-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxycycloartane (**2**), 3-O-β-D-xylopyranosyl-3β,6α,16β,20(*S*),24(*S*),25-hexahydroxycycloartane (**3**) by the extensive use of 1D and 2D-NMR techniques and mass spectrometry.

1. Introduction

Among Turkish flora, the *Astragalus* genus is represented by 445 species, of which 224 are endemic to Turkey (Davis, 1982; Aytaç, 2000). Polysaccharides and saponins are the major classes of chemical compounds that have been isolated from *Astragalus* species. The polysaccharides are reported to possess cytotoxic and immunostimulating effects (Bedir et al., 2000b; Rios and Waterman, 1997), but the most thoroughly investigated constituents of *Astragalus* are the saponins.

In the course of studies on Turkish *Astragalus* species, several cycloartane- and oleanane-type triterpene glycosides were isolated and their structures elucidated (Calis et al., 1997, 1999; Bedir et al., 1999; Polat et al., 2009, 2010; Horo et al., 2010; Gülcemal et al., 2011).

Cycloartane- and oleanane-type glycosides from *Astragalus* species have shown interesting biological properties, including immunostimulating (Bedir et al., 2000b; Calis et al., 1997; Yesilada et al., 2005), anti-protozoal (Özipek et al., 2005), antiviral (Gariboldi et al., 1995), wound healing (Sevimli-Gur et al., 2011), adjuvant (Nalbantsoy et al., 2011) and cytotoxic activities (Radwan et al., 2004).

In our continuing studies of the constituents of *Astragalus* species, we investigated the roots of *A. schottianus*. This paper describes the isolation and structure elucidation of three new cycloartane-type glycosides.

2. Results and discussion

High-resolution electrospray ionization mass spectrometry (HRESIMS) of **1** showed a base peak at *m/z* 807.46042 $[M+Na]^+$. This result supported a molecular formula of $C_{41}H_{68}O_{14}$ (*m/z* 807.45068).

The ^1H NMR spectrum of **1** showed characteristic signals due to cyclopropane–methylene protons as an AX system (δ 0.12 and 0.49, J_{AX} = 4.0 Hz; H_2-19) and seven tertiary methyl groups. Additionally, the resonances for two anomeric protons were observed at δ 4.90 (*d*, *J* = 7.2 Hz) and 4.88 (*d*, *J* = 7.6 Hz). Thus, compound **1** was considered to be a cycloartane-type triterpene diglycoside (Fig. 1). The NMR signals were analyzed by the use of COSY and HMQC. The ^1H and ^{13}C NMR data supported the assignment of the sugar moieties in **1** as β-xylopyranose and β-glucopyranose. The remaining carbon and proton resonances were consistent with $C_{30}H_{50}O_5$ for the aglycon moiety. This result indicated six saturated ring systems because there were no olefinic protons. Additional functionalities on the aglycon included four geminal methine protons on the oxygen-bearing carbon atoms (H-3, H-6, H-16, and H-24). The resonances for the oxygenated carbons also indicated the presence of four oxymethine carbons (δ 89.2, 68.7, 74.8, 74.7; C-3, C-6, C-16, and C-24, respectively) and two oxygenated quaternary carbons (δ 79.9 and 75.5, C-20 and C-25, respectively). HMBC was used to clarify the intermolecular

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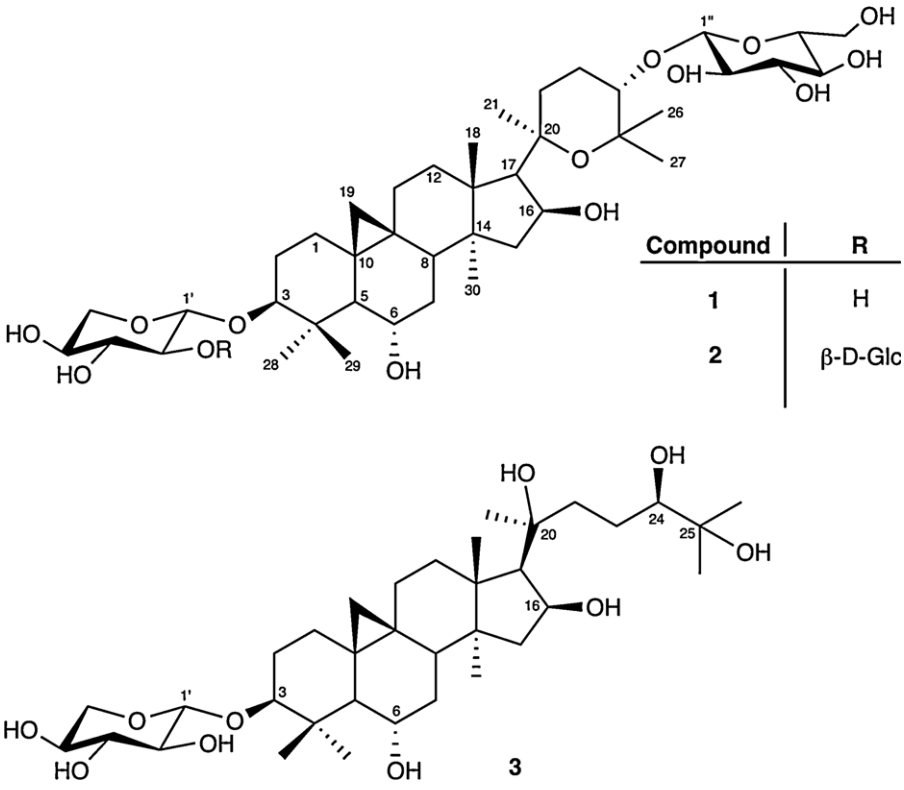

Fig. 1. Structures of **1**–**3**.

connectivity of the partial structures in **1**. This experiment revealed not only connectivity but also interglycosidic linkages. These data suggested the presence of a monohydroxypyran ring as a side chain. This configuration is unusual in the plant kingdom, so far it has been reported only from *Astragalus* spp. Based on these data, it was deduced that compound **1** was a cycloartane diglycoside with cyclocephalogenol as the aglycon previously reported by our group (Bedir et al., 1998). Additionally, the results of the HMBC experiment suggested that compound **1** was a bisdesmosidic saponin in which the sugar residues were linked to C-3 and C-24 of cyclocephalogenol. Key correlation peaks observed in the HMBC spectrum of **1** between H-1′ of the xylosyl at δ 4.90 (*d*, *J* = 7.2 Hz) and C-3 (δ 89.2) of the aglycon and between H-1″ of the glucosyl at δ 4.88 (*d*, *J* = 7.6 Hz) and C-24 (δ 74.7) allowed the saccharide residues at C-3 and C-24. The D configuration of the xylose and glucose units was determined after acidic hydrolysis of **1** followed by gas chromatography analysis. Consequently, the structure of **1** was established as 20(*R*),25-epoxy-3-*O*-β-D-xylopyranosyl-24-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxycycloartane.

The structure of **1** was previously reported by Semmar et al. (2001) as a new natural product. However, on the basis of our detailed inspections, we concluded that the researchers may have misinterpreted their NMR data and proposed an incorrect structure. The correct structure should have been determined as cyclocephaloside I (Bedir et al., 1998). Our conclusion is supported by the following facts: In the case of glycosylation at C-6 position, C-7 gives a resonance at about 33.0–34.0 ppm as a result of glycosylation's up field shift on β-carbon. If there is no such linkage, C-7 is observed around 38.5–39.0 ppm (Agzamova and Isaev, 1999; Bedir et al., 1998). In Semmar's paper; the carbon signal at 32.0 ppm was assigned to C-7, whereas our NMR spectra of **1** indicate C-7 at 39.3 ppm. Same pattern should also be considered for C-23 resonance due to glycosylation at C-24 position ($\delta_{C\text{-}23}$ = 26.8 for Semmar; $\delta_{C\text{-}23}$ = 21.8 for **1**). Additionally, C-6 and C-24 resonances were reported at δ 70.4 and 78.7. In the case of glycosylation at position 6, the carbon resonance was observed around 79.0 ppm that is supposed to provide a long-range correlation with the anomeric proton in the HMBC spectrum. Interestingly, the authors state that the HMBC correlation between 78.7 ppm (assigned to C-24) and 4.86 ppm (anomeric proton of β-glucose) signals facilitates the sugar residue being attached to C-24(*O*) position. But in Semmar's HMBC table providing evidence for the proposed structure, key interactions which should be observed between C-24 (or H-24) and CH_3-26, CH_3-27, CH_2-23, CH_2-22, and from C-6 (or H-6) to CH-5, CH_2-7 and CH_2-8 were not mentioned, whereas we observed and reported these very essential long range correlations for compound **1**. The data indicated that the C-6 (H-6) and C-24 (H-24) resonances were interpreted incorrectly by Semmar and his coworkers implying a known structure, cyclocephaloside I, reported by our group in 1998 (Bedir et al., 1998).

The molecular formula of **2**, was established as $C_{47}H_{78}O_{20}$ by HRESIMS analysis (*m*/*z* 981.48275 $[M\text{-}Cl]^-$, calcd for $C_{47}H_{78}O_{20}Cl$, 981.48258).

The ^{1}H NMR spectrum of **2** showed three anomeric proton resonances at δ 4.90 (*d*, *J* = 8.0 Hz), 4.92 (*d*, *J* = 7.2 Hz), and 5.40 (*d*, *J* = 7.8 Hz) correlated by HSQC to the resonances at δ 106.0, 105.6 and 100.7, respectively. The three sugar units were identified using a combination of COSY and HSQC as two terminal β-glucopyranoses, and glycosylated β-xylopyranose. The D-configurations of the sugar residues were determined by acid hydrolysis. The ^{13}C NMR resonances arising from the sapogenol moiety were identical to those of **1**, exhibiting same glycosylation pattern on the aglycone. These results suggested a bidesmosidic structure for **2** in which the two sugar units were attached to the hydroxyl groups at C-3 and C-24. HMBC experiment performed on **2** established the glycosylation sites showing significant cross-peaks, due to $2J_{C\text{–}H}$ correlations, between C-3 (δ 88.6) and $Xyl_{H\text{-}1'}$ (δ 3.55), between $Xyl_{C\text{-}2'}$ (δ 83.3) and $Glu\text{-}I_{H\text{-}1''}$ (δ 4.90), and between C-24 (δ 74.6) and $Glu\text{-}II_{H\text{-}1'''}$ (δ 5.40). Consequently, the structure of **2** was established

as 20(*R*),25-epoxy-3-*O*-[β-D-glucopyranosyl(1→2)]-β-D-xylopyranosyl-24-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxycycloartane, a new natural product.

HRESIMS [ESI(+)MicroTOF] of **3** (*m*/*z* 675.38677) $[M\text{-}Cl]^-$, calcd for $C_{35}H_{60}O_{10}Cl$, 675.38750) provided a molecular formula of $C_{35}H_{60}O_{10}$.

Taking into account the results of our ^1H and ^{13}C NMR studies, the main features of a cyclopropane-type triterpene possessing an acyclic side chain were evident for compound **3**: characteristic signals due to cyclopropane–methylene protons as an AX system (δ 0.28, 0.58, J_{AX} = 4.0 Hz, H_2-19), and seven tertiary methyl groups (δ 2.02, 1.79, 1.03, 1.35, 1.57, 1.54, 1.68; respectively, H_3-29, H_3-18, H_3-30, H_3-28, H_3-27, H_3-26, H_3-21). Inspection of the ^1H NMR spectral data of **3** showed seven tertiary methyl groups (δ 2.02, 1.79, 1.03, 1.35, 1.57, 1.54, 1.68; respectively, H_3-29, H_3-18, H_3-30, H_3-28, H_3-27, H_3-26, H_3-21). Additionally, the resonance for an anomeric proton was observed at δ 4.61 (J = 7.2 Hz) ($H\text{-}1_{xyl}$), indicative of the presence of a β-linked sugar unit. Thus, compound **3** was considered to be a cycloartane-type triterpene monoglycoside.

The ^{13}C NMR spectrum of **3** contained 35 signals. Five signals were assigned to the sugar unit, which were in good accordance with the presence of β-xylopyranose. On acidic hydrolysis of **3**, D configuration of the xylose unit was confirmed. The remaining 30 resonances were consistent with a $C_{30}H_{52}O_6$ triterpene framework, indicating the presence of 5 degrees of unsaturation. This implied five saturated ring systems because there were no olefinic protons (A–D rings and a cyclopropane ring). Thus, compound **3** was readily deduced to be a cycloartane possessing an acyclic side chain. ^{13}C- and DEPT spectra showed that the resonances assigned to the aglycon moiety consist of 7 methyl, 9 methylene, 7 methine; four of which were oxygen-bearing (δ 67.7, 75.5, 79.6 and 88.4), and 7 quaternary carbons; two of which were oxygen-bearing (δ 72.6 and 76.7). Full assignment of the ^1H and ^{13}C signals of the aglycon part of **3** accomplished by COSY and HMQC spectra. From the DQF-COSY and HMQC experiments, it was possible to establish 5 sequences excluding the signals ascribable to the cyclopropane ring (H_2-19):–CH_2–CH_2–CH(O),–CH–CH(O)–CH_2–CH–,–CH_2–CH_2–,–CH_2–CH(O)–CH–,–CH_2–CH_2–CH(O)–, corresponding to the C-1 → C-3, C-5 → C-8, C-11 → C-12, C-15 → C-17 and C-22 → C-24 moieties, respectively (Fig. 2). All connectivity within **3** was also confirmed by HMBC spectrum (Fig. 2), which showed a long-range correlation between the anomeric proton signal at δ 4.61 (*d*, J = 7.2 Hz) and the carbon resonance at δ 88.4 (C-3), confirming the location of glycosidation. Additionally, the oxygenated quaternary carbons were located at C-20 and C-25 based on the major cross peaks between C-20 → H_3-21 and C-25 → H_3-26/H_3-27 (Fig. 2).

Analyses of H–H vicinal coupling constants and NOE interactions showed that **3** had the same relative configurations at C-3 (β-OH), C-6 (α-OH) and C-16 (β-OH) positions as in **1** and **2**. It has to be mentioned that the ^{13}C NMR data can be regarded as characteristic parameters in the determination of absolute configurations of C-20 and C-24. ^{13}C NMR data of **3** are comparable to those reported for analogous compounds having a 20(*S*), 24(*R*) configuration (Duc et al., 1994; Fadeev et al., 1987; Rong-Qi et al., 1991; Sun and Chen, 1997). In the case of 20(*S*) configuration $\delta_{C\text{-}21}$ gives resonance at 27.0–28.0 ppm (Duc et al., 1994; Rong-Qi et al., 1991; Sun and Chen, 1997; Yalçın et al., 2012), while 20(*R*) configuration gives $\delta_{C\text{-}21}$ at 22.0–22.5 ppm (Duc et al., 1994) ($\delta_{C\text{-}21}$ = 28.7 for **3**). In the case of 24(*R*) configuration $\delta_{C\text{-}24}$ gives resonance around 80.0 ppm (Duc et al., 1994; Fadeev et al., 1987), whereas for the 24(*S*) configuration $\delta_{C\text{-}24}$ resonates at 77.0–77.2 ppm (Bedir et al., 2000a; Duc et al., 1994) ($\delta_{C\text{-}24}$ = 79.9 for **3**). Additionally, in the ROESY spectrum, a correlation between H-16 and H_3-21 demonstrated their co-facial nature (alpha orientation), and verified 20(*S*) absolute configuration.

Based on these evidence, the structure of **3** was established as 3-O-β-D-xylopyranosyl-3β,6α,16β,20(*S*),24(*R*),25-hexahydroxycycloartane (Fig. 1). This compound represents the second entry of the series of cycloartane-type compound possessing a 20-OH functional group in *Astragalus* genus. In nature, only five compounds, obtained from *Oxytropis* bicolor and *Astragalus stereocalyx*, were reported to have such a substitution at C-20 and 3β,16β,20(*S*),24(*S*),25-pentahydroxycycloartane framework (Rong-Qi et al., 1991; Sun and Chen, 1997; Yalçın et al., 2012).

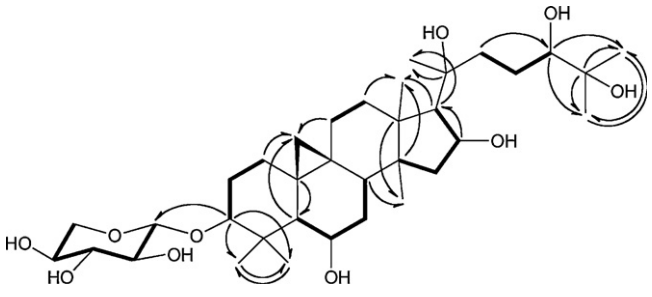

Fig. 2. Spin systems (bold lines) and key long-range correlations (arrows from C to H) of **3** deduced from DQF-COSY/HSQC and HMBC spectra, respectively.

3. Experimental

3.1. General

HR-MS spectra were obtained by microToF-Q II mass spectrometer from Bruker (Bremen, Germany), in positive and negative ESI mode. The 1D and 2D NMR spectra were obtained on Varian Oxford AS400 at 400 (^1H) and 100 MHz (^{13}C) instruments. Proton and carbon chemical shifts were reported relative to TMS. 2D NMR spectra (COSY, ROESY, HMQC, HMBC) were run using standard Varian pulse programs. GC analysis was performed on a Termo Finnigan Trace GC apparatus using an L-Chirasil-Val column (0.32 mm × 25 m). Column chromatography was carried out on silica gel (JT Baker, 40 μm), Sephadex LH-20 (Amersham Biosciences, 17-0090-02) and RP (C-18, 40 μm) (Merck). TLC analyses were carried out on silica gel 60 F_{254} (Merck) and RP-18 F_{254s} (Merck) plates. Compounds were detected by UV and 20% H_2SO_4/water spraying reagent followed by heating at 105 °C for 1–2 min.

3.2. Plant material

Astragalus schottianus Boiss. was collected from Zara Village, 10 km from Erzincan-Sivas highway, Central Anatolia, Turkey in July 20, 2008. Voucher specimen has been deposited in the Herbarium of Bionorm Natural Products Company, Izmir, Turkey (BNH-210708-1).

3.3. Extraction and isolation

The air-dried and powdered plant material of *Astragalus schottianus* Boiss (whole plant; 400 g) was extracted with MeOH (2× 5 L) at 70 °C for 12 h, under reflux. After filtration, the solvent was removed by rotary evaporation to yield 28.37 g of crude extract. The MeOH extract was subjected to vacuum liquid chromatography (VLC) on reversed-phase material (Lichroprep RP-18, 25–40 μm, 200 g) employing H_2O (350 mL), H_2O–MeOH (85:25, 1000 mL; 25:75, 600 mL), and MeOH (1300 mL) to give ten main fractions (A1–A10). After TLC analysis, fractions A3–A10 were found to be rich in saponins. Frs. A3–A10 (17.09 g) was combined and applied to open column chromatography using silica gel (500 g) as stationary phase. Elution was carried out with $CHCl_3$–MeOH mixtures (95:5, 3000 mL; 90:10, 400 mL; 85:15, 10,000 mL;

80:20, 3000 mL; 70:30, 2000 mL) to give fifty-nine fractions (B). Fractions B28–B32 were pooled together and chromatographed over a Biotage Flash Chromatography using reversed-phase 25 M column. Elution was performed by using H_2O–MeOH gradient [70% MeOH (350 mL), 70 → 80% (100 mL) and 80 → 85% (100 mL)] to give seventy-six fractions (C). Compound **1** was obtained from fraction C60–C61 (54.2 mg). The fraction B51–B54 (716.3 mg) was applied to Biotage Flash Chromatography using reversed-phase 25 M column. Elution was carried out with a gradient system: 100% H_2O → 20% MeOH (80 mL), 20% → 60% MeOH (90 mL), 60%–80% MeOH (50 mL), and 80% MeOH (50 mL) to give ninety-three fractions (D). Fractions D67–D70 (23.9 mg) yielded compound **2**. The fraction B18–B22 (379.7 mg) was subjected to vacuum liquid chromatography (VLC) on reversed-phase material (Lichroprep RP-18, 25–40 μm, 25 g) employing, H_2O–MeOH (75:25, 500 mL; 60:40, 600 mL), and MeOH (1300 mL) to give fifty main fractions (E). Fraction E14–E28 (36.4 mg) was subjected to open column chromatography using silica gel (15 g) as the stationary phase. Elution was carried out with $CHCl_3$–MeOH–H_2O mixtures (85:15:0.5, 500 mL; 80:20:1 400 mL) to give fifty-eight fractions (F). Fraction F33–F58 yielded compound **3** (7.2 mg).

3.3.1. *20(R),25-epoxy-3-O-β-D-xylopyranosyl-24-O-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxycycloartane (1)*

Amorphous powder; ^{13}C NMR (100 MHz, Pyridin-d_6): δ_C 108.1 (*d*, C-1′), 101.3 (*d*, C-1″), 89.2 (*d*, C-3), 79.9 (*s*, C-20), 79.1 (*d*, C-3′), 79.0 (*d*, C-5″), 78.8 (*d*, C-3″), 76.2 (*d*, C-2′), 75.8 (*d*, C-2″), 75.5 (*s*, C-25), 74.8 (*d*, C-16), 74.7 (*d*, C-24), 74.1 (*d*, C-4″), 71.8 (*d*, C-4′), 68.7 (*d*, C-6), 67.6 (*d*, C-5′), 63.5 (*t*, C-6″), 61.3 (*d*, C-17), 54.7 (*d*, C-5), 47.8 (*d*, C-8), 47.7 (*s*, C-13), 47.2 (*s*, C-4), 46.5 (*t*, C-15), 43.3 (*s*, C-10), 39.3 (*t*, C-7), 34.4 (*s*, C-14), 33.0 (*t*, C-2), 30.9 (*t*, C-12), 30.0 (*t*, C-1), 29.5 (*t*, C-19), 29.3 (*q*, C-26), 29.0 (*q*, C-28), 28.9 (*q*, C-27), 28.6 (*q*, C-21), 27.6 (*t*, C-22), 26.9 (*t*, C-11), 21.8 (*t*, C-23), 21.5 (*q*, C-18), 20.8 (*q*, C-30), 19.5 (*s*, C-9), 17.2 (*q*, C-29). ^{1}H NMR (400 MHz, Pyridin-d_6): δ 4.90 (1H, *d*, *J* = 7.2 Hz, H-1′), 4.88 (1H, *d*, *J* = 7.6 Hz, H-1″), 4.82 (1H, dd, H-16), 4.56 (1H, dd, *J* = 12.2, 2.0 Hz, H-6″a), 4.36 (1H, dd, *J* = 11.2, 5.2 Hz, H-5′a), 4.35 (1H, dd, H-5″b), 4.18 (1H, m, H-4′), 4.15 (1H, t, *J* = 8.4 Hz, H-3′), 4.21 (1H, m, H-3″), 4.16 (1H, m, H-4″), 3.96 (1H, m, H-5″), 4.04 (1H, m, H-2″), 4.06 (1H, m, H-2′), 3.84 (1H, brs, H-24), 3.74 (1H, m, H-6), 3.71 (1H, †, H-5′b), 3.61 (1H, m, H-3), 1.96 (1H, m, H-17), 1.80 (1H, dd, *J* = 12.0, 4.0 Hz, H-8), 1.53 (1H, s, H-21), 1.72 (1H, *d*, *J* = 9.2 Hz, H-5), 0.12 and 0.49 (each 1H, *d*, J_{AB} = 4.0 Hz, H-19a and H-19b, respectively); HRESIMS [ESI(+)MicroTOF]: (*m*/*z* 807.46042) $[M+Na]^+$ (calcd for $C_{41}H_{68}O_{14}Na$, 807.45068).

3.3.2. *20(R),25-epoxy-3-O-[β-D-glucopyranosyl(1→2)]-β-D-xylopyranosyl-24-O-β-D-glucopyranosyl-3β,6α,16β, 24α-tetrahydroxycycloartane (2)*

Amorphous powder; ^{13}C NMR: 981.48258. ^{13}C NMR (100 MHz, Pyridin-d_6) δ 106.0 (*d*, C-1″), 105.6 (*d*, C-1′), 100.7 (*d*, C-1‴), 88.6 (*d*, C-3), 83.3 (*d*, C-2′), 79.4 (*s*, C-20), 78.6 (*d*, C-3‴), 78.5 (*d*, C-3″), 77.9 (*d*, C-5″), 77.8 (*d*, C-5‴, C-3′), 76.9 (*d*, C-2‴), 75.2 (*d*, C-2″), 74.9 (*s*, C-25), 74.6 (*d*, C-24), 74.1 (*d*, C-16), 71.8 (*d*, C-4‴), 71.7 (*d*, C-4″), 70.9 (*d*, C-4′), 67.8 (*d*, C-6), 66.6 (*d*, C-5′), 63.0 (*t*, C-6″), 62.7 (*t*, C-6‴), 60.8 (*d*, C-17), 54.0 (*d*, C-5), 47.2 (*t*, C-15), 46.7 (*d*, C-8), 46.6 (*s*, C-14), 45.9 (*s*, C-13), 42.8 (*s*, C-4), 38.6 (*t*, C-7), 33.9 (*t*, C-12), 32.3 (*t*, C-1), 30.5 (*t*, C-19), 29.8 (*s*, C-10), 29.3 (*t*, C-2), 29.3 (*q*, C-28), 28.6 (*q*, C-26), 28.0 (*q*, C-21), 26.8 (*q*, C-27), 26.3 (*t*, C-22), 22.9 (*t*, C-11), 21.2 (*t*, C-23), 20.9 (*s*, C-9), 20.2 (*q*, C-18), 18.9 (*q*, C-30), 16.5 (*q*, C-29); ^{1}H NMR (400 MHz, Pyridin-d_6) δ 5.40 (1H, *d*, *J* = 7.8 Hz, H-1″), 4.92 (1H, *d*, *J* = 7.2 Hz, H-1′), 4.90 (1H, *d*, *J* = 8.0 Hz, H-1‴), 4.81 (1H, m, H-16), 4.60 (1H, dd, *J* = 12.4, 2.0 Hz, H-6‴a), 4.52 (1H, dd, *J* = 11.6, 3.2 Hz, H-6″a), 4.47 (1H, dd, *J* = 12.0, 3.6 Hz, H-6″b), 4.38 (1H, dd, *J* = 12.4, 5.6 Hz, H-6‴b), 4.32 (1H, m, H-4″), 4.26 (1H, m, H-3′), 4.26 (1H, m, H-5′a), 4.25 (1H, m, H-3″), 4.23 (1H, m, H-2′), 4.21 (1H, m, H-3‴), 4.20 (1H, m, H-4′), 4.18 (1H, m, H-4‴), 4.14 (1H, t, H-2‴), 4.05 (1H, m, H-2″), 3.98 (1H, m, H-5‴), 3.98 (1H, m, H-5″), 3.84 (1H, brs, H-24), 3.74 (1H, m, H-6), 3.66 (1H, dd, *J* = 10.8, 10.0 Hz, H-5′a), 3.55 (1H, dd, *J* = 11.6, 4.4 Hz, H-3), 2.09 (1H, m, H-17), 1.53 (1H, s, H-21), 1.92 (1H, m, H-8), 1.69 (1H, *d*, *J* = 9.6 Hz, H-5), 0.51 and 0.12 (each 1H, *d*, J_{AB} = 4.4 Hz, H-19a and H-19b, respectively); HRESIMS [ESI(+)MicroTOF]: (*m*/*z* 981.48275 $[M-Cl]^-$ (calcd for $C_{47}H_{78}O_{20}Cl$).

3.3.3. *3-O-β-D-xylopyranosyl-3β,6α,16β,20(S),24(R),25-hexahydroxycycloartane (3)*

Amorphous powder; ^{13}C-NMR (100 MHz, Pyridin-d_6): δ 107.6 (*d*, C-1′), 88.4 (*d*, C-3), 79.6 (*d*, C-24), 78.4 (*d*, C-3′), 76.7 (*s*, C-20), 75.5 (*d*, C-16), 73.8 (*d*, C-2′), 72.6 (*s*, C-25), 71.0 (*d*, C-4′), 67.7 (*d*, C-6), 66.8 (*d*, C-5′), 56.4 (*d*, C-17), 54.0 (*d*, C-5), 48.6 (*t*, C-15), 46.9 (*s*, C-14), 46.5 (*s*, C-13), 46.4 (*d*, C-8), 42.5 (*s*, C-4), 39.8 (*t*, C-22), 38.4 (*t*, C-7), 33.5 (*t*, C-12), 32.2 (*t*, C-1), 30.1 (*t*, C-2), 29.8 (*s*, C-10), 29.1 (*q*, C-28), 28.7 (*q*, C-21), 28.4 (*t*, C-19), 26.3 (*t*, C-11, C-23), 26.1 (*q*, C-27), 25.7 (*q*, C-26), 20.9 (*q*, C-18), 20.3 (*s*, C-9), 20.1 (*q*, C-30), 16.2 (*q*, C-29); ^{1}H NMR (400 MHz, Pyridin-d_6): δ 4.95 (1H, m, H-16), δ 4.61 (1H, *d*, *J* = 7.2 Hz, H-1′), 4.38 (1H, dd, *J* = 5.6, 11.6 Hz, H-5′a), 4.26 (1H, m, H-4′), 4.18 (1H, dd, *J* = 8.8, 8.0 Hz, H-3′), 4.09 (1H, dd, *J* = 8.0, 7.2 Hz, H-2′), 3.93 (1H, brd, *J* = 10.0 Hz, H-24), 3.76 (1H, m, H-6), 3.74 (1H, m, H-5′b), 3.58 (1H, dd, *J* = 10.4, 5.2 Hz, H-3), 2.38, (1H, m, H-22a), 2.34 (1H, m, H-2a), 2.26 (1H, m, H-22b), 2.11 (1H, m, H-15a), 2.11 (1H, *d*, *J* = 6.8 Hz, H-17), 2.02 (1H, m, H-12a), 2.02 (3H, s, H-29), 1.94 (2H, m, H-2b and H-8), 1.91 (1H, m, H-23a), 1.86 (1H, m, H-23b), 1.79 (1H, m, H-15b), 1.79 (3H, s, H-18), 1.75 (1H, m, H-7a), 1.68 (1H, m, H-12b), 1.68 (1H, s, H-21), 1.67 (1H, m, H-5), 1.57 (3H, s, H-27), 1.55 (1H, m, H-7b), 1.54 (3H, s, H-26), 1.35 (3H, s, H-28), 1.03 (3H, s, H-30), 0.58 and 0.28 (each 1H, *d*, J_{AB} = 4.0 Hz, H-19a and H-19b, respectively); HRESIMS [ESI(+)MicroTOF]: (*m*/*z* 675.38677) $[M-Cl]^-$ (calcd for $C_{35}H_{60}O_{10}Cl$, 675.38750).

3.3.4. *Acid hydrolysis of compounds* 1–3

A solution of each compound (1.0 mg) in 2 N HCl (1 mL) was refluxed for 3 h. Under a stream of N_2, the solution was concentrated. The residue was dissolved in 1-(trimethylsilyl)-imidazole and pyridine (0.1 ml), and the solution was stirred at 60 °C for 5 min. After drying under a stream of N_2, the residue was partitioned between H_2O and $CHCl_3$ (1 ml, 1:1 v/v). The $CHCl_3$ layer was analyzed by gas chromatography with an L-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C. A temperature program was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. The chromatographic peaks of the hydrolysate of **1** and **2** were detected at 14.64 and 14.72 min, respectively, for D-glucose, and 10.96/12.02 and 10.99/12.04 min, respectively for D-xylose. The peaks of D-xylose (10.96 and 12.01 min) were detected in the hydrolysate of **3**. Retention times for authentic samples were found at 14.71 min (D-glucose), and 10.98 and 12.01 min (D-xylose).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2012.02.011.

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