DOI 10.1007/s10600-023-03984-y
Chemistry of Natural Compounds, Vol. 59, No. 2, March, 2023

TWO NEW OLEANOLIC SAPONINS FROM STEM BARKS OF *Xeromphis nilotica*

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Phytochemical investigation of the stem bark of Xeromphis nilotica *led to the isolation of two new triterpenoid saponins; 3-*O-β-*D-glucopyranosyl (1→3)-*β-*D-glucopyranosyl-olean-12-ene-23,27-diol (**1**) and 3-*O-*{*β-*D-glucopyranosyl-(1→3)-*O-*[*β-*D-glucopyranosyl-(1→3)]-*β-*D-glucopyranosyl}-oleanolic acid (**2**). Their structures were elucidated through extensive chromatographic separations and spectroscopic methods [1D, 2D NMR and HR-ESI-MS]. Sugar moieties were identified through TLC after acid hydrolysis.*

Keywords: triterpene saponins, *Xeromphis nilotica*, Rubiaceae.

Xeromphis nilotica (Stapf) Keay (local name, Shagarat Almarfaein) is a Sudanese medicinal plant, commonly used in western Darfur and the Nuba Mountains area for treatment of jaundice and as a fish poison [1, 2]. It is also reputed to have a medicinal value for the treatment of various diseases including epilepsy, pain, mental disorder and fever [3, 4]. Pharmacological investigation of *X. nilotica* material reveals important biological activities such as antinociceptive, anti-inflammatory [5], molluscicidal [6], antischistosomal [7], and CNS activities [8, 9]. Previous phytochemical investigation of *X. nilotica* roots, stem barks and leaves resulted in the isolation of a number of aromatic compounds, particularly simple coumarin derivatives, iridoid [2], flavonoids, carbohydrates, alkaloids and triterpenoid saponins [1, 6, 10].

Our previous investigations of this species led to the isolation and characterization of coumarin glycosides [11] and triterpene saponins [12]. The following contribution is based on our continuing investigations on the chemical constituents of the stem barks of *X. nilotica.* In this paper, we report the structural determination of two new oleanolic saponins; 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosylolean-12-ene-23,27-diol (**1**) and 3-*O*-{*β*-D-glucopyranosyl-(1→3)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl}-oleanolic acid (**2**).

Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was found to be $C_{42}H_{70}O_{13}NH_4$ via the positive HR-ESI-MS ion at *m/z* 800.4463 $[M + NH_4]^+$. The ^{1}H NMR experiment data of **1** (Table 1), revealed six tertiary methyl protons at δ 0.83 (3H, s, H-24), 1.04 (3H, s, H-25), 1.14 (3H, s, H-26), 0.80 (3H, s, H-28), 0.89 (3H, s, H-29), and 0.94 (3H, s, H-30); one olefinic proton at δ 5.22 (1H, br.s, H-12); two anomeric protons at δ 4.36 (1H, d, J = 7.8 Hz) and 4.55 (1H, d, J = 7.8 Hz) together with one signal at δ 2.84–2.86 (1H, m, H-18) and one signal at δ 3.18 (1H, dd, J = 11.7, 4.4 Hz, H-3). ^{13}C NMR spectrum data of **1** (Table 2) showed 42 carbon resonances, 30 of the 42 carbons were assigned to the triterpenoid skeleton and 12 to the two sugars moiety. They included six tertiary carbons at δ 14.5 (C-24), 15.5 (C-25), 16.3 (C-26), 32.1 (C-28), 33.3(C-29), and 22.5 (C-30); two olefinic carbons at δ 122.1 (C-12) and 143.4 (C-13), and two anomeric protons at δ 104.4 (C-1′) and 103.7 (C-1″), together with three oxygenated methine carbons at δ 62.6 (C-27), 63.6 (C-23), and 89.4 (C-3).

Overall ^{1}H and ^{13}C NMR data of **1** (Tables 1 and 2) showed great similarity to olean-2-en-27-ol [13], except that C-23 and an C-3 were attached with a hydroxyl group and sugar chain, respectively, in **1**. Acid hydrolysis of 1 afforded sugar moiety which was identified by TLC as glucose. The HMBC spectrum (Fig. 1), showed the correlation between H-1″ (δ 4.55) of the terminal glucose with C-3′ (δ 86.4) of the inner glucose and H-1′ (δ 4.36) of the inner glucose with C-3 (δ 89.4) of aglycone.

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TABLE 1. ^{1}H NMR Data of Compounds **1** and **2** (400 MHz, MeOH-d_4, δ, ppm, J/Hz)

C atom	1	2	C atom	1	2
1	1.04–1.06 (m); 1.55–1.57 (m)	1.05–1.07 (m); 1.56–1.58 (m)	29	0.89 (s)	1.23 (s)
2	1.28–1.30 (m); 1.99–2.01 (m)	1.27–1.29 (m); 1.98–2.00 (m)	30	0.94 (s)	1.24 (s
3	3.18 (dd, J = 11.7, 4.4)	3.17 (dd, J = 11.6, 4.5)	1′	4.36 (d, J = 7.8)	4.34 (d, J = 7.8)
5	1.21–1.23 (m)	1.22–1.24 (m)	2′	3.25–3.27 (m)	3.33–3.35
6	1.96–1.98 (m); 1.77–1.79 (m)	2.00–2.02 (m); 1.80–1.83 (m)	3′	3.62 (t, J = 5.7)	3.50 (t, J = 8.8)
7	1.49–1.51 (m); 1.29–1.30 (m)	1.52–1.54 (m); 1.37–1.40 (m)	4′	3.28–3.30 (m)	3.25–3.27 (m)
9	1.84–186 (m)	1.86–1.88 (m)	5′	3.41 (1H, m)	3.35–3.37 (m)
11	1.97–1.99 (m); 1.98–2.00 (m)	1.93–1.95 (m); 1.97, 1.99 (m)	6′	3.84–3.86 (m)	3.86–3.88 (m)
12	5.22 (br.s)	5.19 (br.s)	1″	4.55 (d, J = 7.8)	4.57 (d, J = 7.8)
15	1.20–1.22 (m); 1.93–1.94 (m)	1.23–1.25 (m); 1.91–1.93 (m)	2″	3.37–3.39 (m)	3.28–3.30 (m)
16	1.22–1.23 (m); 1.88–1.90 (m)	1.24–1.26 (m); 1.90–1.92 (m)	3″	3.51 (t, J = 8.9)	3.51 (t, J = 8.4)
18	2.84–2.86 (m)	2.87–2.89 (m)	4″	3.31–3.33 (m)	3.57–3.29 (m)
19	1.01–1.03 (m); 1.67–1.69 (m)	1.11–1.13 (m); 1.66–1.68 (m)	5″	3.39–3.41 (m)	3.36–3.38 (m)
21	1.53–156 (m); 1.80–182 (m)	1.54–156 (m); 1.82–1.84 (m)	6″	4.05–4.07 (m)	3.96–3.98 (m)
22	1.24–1.26 (m); 1.45–1.47 (m)	1.71–1.73 (m) ; 1.91-1.93, m		3.66–3.68 (m)	3.69–3.71 (m)
23	3.66 (d, J = 11.2)	1.13 (s)	1‴		5.16 (d, J = 7.6)
	3.27 (d, J = 11.1)		2‴		3.63–3.65 (m)
24	0.83 (s)	0.82 (s)	3‴		3.53 (t, J = 8.8)
25	1.04 (s)	0.93 (s)	4‴		3.34–3.35 (m)
26	1.14 (s)	1.04 (s)	5‴		3.35–3.37 (m)
27	3.36 (d, J = 10.2)	1.88 (s)	6‴		3.94 (overlapped)
	3.66 (d, J = 10.4)				3.82–3.84 (m)
28	0.80 (s)	–			

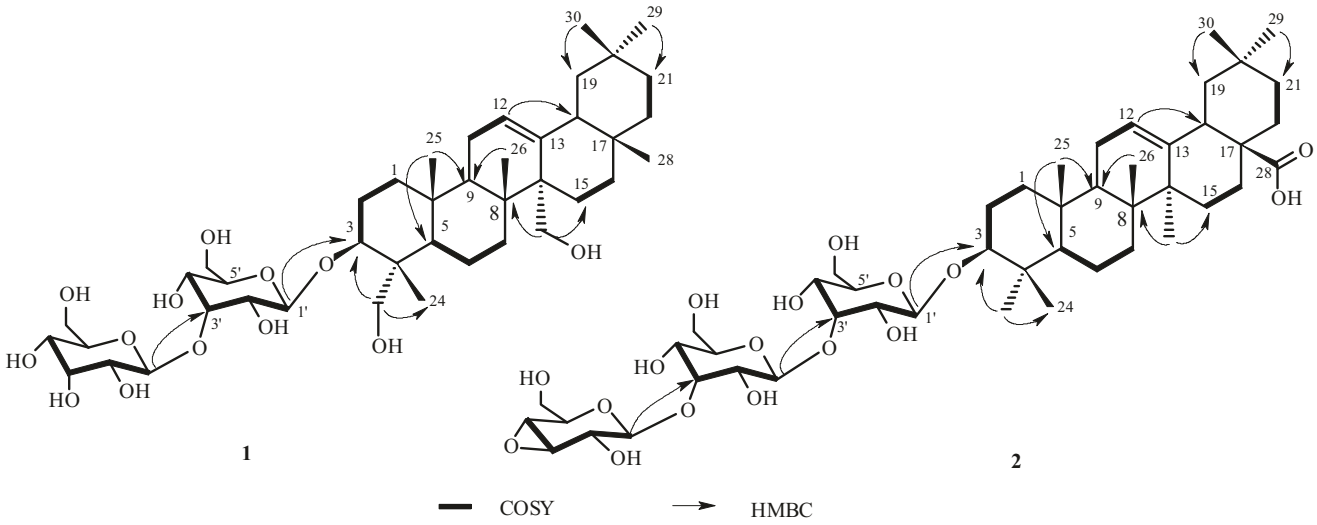

Fig. 1. The key HMBC and ^{1}H–^{1}H COSY correlations observed in compounds **1** and **2**.

The configuration of the sugars was established to be *β* glucose units characterized by the large coupling constants [14, 15], starting from the anomeric proton at δ 4.36 (d, J = 7.8 Hz) and the other anomeric proton resonating at δ 4.55 (d, J = 7.8 Hz). On the basis of all the foregoing information, compound **1** was identified as 3-*O*-*β*-D-glucopyranosyl (1→3)-*β*-D-glucopyranosylolean-12 ene-23,27-diol.

Compound **2** was obtained as a white amorphous powder. HR-ESI-MS, showed peak at *m/z* 965 $[M + Na]^+$, which corresponds to the molecular formula $C_{48}H_{78}O_{18}$. Detailed analysis of the ^{1}H NMR spectrum data of **2** (Table 1) exhibited seven methyl protons singlets at δ 1.13 (3H, s, H-23), 0.82 (3H, s, H-24), 0.93 (3H, s, H-25), 1.04 (3H, s, H-26), and 1.88 (3H, s, H-27), 1.23 (3H, s, H-29), 1.24 (3H, s, H-30); an olefinic proton as a broad singlet at δ 5.19 (1H, br.s, H-12); three anomeric protons δ 4.34 (d, J = 7.8 Hz, H-1′); 4.57 (d, J = 7.8 Hz, H-1″) and 5.16 (d, J = 7.6 Hz, H-1‴) together with a multiplet of one proton at δ 2.87–2.89 (1H, m, H-18) and one signal at δ 3.17 (1H, dd, J = 11.6, 4.5 Hz, H-3).

TABLE 2. ^{13}C NMR Data of Compounds **1** and **2** (400 MHz, MeOH-d_4, δ, ppm)

C atom	**1**	**2**	C atom	**1**	**2**
1	38.6 (CH_2)	38.3 (CH_2)	25	15.5 (CH_3)	14.6 (CH_3)
2	27.4 (CH_2)	28.5 (CH_2)	26	16.3 (CH_3)	16.8 (CH_3)
3	89.4 (CH)	89.6 (CH)	27	62.6 (CH_2)	26.2 (CH_3)
4	41.4 (C)	54.2 (C)	28	32.1 (CH_3)	183.8 (C)
5	55.8 (CH)	55.7 (CH)	29	33.3 (CH_3)	32.8 (CH_3)
6	17.9 (CH_2)	18.0 (CH_2)	30	22.5 (CH_3)	23.5 (CH_3)
7	32.3 (CH_2)	33.0 (CH_2)	1′	104.4 (CH)	104.9 (CH)
8	39.0 (C)	39.2 (C)	2′	74.0 (CH)	74.6 (CH)
9	47.6 (CH)	48.4 (CH)	3′	86.67 (CH)	86.5 (CH)
10	38.2 (C)	38.4 (C)	4′	70.1 (CH)	70.8 (CH)
11	23.1 (CH_2)	25.6 (CH_2)	5′	76.7 (CH)	76.6 (CH)
12	122.1 (CH)	121.2 (CH)	6′	61.1 (CH_2)	61.1 (CH_2)
13	143.4 (C)	145.1 (C)	1″	103.7 (CH)	103.7 (CH)
14	41.3 (C)	41.6 (C)	2″	73.5 (CH)	73.7 (CH)
15	27.0 (CH_2)	27.8 (CH_2)	3″	76.2 (CH)	82.6 (CH)
16	24.9 (CH_2)	25.1 (CH_2)	4″	68.6 (CH)	68.7 (CH)
17	46.2 (C)	46.6 (C)	5″	75.8 (CH)	75.9 (CH)
18	41.5 (CH)	42.0 (CH)	6″	61.3 (CH_2)	61.3 (CH_2)
19	45.87 (CH_2)	46.5 (CH_2)	1‴		101.2 (CH)
20	30.2 (C)	30.4 (C)	2‴		72.6 (CH)
21	32.6 (CH_2)	34.9 (CH_2)	3‴		74.6 (CH)
22	36.4 (CH_2)	36.6 (CH_2)	4‴		68.7 (CH)
23	63.6 (CH_2)	29.3 (CH_3)	5‴		76.6 (CH)
24	14.5 (CH_3)	15.7 (CH_3)	6‴		62.9 (CH_2)

The ^{13}C NMR spectrum data (Table 2) showed seven methyl carbons signals at δ 29.3 (C-23), 15.7 (C-24), 14.6 (C-25), 16.8 (C-26), 26.2 (C-27), 32.8 (C-29), and 23.5 (C-30); two olefinic carbon signals at δ 121.2 (C-12) and 145.1 (C-13); downfield signal at δ 183.8 (C-28), which presented carboxylic carbon and three anomeric carbons resonating at δ 104.9 (C-1′), 103.7 (C-1″), and 101.2 (C-1‴) attributed to three sugar residues. Acid hydrolysis suggested that the monosaccharide of this compound is D-glucose, which was identified by TLC analysis of its R_f value [16]. The aglycone was further recognized to be oleanolic acid by comparison of its ^1H and ^{13}C NMR data with those reported in the literature [14] which were in good agreement. The *β*-configuration of the anomeric position of the glucosyl units was deduced from the large values of the coupling constant of the H-1′, H-1″, and H-1‴ (J = 7.8 Hz; J = 7.8 Hz, and J = 7.6 Hz), respectively, the downfield shifts of C-3′ (86.5) and C-3″ (82.6) of the first and second glucosyl moieties suggested the bonding points of the trisaccharide chain [17, 18]. The key HMBC spectrum data (Fig. 1), showed correlations between the anomeric proton at δ_H 5.16 (H-1‴) of the terminal glucose with inner glucose at δ_C 82.6 (C-3″); the anomeric proton of the inner glucose at δ_H 4.57 (H-1″) with the first glucose at δ_C 86.5 (C-3′) and anomeric proton of the first glucose at δ 4.34 (H-1′) with the aglycone part at δ_C 89.6 (C-3). On the basis of this evidence, the structure of compound **2** elucidated as 3-*O*-{*β*-D-glucopyranosyl-(1→3)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl}-oleanolic acid.

EXPERIMENTAL

General Experimental Procedures. Melting points were recorded on an X-4 type micro-melting point apparatus, which was uncorrected. The NMR spectra were measured on a Bruker-DRX-400-NMR spectrometry and with TMS as internal standard, HR-ESI-MS were obtained with API QSTAR Pulsari System Mass Spectrometer. Column chromatography was carried out on silica gel (Merck Kiesel gel 300–400 mesh), TLC was performed with silica gel GF_{254} (Merck). All the chemicals and solvents were commercial grade and used after further purification.

Plant Material. "Shagarat Almarfaein" stem barks were collected from the Zalingei area, central Darfur State, Sudan, in April of 2017, and identified by Prof. G. A. Yagoub, Faculty of Agriculture, University of Zalingei. Voucher specimens (No. 20171013), was deposited in the herbarium of the author's laboratory.

Extraction and Isolation. The dried stem bark of *Xermophis nilotica* (1.5 kg) was powdered and extracted with 95% EtOH at room temperature (every 7 days × 3 L). After evaporation of the solvent under vacuum (Rotary evaporation), the dried residue (355 g) was suspended in H_2O and then partitioned with EtOAc. The EtOAc fraction (120 g) was chromatographed over silica gel (Merck Kiesel gel 300–400 mesh) using ($CHCl_3$–MeOH) gradually, first eluted with pure $CHCl_3$, then checked by thin layer chromatography (TLC) using the $CHCl_3$–MeOH (10:1, 10:2, 10:3) then with $CHCl_3$–MeOH (10:1–1:1) and finally eluted with pure MeOH (33 fractions were collected) as mobile phases. Fractions showing similarities on TLC were combined together to provide three fractions (F_I, F_{II}, and F_{III}). Fraction F_{III} (35 g) was loaded on a silica gel column eluting with EtOAc, increasing polarity by adding MeOH, to yield two subfractions (F_i, F_{ii}). Subfraction F_i was further subjected to column chromatography (CC) again using EtOAc and MeOH mixtures with increasing polarities to give compound **1**, while subfraction F_{ii} was re-chromatographed over silica to obtain compound **2**.

3-*O*-*β*-D-Glucopyranosyl (1→3)-*β*-D-glucopyranosylolean-12 ene-23,27-diol (1), white amorphous powder. UV 365 nm showed blue color, deep purple color after spraying 7% H_2SO_4 reagent, molecular formula $C_{42}H_{70}O_{13}NH_4$. HR-ESI-MS *m/z* 800.4463 $[M + NH_4]^+$. ^1H (400 MHz, MeOH-d_4) and ^{13}C (100 MHz, MeOH-d_4) NMR spectral data, see Tables 1 and 2.

3-*O*-{*β*-D-Glucopyranosyl-(1→3)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl}-oleanolic acid (2), white amorphous powder. UV 365 nm showed blue color and deep purple color after spraying 7% H_2SO_4 reagent. HR-ESI-MS, showed peaks at *m/z* 965.5073 $[M + Na]^+$, corresponding to formula $C_{48}H_{78}O_{18}Na$. ^1H (400 MHz, MeOH-d_4) and ^{13}C (100 MHz, MeOH-d_4) NMR spectral data, see Tables 1 and 2.

Acid Hydrolysis of the Saponins. 10 mg each of compound **1** and **2** was added to a solution of 10% AcOH–EtOH (10 mL). The mixture was stirred for 6 h. After cooling, the solution was removed under reduced pressure. The residue was diluted with H_2O (5 mL), and the resulting precipitate was collected and chromatographed on a silica gel column (35 g, Si gel). Elution of the column with 10% MeOH in $CHCl_3$ afforded the aglycones. The aqueous phase was neutralized by $NaHCO_3$ and analyzed for sugars using PC. The solvent system used was *n*-BuOH–AcOH–H_2O, 4:1:5 [15].

ACKNOWLEDGMENT

The authors are grateful to the China Scholarship Council (CSC) for the research scholarship, and to the Department of Botany, Faculty of Agriculture, University of Zalingei for facilitating the collection and authentication of the plant material.

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