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Osteosaponins 1 and 2: two new saponin glycosides from *Osteospermum vaillantii*

Bahar Ahmed[a]*, Riaz A. Khan[b], Tawfeq A. Al-Howiriny[c] and Adnan J. Al-Rehaily[c]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110 062, India;* [b]*Department of Chemistry, Manav Rachna International University (MRIU), Aravali Hills, Faridabad, HR, India;* [c]*Medicinal, Aromatic and Poisonous Plants Research Center, Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*

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Osteospermum vaillantii (Decne) T. Norl., collected from southern Saudi Arabia, yielded two new saponins characterised as 3-*O*-*β*-D-glucopyranosyl-(2′ → 1″)-*β*-D-glucopyranosyl-(3′ → 1‴)-*O*-*β*-D-galactopyranosyl-oleanolic acid, designated as osteosaponin (**1**), and 3-*O*-*β*-D-glucopyranosyl-(2′ → 1″)-*β*-D-glucopyranosyl-(2″-1⁗)-*β*-D-glucopyranosyl-(3′ → 1‴)-*O*-*β*-(-D-galactopyranosyl-oleanolic-acid-28-C(O)-*O*-*β*-D-(1⁗)-glucopyranosyl ester, designated as osteosaponin (**2**). The sugar attachments and configurations were confirmed by spectroscopic methods, that is, ^{1}H, ^{13}C-NMR, COSY, HSQC and HMBC-NMR experiments.

Keywords: *Osteospermum vaillantii*; Asteraceae; saponins; oleanolic acid; tri and penta-glycosides; *β*-D-galactopyranose derivatives

1. Introduction

Osteospermum vaillantii is a medicinal herb frequently used in Africa and Mediterranean desert regions as folk medicine by nomadic tribes and Arabian Bedouins for fever, stomach ailments and liver disorders. *Osteospermum* is the largest genus native to South Africa, with about 70 species in the tribe Calendulaceae and the family Asteraceae, and is commonly known as 'African daisy'. Only a few species have been chemically examined, with reports of sandaracopimarene, cassane diterpenes (Bohlmann & Zdero, 1975), tridecapentayene, sesquiterpene glycosides, trachylobane derivatives, triterpenes and saponins from different and representative species of this genus, that is, *Osteospermum imbricatum*, *Osteospermum microcarpum* subsp. *septentrionale*, *Osteospermum corymbosum*, *Osteospermum rigidum* var. *elegans*, and *Osteospermum caulescens*, distributed mainly in South Africa and Nigeria (Bohlmann, Wallmeyer, Jakupovic, & Ziesche, 1983; Bohlmann, Weikgenannt, & Zdero, 1973; Jakupovic, Zdero, Paredels, & Bohlmann, 1988). More recently, *O. vaillantii* from southern parts of the Arabian Peninsula have been found to contain triterpene

*Corresponding author. Email: baharchem@yahoo.com

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glycosides (Abdel-Sattar, 2001). We now report the presence of a triglycoside and a pentaglycoside derivative of an oleanolic acid triterpene (**1**, **2**) for the first time from *O. vaillantii* collected from southern Saudi Arabia.

2. Results and discussion

Compound **1** was obtained as a white solid and showed the presence of typical hydroxyls (ν_{max} 3400–3500 cm^{-1}) and carboxyl functionalities (1710 cm^{-1}) in its infrared (IR) spectrum. The proton NMR spectrum exhibited seven distinguishable signals of three protons each attributed to seven methyls (δ_H 0.84, 0.99, 0.97, 1.00, 1.16, 1.22 and 1.31), one double bond methine (δ 5.4, 1H, d, $J = 5.5$ Hz), 10 methylenes (δ 1.32–2.2, 10 × CH_2), three methines (δ 1.42, 1.45, 2.48) and a carbinolic proton (δ 3.10, 1H, dd, $J = 5.5$, 6.8 Hz) due to a triterpene moiety. Moreover, three anomeric protons (δ_H 5.00, dd, $J = 8.0$ Hz; 5.05, dd; $J = 7.5$ Hz, and 5.11, dd, $J = 7.6$ Hz) along with 21 other sugar protons in the region (δ_H 2.85–5.1) were also observed due to three sugar moieties in the molecule. The anomeric carbons at δ 104.4, 104.5 and 105.1 also substantiated the unambiguous presence of three sugar units, a double bond assignable to C-12 (δ_C 122.4, C-12; 144.4, C-13) and a carboxyl functionality attributable to C-17 (δ_C 180.1) in the molecule (Tables 1 and 2). The NMR observations coupled to the hydrolysis information suggested an oleanolic acid saponin with C-3 glycosylation by three interlinked sugars and an acid-free moiety at C-17 of the triterpene unit (^{13}C-NMR downward chemical shifts and DEPT analysis, Tables 3 and 4). A closer look at the structure was possible by its peracetylation with Ac_2O-pyridine/RT/12 h, which yielded acetate (**1a**). Its ^{13}C-NMR spectrum exhibited three anomeric carbons at δ_C 103.55, 99.32, 99.47 exhibiting connectivities with triterpenoidal C-3 and with internal sugar linkages to glucose units, Glu-I, Glu-II and a galactose moiety Gal-I. The presence of three glycosyl methylenes (sugar's sixth position-CH_2) at δ_H 4.15, dd, $J = 12.0$, 12.0,

Table 1. Aglycone part: ^{13}C-NMR chemical shifts for compounds (**1**) and (**2**).

Carbon position	DEPT	(**1**)	(**2**)	Carbon position	DEPT	(**1**)	(**2**)
1	CH_2	38.6	38.6	16	CH_2	23.7	23.4
2	CH_2	26.4	26.3	17	C	46.6	46.6
3	CH–OR	88.5	88.6	18	CH	41.0	41.3
4	C	39.2	39.4	19	CH_2	46.5	46.2
5	CH	55.7	55.4	20	C	31.1	30.2
6	CH_2	18.4	18.5	21	CH_2	34.1	33.6
7	CH_2	33.2	33.0	22	CH_2	33.2	32.2
8	C	39.8	39.5	23	CH_3	21.1	21.1
9	CH	48.1	47.6	24	CH_3	23.2	23.1
10	C	36.6	36.4	25	CH_3	15.4	15.3
11	CH_2	23.4	23.4	26	CH_3	17.4	17.2
12	CH=C	122.4	122.4	27	CH_3	26.3	25.8
13	CH=C	144.4	142.7	28	C=O	180.1	176.6
14	C	42.1	41.7	29	CH_3	27.9	28.0
15	CH_2	28.2	27.9	30	CH_3	28.0	28.0

Table 2. Aglycone part: DEPT analyses of acetylated derivatives (**1a**) and (**2a**).

Acetates	Carbon types	CMR values (in δ)
(**1a**)	$7 \times CH_3$	15.3, 16.29, 16.82, 24.0., 25.9, 27.7, 33.0
	$10 \times CH_2$	18.2, 23.0, 23.4, 25.8, 27.6, 32.4, 32.79, 33.8, 38.5, 45.9
	$5 \times CH$	41.08, 47.63, 55.64, 80.13, 122.57
	$7 \times C$	30.68, 36.71, 39.1, 39.3, 41.6, 46.49, 142.8
	$1 \times C{=}O$	182.61
	Total	Total aglycone carbon signals 30
(**2a**)	$7 \times CH_3$	16.5, 23.5, 27.5, 31.0, 28.0. 33.0, 37.0
	$10 \times CH_2$	18.2, 22.8, 23.4, 25.7, 27.69, 31.7, 32.9, 33.75, 38.85, 45.76
	$5 \times CH$	41.0, 47.58, 55.51, 81.09, 122.90
	$7 \times C$	30.6, 36.69, 38.8, 39.3, 41.69, 46.7, 142.87
	$1 \times C{=}O$	175.59
	Total	Total aglycone carbon signals 30

Table 3. Glycone part: DEPT analyses of acetylated derivatives **1a** and **2a** and carbon accountability.

Compounds	Carbon types	CMR values (in δ)
(**1a**)	$10 \times CH_3$	20.56, 20.60(×2), 20.66(×2), 20.76, 20.81, 20.84, 21.05, 21.11
	$3 \times CH_2$	60.77, 62.0, 62.75
	$12 \times CH$	66.8, 68.3, 68.5, 69.5, 70.7, 71.3, 71.5, 71.8, 72.0, 73.1, 77.7, 80.1
	$3 \times CH$, anomerics	103.6, 99.5, 99.3
	$10 \times C{=}O$	168.87, 169.18, 169.30, 169.39, 170.14, 170.16, 170.20, 170.39, 170.66, 170.69
	Total	Sugar units 3, glycone signals 38, aglycone signals 30, total carbon signals 68
(**2a**)	$17 \times CH_3$	20.44, 20.52, 20.59(×2), 20.60(×2), 20.65, 20.69(×2), 20.72(×2), 20.78, 20.82, 20.85(×2), 21.06, 21.34
	$5 \times CH_2$	60.91, 61.03, 61.5, 62.4, 62.65
	$20 \times CH$	66.8, 66.9, 67.9, 68.5, 69.0, 69.3, 69.9, 70.4, 70.5, 70.9, 71.0, 71.4, 71.7, 72.5, 72.8, 73.3, 74.5, 77.2, 78.5, 81.1
	$5 \times CH$, anomeric	104.6, 103.0, 101.4, 101.2, 91.6
	$17 \times C{=}O$	168.76, 168.94, 169.27, 169.34 × 2, 169.44 × 2, 170.13, 170.15, 170.22, 170.34, 170.44 × 2, 170.63 × 2, 170.74, 170.76
	Total	Sugar units 5, glycone signals 64, aglycone signals 30, total carbon signals 94

2.0 Hz, δ_C 60.77; 4.25, dd, $J = 11.8$, 11.8, 1.8 Hz, 62.05; and 4.32, dd, $J = 11.5$, 11.5, 1.5 Hz, 62.75 as well as other carbon signals for glycosyl's skeletal carbons between 68.31 and 90.82, 12 carbons also confirmed the presence of three hexose sugar units, wherein two were identified as 3-*O*-*β*-D-glucopyranosyl and one was found to be 3-*O*-*β*-D-galactopyranosyl based on the sugar hydrolysis data and NMR chemical shifts

Table 4. ^{1}H and ^{13}C-NMR methyl data, glycone units, acetylated compounds (**1a**) and (**2a**).

(**1a**)			(**2a**)		
^{1}H (CH_3)	^{13}C (CH_3)	^{13}C (C=O)	^{1}H (CH_3)	^{13}C (CH_3)	^{13}C (C=O)
2.00	20.56	168.87	1.69	20.44	168.76
2.02(×2)	20.60(×2)	169.18	1.72	20.52	168.94
2.07	20.66(×2)	169.30	1.79	20.59(×2)	169.27
2.09(×2)	20.76	169.39	1.794	20.60(×2)	169.34(×2)
2.10(×2)	20.81	170.14	1.796	20.65	169.44(×2)
2.14	20.84	170.16	1.84	20.69(×2)	170.13
2.22	21.05	170.20	1.841	20.72(×2)	170.18
	21.11	170.39	1.85	20.78	170.22
		170.66	1.855	20.82	170.34
		170.69	1.857	20.85(×2)	170.44(×2)
			1.868	21.06	170.63(×2)
			1.871	21.34	170.74
			1.879		170.76
			1.891		
			1.892		
			1.93		
			1.97		

for sugars (Table 5). The triterpene skeletal carbons for a C-12 double bond at δ_C 122.4, 143.4 ($_{12}CH{=}C_{13}$), and one carbinolic carbon at δ_C 80.1 confirmed the C-3 glycone attachment to the triterpene nucleus and the C_{12} double bond position in the triterpene framework, further supplemented by inputs from HSQC, HMBC and ^{1}H-^{1}H COSY data (Table 6). In addition, *O*-acetylated glycosyl methines, 10 more methylene carbons, 7 methyls, 7 quaternary carbons, 5 methines and one carboxyl carbon observed in its ^{13}C-DEPT-135 analyses and ^{13}C-NMR spectrum confirmed the aglycone moiety's structure as oleanolic acid. The connectivity of C-3 with a sugar moiety and the placement of the C-12/13 double bond as well as a carboxyl at C-17 was further substantiated by 2D NMR (HSQC, HMBC and HOMO-COSY) observations (Bock & Pedersen, 1983; Bock & Thorgersen, 1982; Bradbury & Jenkins, 1984; Cai, Xiao, & Wei, 1982). The sugar connectivity between Glu-I, Glu-II and Gal-I were deduced based on the downfield shift of C-2′ and C-3′ of the Glu-I sugar unit. The observance of the carboxyls' C=O functionality at δ_C 180.1 did not show any long range correlation in the HMBC spectrum to any of the sugar carbons, which indicated the free nature of the oleanolic acid with C-17 carboxyl group, whereas C-3 attachment of three hexose sugars with internal glycosyls connectivities as (Glu-$I_{C\text{-}2'} \rightarrow {}_{C\text{-}1''}$Glu-II) and (Glu-$I_{C\text{-}3'} \rightarrow {}_{C\text{-}1'''}$Gal-I) was confirmed by HMBC, HSQC and ^{1}H-^{1}H COSY spectra (Figure 1). Thus, the structure of compound (**1**) was deduced as 3-*O*-*β*-D-glucopyranosyl-(2′ → 1″)-*β*-D-glucopyranosyl-(3′ → 1‴)-*O*-*β*-Dgalactopyranosyl oleanolic acid and has been designated as osteosaponin **1** (Figure 2(a)). The 3D structure and critical correlations of acetyl derivative **1a** are shown in Figure 3.

Compound **2** was obtained as a white solid and showed the typical hydroxyls and carboxyl functionalities (3400–3500 and 1710 cm^{-1}) in the IR spectrum, while the proton NMR exhibited distinguishable signals attributed to seven methyls (δ_H 0.84, 0.92, 0.94, 1.02, 3 × 1.19), a double bond methine (δ 5.40, 1H, d, $J = 5.5$ Hz), 10

Table 5. Glycone part: chemical shifts of free and acetylated derivatives **1**, **2** and **1a**, **2a**.

Unit	Carbon no.	(**1**)	(**1a**)	(**2**)	(**2a**)
Glu-I	1′	103.4	103.5	104.1	104.6
	2′	79.9	80.1	79.8	80.1
	3′	77.7	77.7	78.0	78.5
	4′	68.5	68.5	67.2	68.5
	5′	72.9	73.0	73.0	74.5
	6′	62.0	62.8	62.0	62.5
Glu-II	1″	99.0	99.3	103.6	103.0
	2″	69.2	69.5	76.7	77.2
	3″	71.0	71.5	71.6	71.4
	4″	66.1	66.8	68.3	69.0
	5″	71.6	72.0	72.6	73.3
	6″	62.1	62.0	61.1	61.0
Gal-I	1‴	99.1	99.5	101.1	101.4
	2‴	70.5	70.7	70.4	70.4
	3‴	71.7	71.3	70.7	71.0
	4‴	67.2	68.3	67.2	67.9
	5‴	71.3	71.8	71.6	72.8
	6‴	60.3	60.8	61.3	61.5
Glu-III	1⁗			91.6	91.6
	2⁗			68.9	69.3
	3⁗			70.4	70.5
	4⁗			66.3	66.8
	5⁗			71.5	71.7
	6⁗			60.3	60.9
Glu-IV	1′′′′′			101.2	101.2
	2′′′′′			70.0	69.9
	3′′′′′			70.0	70.5
	4′′′′′			66.7	66.8
	5′′′′′			71.0	71.7
	6′′′′′			62.2	62.7

Table 6. Major correlation connectivities for aglycone parts (**1**) and (**2**).

	(**1**)			(**2**)		
No.	HSQC	HMBC*	COSY	HSQC	HMBC*	COSY
3	88.5	1′, 5, 2, 23, 24	H_2-2	88.6	1′, 5, 2, 23, 24	H_2-2
2′	79.9	1′, 1″, 3′	H-1′, H-3′	79.8	1′, 1″, 3′	H-1′, H-3′
3′	77.7	5′, 1‴	H-4′, H-2′	78.0	5′, 1‴	H-4′, H-2′
5′	72.9	4′, 6′	H-4′, H_2-6′	73.0	4′, 6′	H-4′, H_2-6′
12	122.4	11, 18, 13	H-11	122.4	11, 18, 13	H-11
13	144.4	–	–	142.7	–	–
17	46.6	–	–	46.6	–	–
28	181.1	–	–	176.6	–	–
1′	103.4	3, 3′, 2′	H-2′	104.1	3, 3′, 2′	H-2′
1″	99.1	3″, 2″, 2′	H-2′, H-2″	101.1	3″, 2″, 2′	H-2″
1‴	99.0	3‴, 2‴, 3″	H-2‴	103.6	3‴, 2‴, 3′	H-2‴
1⁗	–	–	–	91.6	2⁗, 3⁗	H-2⁗
1′′′′′	–	–	–	101.2	H-2″, H-2′′′′′, H-3′′′′′	H-2′′′′′

Note: *Correlations of protons/hydrogen are shown to carbon in HMBC.

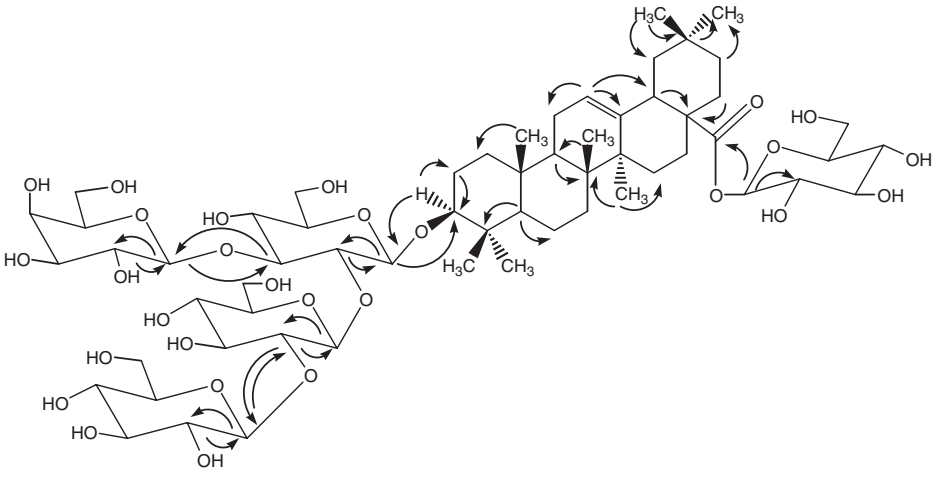

Figure 1. Significant HMBC for osteosaponin **1**.
Note: Correlations of protons/hydrogen are shown to carbon.

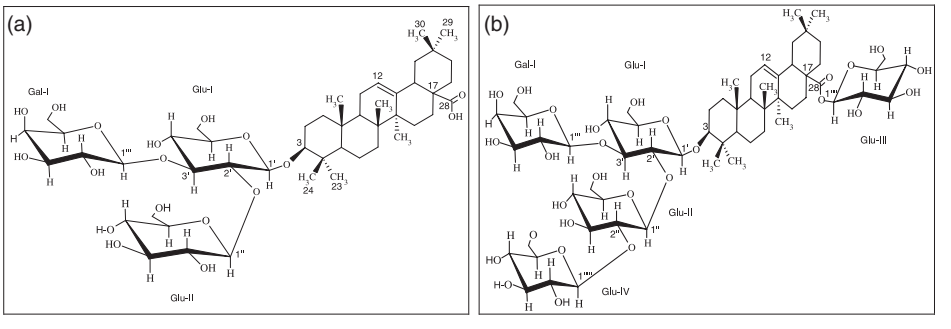

Figure 2. (a) Osteosaponin **1** and (b) osteosaponin **2**.

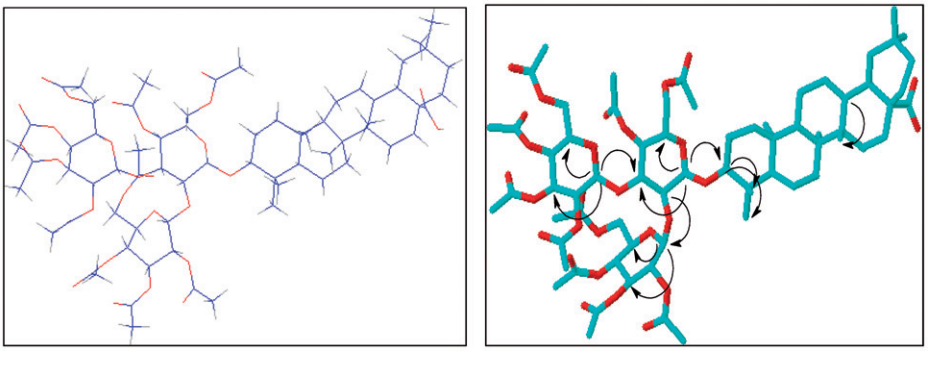

Figure 3. 3D structure and critical correlations for acetyl osteosaponin (**1a**).

methylenes (δ 1.23–2.22, $10 \times CH_2$), three methines (δ 1.40, 1.43, 2.26) and a carbinolic proton (δ 3.11, dd, $J = 5.6$, 6.5 Hz) due to a triterpene skeleton, and five anomeric protons (δ 5.01, dd, $J = 7.80$ Hz; 5.51, dd, $J = 7.90$ Hz; 5.50, dd, $J = 7.60$ Hz; 2×5.72, dd, $J = 7.90$ Hz) and other typical sugar protons in the region (δ 3.6–4.6, 35 H) due to five sugar moieties in the molecule. The positions and assignments of carbon-13 signals of aglycone moieties and sugar units were correlated with expected reported values of the relevant compounds in the literature (Gafner, Msonthi, & Hostettmann, 1985; Ikuta et al., 1997; Laffite et al., 1978; Pizza, Zhong-Liang, & de Tommasi, 1987; Texier, Ahond, Regerat, & Pourrat, 1984). The five anomeric carbon signals (δ_C 106.2, 104.6, 105.1, 102.6 and 95.6) also revealed the unambiguous presence of five sugar moieties, one methine carbon (C-12 at δ 122.4, C_{13} at δ143.8 for $_{12}CH{=}C_{13}$) exhibiting one double bond, and one carbonyl carbon (C=O at δ 176.4) showed the presence of one carbonyl function in the molecule (Tables 1 and 2). The ^{13}C-NMR observations coupled to the hydrolysis information suggested an oleanolic acid saponin as the tentative structure, with C-3 glycosylation by at least four interlinked sugars, and an esterified carboxyl moiety at C-17 of the triterpene unit attached with one glucose moiety (^{13}C downward chemical shifts and DEPT analysis, Tables 4 and 5). A closer look at the structure was possible by its per-acetylation with Ac_2O-pyridine/RT/12 h, which yielded (**2a**), exhibiting five anomeric carbons in its ^{13}C NMR spectrum at δ 104.61, 103.04, 101.39, 101.22 and 91.56, showing connectivities with C-3, C-17, C-28 and with internal glycosyl linkages to glucose units, Glu-I, Glu-II, Glu-III, Glu-IV and Gal-I, respectively. The presence of five glycosyls methylenes (sugar's sixth position-CH_2) at δ_H, 4.10, dd, $J = 12.0$, 12.0, 2.0 Hz, δ_C 62.6; 4.20, dd, $J = 11.8$, 11.8, 2.0 Hz, 62.4; 4.30, dd, $J = 11.5$, 11.5, 1.5 Hz, 61.0; 4.35, dd, $J = 12.0$, 12.0, 2.0 Hz, 60.9 and 4.40, dd, $J = 11.8$, 11.8, 1.8 Hz, 61.5 as well as other carbons signal between 68.31–90.43, 20 carbons also substantiated the presence of five hexose sugar units, of which four were 3-*O*-β-D-glucopyranosyls and one was found to be 3-*O*-β-D-galactopyranosyl based on NMR chemical shifts and hydrolysis findings (Kasai, Suzuo, Asakawa, & Tanaka, 1977; Kochetkov, Chizhov, & Shashkov, 1984). The triterpene skeletal carbons for a C-12 double bond at 122.90, 142.87 ($_{12}CH{=}C_{13}$) and one carbinolic carbon at δ 80.1 confirmed the sugar connectivities and double bond position in the triterpene framework by inputs from HSQC, HMBC and ^{1}H HOMO-COSY data (Table 6). In addition, *O*-acetylated sugar methines, 10 more methylene carbons, seven methyls, seven quaternary carbons, five methines and one carboxyl carbon found in the skeletal region of triterpene and its ^{13}C-DEPT-135 analyses, and ^{13}C-NMR spectral observation confirmed the aglycone moiety's structure as substituted oleanolic acid. The sugar connectivity between Glu-I, Glu-II, Glu-IV and Gal-I were deduced based on the downfield shift of the $C_{2'}$ (Glu-I $_{C\text{-}2'} \rightarrow {}_{C\text{-}1''}$ Glu-II) and $C_{3'}$ carbons of the Glu-I sugar unit as (Glu-I $_{C\text{-}3'} \rightarrow {}_{C\text{-}1'''}$ Gal-I) and Glu-IV attachment to Glu-II (Glu-II $_{C\text{-}2''} \rightarrow {}_{C\text{-}1''''}$ Glu-IV) by its ^{13}C-NMR spectral downfield shift for the Glu-II$_{C\text{-}2''}$ position (Table 3) (Tori, Sea, Yoshimura, Arita, & Tomita, 1977; Tori, Seo, Shimaoka, & Tomita, 1974; Tori et al., 1976). Thus, the structure of compound (**2**) was deduced as 3-*O*-β-D-glucopyranosyl-(2′ → 1″)-β-D-glucopyranosyl-(2″-1‴′)-β-D-glucopyranosyl-(3′ → 1‴)-*O*-β-(D-galactopyranosyl-oleanolic-acid-28-C(O)-*O*-β-D-(1⁗′)-glucopyranosyl ester, and has been designated as osteosaponin **2** (Figure 1(b)). The 3D structure and critical correlations of acetyl derivative **2a** are shown in Figure 4.

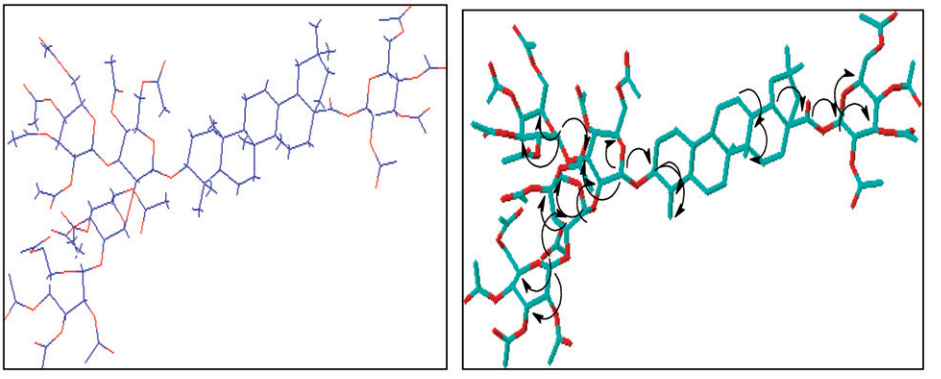

Figure 4. 3D structure and critical correlations for acetyl osteosaponin (**2a**).

3. Experimental

3.1. *General*

The IR spectra were recorded as KBr pellets on a PYE 130 UNICAM spectrophotometer, mass spectra on a Finnegan MAT 300 mass spectrophotometer, 1D and 2D NMR on a Bruker DRX 400 spectrometer in DMSO-d_6 using TMS as an internal standard reference; chemical shift in δ (ppm) coupling constants (J values) are in Hertz. Column chromatography was performed on silica gel (230–400 mesh) as an adsorbent. Thin layer chromatography was performed on silica gel G.

3.2. *Plant material*

The aerial parts of *O. vaillantii* (Decne) T. Norl. were collected from the Southern Abha regions of the Kingdom of Saudi Arabia in March 2006. A voucher specimen has been deposited in the Herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

3.3. *Extraction and isolation of chemical constituents*

The shade dried powdered aerial parts of *O. vaillantii* (5.0 kg) were defatted with petroleum ether (b.p. $\sim 60^{\circ}$C) and exhaustively extracted in a Soxhlet apparatus with 95% ethanol for 20 h. The ethanol was evaporated under reduced pressure ($<40^{\circ}$C) to obtain a gummy residue (600 g), which was re-suspended in 1.5 L of water and then partitioned by successive extraction between ethyl acetate and *n*-butanol to give 170 and 290 g fractions as semi-solid red-brown jellies, respectively. The *n*-butanol (200 g) extract was adsorbed on silica gel and chromatographed over silica gel column (5×100 cm) using a $CHCl_3$–MeOH mixture in increasing polarity as eluent; 250 mL fractions of each were cut. The eluent of the $CHCl_3$–MeOH mixture (75/25 and 60/40 onwards) afforded compounds **1** (200 mg) and **2** (300 mg), respectively. The compounds were further purified by re-column chromatography and crystallised from benzene–methanol mixtures, which afforded four saponin glycosides, of which two were known products (Abdel-Sattar, 2001).

3.4. *Acid hydrolysis*

The acid hydrolysis in 5% aq. HCl of compounds (**1** and **2**) afforded oleanolic acid as an aglycone moiety identified by comparison with authentic samples, while the aqueous phase yielded β-D-glucose and β-D-galactose as glycone moieties authenticated by comparisons with authentic samples.

3.5. *Acetylation of compounds 1 and 2*

The saponins (**1** and **2**, 50 mg each) were acetylated with Ac_2O-pyridine by storing overnight and then by refluxing on a water bath for about 6 h. The crude products were purified by silica gel CC (columns 2.5×100 cm) in petroleum ether-$CHCl_3$ with increasing polarity as eluent and then re-crystallised from methanol, which yielded acetates **1a** (45 mg) and **2a** (43 mg), respectively.

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