Two New Oleanane Triterpene Glycosides from the Bark of *Terminalia arjuna*

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Two new oleanane triterpene glycosides designated as termiarjunoside I (**1**) and termiarjunoside II (**2**), isolated from the bark of *Terminalia arjuna* (Combretaceae), have been characterized as olean-1α,3β,9α,22α-tetraol-12-en-28-oicacid-3β-D-glucopyranoside (**1**) and olean-3α,5α,25-triol-12-en-23,28-dioic acid-3β-D-glucopyranoside (**2**), respectively, on the basis of chemical and spectral data evidences.

Key words: Terminalia arjuna, Combretaceae, Termiarjunoside I, Termiarjunoside II

Introduction

Terminalia arjuna (Roxb.) Wight and Arnot. (*Combretaceae)*, is a deciduous tree found throughout India. Its stem bark has found extensive application in Indian system of medicine as a cardiac tonic with particular efficacy against heart failure, ischaemic cardiomyopathy, artherosclerosis and coronary artery ailments [1]. The cardioprotective activities of the bark have also been substantiated by various pharmacological evaluations and clinical trials [2]. A number of terpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid and phytosterols have been isolated from the bark [3], but the issue of active principles and the mechanism of therapeutic activity of *T. arjuna* remain to be elucidated. In the present study, we have isolated two new oleanane type triterpene glycosides named Termiarjunoside I (**1**) and Termiarjunoside II (**2**) from ethanolic extract of *T. arjuna* bark.

Results and Discussion

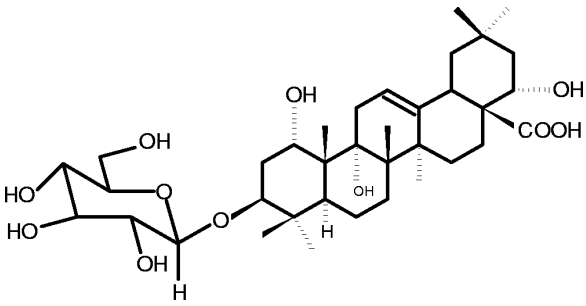
Termiarjunoside I (**1**) was obtained as colourless crystalline solid having an optical rotation through out of $[\alpha]_D^{25} + 27^\circ$(c 0.11 MeOH). The FABMS of **1** displayed a molecular ion peak at m/z = 666 $[M]^+$, consistent with a molecular formula of $C_{36}H_{58}O_{11}$, which was also supported by ^{13}C and DEPT NMR spectra. The other fragment ions appeared at m/z = 504 [M+H-Glc]$^+$ and 239 [M+H-Glc-$C_{16}H_{24}O_3$]$^-$. The HREIMS of aglycone **1** revealed the molecular ions peak at m/z = 503.0160 $[M]^+$ and other important fragment ion peaks due to retro-Diels-Alder fragmentation pattern of α-amyrin-type triterpene at m/z = 264.0018, 240.0321, 222.0216, 204.0141, 186.8132, 145.3280, which are characteristic of an olean-12-ene derivative compound possessing three hydroxyl groups in ring A/B and one hydroxyl group in ring D or E [4].The ^{13}C NMR spectrum showed 36 signals, which indicated that, the isolated triterpene glycoside contains one sugar unit. Absorption bands at 3430, 1707, 1650, 1084 cm^{-1} in the IR spectrum suggested the presence of hydroxyl, carbonyl, olefinic and glycosidic linkage, respectively. The presence of an olefinic proton at $\delta = 5.22$ (d, $J = 5.06$ Hz) (H-12), which corresponded to the signal at $\delta_c = 122.2$ (C-12) in the HMBC NMR spectrum, and the presence of seven tertiary methyls in the ^1H NMR spectrum of **1** displaying signals at δ = 0.91, 0.88, 0.62, 0.69, 1.20, 0.84, and 0.86 for C-23, C-24, C-25, C-26, C-27, C-29, and C-30, respectively, indicated the aglycone being a substituted Δ^{12}-oleanane-type triterpene [5]. The ^1H NMR spectrum showed the four oxygenated methine proton resonances at $\delta = 3.13, 3.09, 3.79, 3.11$ corresponding to C-1, C-3, C-9, C-22, respectively. The ^{13}C and DEPT NMR signals exhibited the presence of seven methyls,

nine methylenes, eleven methines and nine quarternary carbons including the four oxygenated protons resonating at $\delta = 77.66$ (C-1), 82.25 (C-3), 72.37 (C-9), 80.01 (C-22) and a downfield signal at $\delta = 175.75$, which was assigned to C-28 carboxylic carbon in ring E at C-17 position. These analogous data suggested that aglycone **1** was an oleanane type triterpene with the above functionalities [6]. Acid hydrolysis of **1** with 2 *N* HCl gave D-glucose together with the aglycone moiety. The absolute configuration of the sugar was determined by direct comparison of HPLC and optical rotation results with those of a reference compound. The hydroxyl position of C-1, C-3, C-9 and C-22 were determined by the ROESY spectrum which showed the correlation of H-1 with the methyl protons H_3-24 and H_3-25 and of H-3 with H-5, indicating the position of hydroxyl groups to be at $1\alpha,3\beta$, which was further confirmed through the coupling constants between H-1, H-2 (4.4) and H-3, H-2 (9.5), respectively. The coupling constants between H-21, H-22 (4.5, 5.12 Hz), indicated the hydroxyl groups to have 22α orientations, which was further supported by the ROESY correlation of H-22 with H_3-29 [7]. The H-1' anomeric proton was observed at $\delta = 4.96$, which corresponded in turns to the signal at $\delta = 103.5$, indicating the presence of a sugar unit. The anomeric proton resonance at $\delta = 4.96$ correlated with the glucosyl H-6' signal at $\delta = 3.62$ in the TOCSY experiment [8], suggesting that the sugar unit was glucose. In the HMBC spectrum, the anomeric proton signal at $\delta = 4.96$ also correlated with the signal at $\delta = 82.25$ (C-3) and the attendant downfield signal of aglycone at $\delta = 75.60$ suggested that the glucose unit was connected to the C-3 hydroxyl group. The connectivity of the glucose unit and the stereochemistry at the C-3 position was confirmed by a ROESY experiment [9], where a correlation was observed between the glycosyl anomeric proton ($\delta = 4.96$) and Hβ-3 at $\delta = 3.09$. Moreover, the ^{1}H NMR coupling constant of H-3 ($J = 9.53$, 5.5 Hz) confirmed that the stereochemistry of C-3 was in β-position [9]. On the account of these spectral evidences the structure of compound **1** was elucidated as olean-1α,3β,9α,22α-tetraol-12-en-28-oic acid-3β-D-glucopyranoside (Scheme 1, Table 1).

Termiarjunoside II (**2**) was obtained as colourless amorphous powder. It did not show the molecular ion peak in the FABMS and EIMS and the molecular formula, $C_{36}H_{56}O_{12}$, was determined through exact measurement of various mass fragment ions and ^{13}C NMR and DEPT broad band spectra. The FABMS showed the prominent ions peak at m/z = 518 [M+H-Glc]$^+$ and 268 [M-H-Glc-$C_{16}H_{24}O_2$]$^+$. The HREIMS of aglycone **2** revealed the molecular ion peak at m/z = 518.0123 [M+H]$^+$ and other important fragment ion peaks due to the retro-Diels-Alder fragmentation pattern of α-amyrin-type triterpene at m/z = 270.0313,

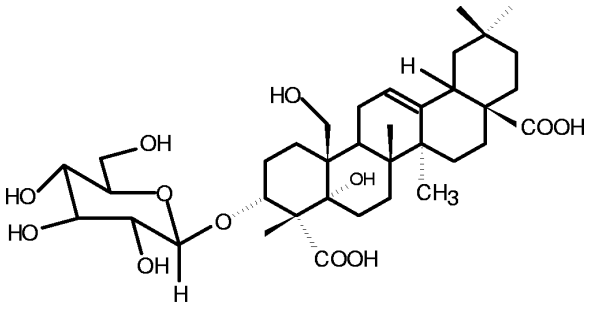
Table 1. ^{13}C NMR data of aglycones and compounds **1** and **2** in DMSO-d_6 (600 MHz).

Carbon	**1**	Aglycone 1	**2**	Aglycone 2
1	77.66	77.60	40.68	40.68
2	27.73	27.78	27.10	27.10
3	82.25	75.60	80.50	80.50
4	38.86	38.32	41.31	41.31
5	54.88	53.96	77.54	77.54
6	18.09	18.25	17.16	17.16
7	32.18	33.50	33.19	33.19
8	41.07	41.30	42.43	42.43
9	72.37	72.20	47.04	47.04
10	45.18	45.65	37.29	37.29
11	23.31	22.56	23.00	23.00
12	122.20	122.35	121.54	121.54
13	143.22	143.56	143.50	143.50
14	47.27	47.60	44.10	44.10
15	28.32	29.10	22.45	22.45
16	26.98	27.20	31.55	31.55
17	48.53	48.65	46.00	46.00
18	80.01	80.53	31.73	31.73
23	28.71	28.85	25.56	25.56
24	16.21	16.78	175.14	175.14
25	16.65	17.20	63.88	63.88
26	16.68	16.50	16.69	16.69
27	24.10	23.90	16.75	16.75
28	175.75	175.80	175.25	175.14
29	28.00	28.50	32.68	32.68
30	24.46	24.10	23.30	23.30
3-O-Glc 1				
1'	103.50		104.50	
2'	67.10		69.52	
3'	69.50		72.33	
4'	72.37		75.80	
5'	76.67		76.61	
6'	60.63		60.65	



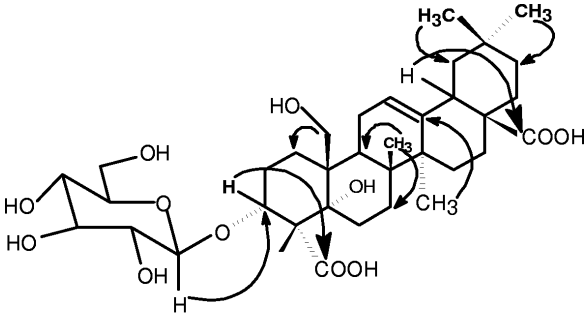
1

Scheme 1. Chemical structure of compound **1**.



2

Scheme 2. Chemical structure of compound **2**.



2

Scheme 3. Selected HMBC of compound **2**.

248.0186, 203.0318, 188.0119 and 145.0163, which are characteristic of an olean-12-ene derivative possessing three hydroxyl groups in rings A/B [4]. Its IR spectrum was consistent with the presence of hydroxyl (3510, 3440 cm^{-1}), carbonyl (1710 cm^{-1}), and olefinic (1655 cm^{-1}) groups and glycosidic linkage (1062 cm^{-1}), respectively. Compound **2** indicated eight double bond equivalents, five of them were adjusted in a pentacyclic carbon frame work and one each in olefinic linkage, carboxylic group and sugar moiety. The presence of an olefinic proton at $\delta = 5.25$ (dd, $J = 5.49$, 4.76 Hz), (H-12) corresponding to the signal at $\delta_c = 121.54$ (C-12) in the HMBC NMR spectrum and the presence of seven tertiary methyl groups in the ^{1}H NMR spectrum of **2** indicated the aglycone being a substituted Δ^{12-}-oleanane-type triterpene. The ^{1}H NMR spectrum showed the three oxygenated methylene protons resonating at $\delta = 3.14$ (C-3), 3.69 (C-5) and 3.41 (C-25), respectively. The ^{13}C NMR signals for oxygenated carbons were observed at $\delta = 80.5$ (C-3), 77.54 (C-5), 63.88 (C-25), respectively. The downfield signals at $\delta = 175.14$ (C-24) and 175.25 (C-28) corresponded to the carboxylic carbon. These analogous data suggested that aglycone **2** was an oleanane-type triterpene attached with the above-mentioned functionalities [6].

The stereochemistry of **2** was determined by analysis of its coupling constants and ROESY data. The coupling constant of H-3 (4.03, 5.09 Hz) indicated that the C-3 hydroxyl group should have 3α orientation, which was further supported by the ROESY correlations of H-3 with H-1β showing the intense cross peak between H_3-24 and the H-25 with H_3-26. Acid hydrolysis of **2** with 2 *N* HCl gave D-glucose together with the aglycone moiety. The absolute configuration of the sugar was determined by direct comparison of HPLC and optical rotation results with those of a reference compound. An anomeric proton observed at $\delta = 5.10$ (d, $J = 5.86$ Hz), corresponding to the signal at $\delta = 104.5$, indicated the presence of a sugar unit. The sugar moiety was confirmed on the basis of HMBC and ROESY correlations and comparison with glucose as mentioned in case of compound **1**. On the account of these spectral evidences the structure of compound **2** was elucidated as olean-3α,5α,25-triol-12-en-23,28-dioic acid-3β-D-glucopyranoside (Scheme 2, 3, Table 1).

Experimental Section

Melting points were determined with the scientific micro-melting point apparatus. UV spectra were recorded on a Perkin Elmer Lamda-20 spectrophotometer. FT-IR spectra were recorded on a Perkin Elmer-377 spectrophotometer using KBr pellets. ^{1}H, ^{13}C NMR, APT, ^{1}H-^{1}H-COSY, HMBC, NOESY, ROESY and TOCSY spectra were recorded on a Brucker 600 MHz spectrometer with TMS used as internal standard. FAB-MS was scanned on a JMS-PX 303 mass spectrometer and HR-EIMS was recorded on a Joel D-300 mass instrument. Optical rotation was measured on a Perkin Elmer-241 polarimeter at 589 nm in MeOH. HPLC was performed on Shimadzu, LC-10 AT-VP using an ODS column (waters NONA-Pak C_{18}, 21.2 mm i.d×250 mm, 7 μm and 4.6 mm i.d×250 mm). HPTLC was recorded on Camag using Linomat-5, in HPTLC plate silica gel H (5 – 7 μm). Column chromatography was carried out with silica gel particle size (60 – 120 mesh), Merck. TLC was conducted on silica gel 60 F_{254} (Merck).

Plant material

The bark of *T. arjuna* was collected from Rishikesh, India, in October 2001. The specimen was identified by Dr. M. P. Sharma (Taxonomist), in the Department of Botany, Jamia

Hamdard. A voucher specimen is deposited in the herbarium of the Phytochemistry Research Laboratory having registration number 03/15/Phytochem, Jamia Hamdard.

Extraction and separation

The air-dried and pulverized bark (2.5 kg) was exhaustively extracted with 95% ethanol at 80 °C in a Soxhlet apparatus for five days. The combined extracts were evaporated to dryness under reduced pressure to yield a dried ethanolic extract (270 g). The residue was sequentially refluxed with solvents of increasing polarity, *viz.*, petroleum ether, dichloromethane, and acetone fraction. Acetone fraction (35 g), was loaded into a column with (60 – 120) mesh silica gel (700 g) and stepwise eluted with $CHCl_3$ and $CHCl_3$-MeOH in the ratios of 98 : 2, 95 : 5, 9 : 1 and 8 : 2 (elution volume, 5 l each) to give 4 corresponding fractions, *viz*, fr. A-1 (3 g), fr. A-2 (4.1 g), fr. A-3 (8.5 g), and fr. A-4 (7.3 g). Fr. (A-1, A-2, A-3) exhibited an identical R_f in TLC plate, so we remixed them together, whereas fr. A-4 showed a different spot on the TLC plate, but was not analyzed further. The pooled fractions were further separated by preparative HPLC using reverse phase, C_{18}-column (Waters, 21.2 mm i.d×250 mm, ODS, 7 μm) for preparative HPLC, the flow rate of 5 ml/min; detection: 260 nm with the mobile phase MeOH-H_2O (8 : 2) afforded compounds **1** (R_t = 12 min, 80 mg) and **2** (R_t = 14.6 min, 72 mg). Both compounds **1** and **2** of the acetone fraction were qualitatively and quantitatively analyzed 0.021% and 0.019%, respectively, using HPTLC methods (solvent system-$CHCl_3$-MeOH-CH_3COOH 95 : 5 : 0.2), after spraying with Liebermann-Burchard reagent at 254 nm UV light.

*Acid hydrolysis of **1** and **2***

A solution of the compound (25 mg) in 10% HCl-60% EtOH (10 ml) was heated on a steam water bath for 6 h. After dilution with water and neutralization with Ag_2CO_3, the solution was extracted with EtOAc. The EtOAc layer was evaporated and chromatographed on a flash silica gel (230 – 400 mesh) column, eluted with hexane-ethyl acetate (7 : 3) to get 15 mg aglycone, which was analyzed by IR, NMR and MS by comparison with compounds **1** and **2**. The water layer was concentrated and passed through a NOVA-Pak C_{18} cartridge (Waters, 4.6 mm i.d×250 mm, silica gel, 5 μm), then separated in several injections by HPLC [HPLC conditions were mobile phase: MeCN-H_2O (3 : 1); flow rate: 0.7 ml/min; detection: refractive index] to afford D-glucose (from **1** and **2**) (R_t = 16.92 and 16.95 min, $[\alpha]_D^{25} + 52.7°$).

*Termiarjunoside I (**1**)*

Amorphous powder; $[\alpha]_D^{25} + 27°$ (c 0.11 MeOH); m. p. 234 – 238 °C. – UV (MeOH): λ_{max} = 263 nm. – IR (KBr): ν_{max} 3430, 1707, 1650, 1084 cm^{-1}. – ^{1}H NMR (DMSO-d_6) δ = 5.22 d (J = 5.06 Hz, H-12), 3.79 d (J = 5.2 Hz, H-9), 3.13 dd (J = 3.66, 4.4 Hz, H-1), 3.11 dd (J = 4.5, 5.12 Hz, H-22), 3.09 dd (J = 9.53, 5.5 Hz, H-3), 0.86 (brs, H-30), 0.84 (brs, H-29), 1.20 (brs, H-27), 0.69 (brs, H-26), 0.62 (brs, H-25), 0.88 (brs, H-24), 0.91 (brs, H-23), 3-O-sugar δ = 4.96 d (J = 4.76 Hz, H-1'), 3.41 dd (J = 5.87, 5.13 Hz, H-2'), 3.10 dd (J = 4.03, 5.50 Hz, H-3'), 3.08 dd (J = 5.49, 5.5 Hz, H-4'), 3.20 d (J = 4.3 Hz, H-5'), 3.60 d (J = 5.87 Hz, H_2-6'a), 3.62 d (J = 5.13 Hz, H_2-6'b). – FABMS m/z = 666 (calcd for $C_{36}H_{58}O_{11}$ $[M]^+$), 504 $[M+H\text{-}Glc]^+$, 239 $[M+H\text{-}Glc\text{-}C_{16}H_{24}O_3]^-$. – ^{1}H NMR of aglycone **1** ($CDCl_3$) δ = 5.10 d (J = 5.63 Hz, H-12), 3.11 dd (J = 4.1, 3.82 Hz, H-1), 3.25 dd (J = 9.81, 5.90 Hz, H-3), 3.79 d (J = 5.1 Hz, H-9), 3.25 dd (J = 4.5, 5.2 Hz, H-22), 0.91 (brs, H-30), 0.86 (brs, H-29), 1.15 (brs, H-27), 0.62 (brs, H-26), 0.68 (brs, H-25), 0.86 (brs, H-24), 0.86 (brs, H-23). – ^{13}C NMR of aglycone and **1**: see in Table 1. – HREIMS (70 eV) (aglycone **1**) m/z (%) = 503.0160 $[M]^+$ (2.9), 484.0189 (5.1), 426.3261 (4.2), 408.0693 (4.3), 264.0018 (60.8), 240.0321 (79.3), 222.0216 (8.0), 204.0141 (100), 186.8132 (28.0), 145.3280 (18.3).

*Termiarjunoside II (**2**)*

Amorphous powder; $[\alpha]_D^{25} + 28°$ (c 0.12 MeOH); m. p. 226 – 228 °C. – UV (MeOH): λ_{max} = 243 nm. – IR (KBr): ν_{max}: 3510, 3440, 1710, 1655, 1062 cm^{-1}. – ^{1}H NMR (DMSO-d_6) δ = 5.25 dd (J = 5.49, 4.76 Hz, H-12), 3.14 dd (J = 4.03, 5.09 Hz, H-3), 3.69 d (J = 5.7 Hz, H-5), 3.41 d (J = 5.13 Hz, H-25), 0.87 (brs, H-30), 0.86 (brs, H-29), 0.90 (brs, H-27), 0.65 (brs, H-26), 1.10 (brs, H-24), 3-O-sugar δ = 5.10 d (J = 5.86 Hz, H-1'), 3.12 dd (J = 7.33, 8.06 Hz, H-2'), 3.11 dd (J = 7.3, 6.60 Hz, H-3'), 3.16 dd (J = 3.66, 4.03 Hz, H-4'), 3.18 dd (J = 5.12, 3.66 Hz, H-5'), 3.44 d (J = 5.87 Hz, H-6'). – FABMS m/z = 680 $[M]^+$, 518 $[M+H\text{-}Glc]^-$, 268 $[M\text{-}H\text{-}Glc\text{-}C_{16}H_{24}O_2]^+$. – ^{1}H NMR of aglycone **2** ($CDCl_3$) δ = 5.10 dd (J = 5.3, 4.62 Hz, H-12), 3.20 dd (J = 4.13, 5.0 Hz, H-3), 3.75 d (J = 5.6 Hz, H-5), 3.31 d (J = 5.2 Hz, H-25), 0.67 (brs, H-30), 0.96 (brs, H-29), 0.80 (brs, H-27), 0.75 (brs, H-26), 1.20 (brs, H-24), – ^{13}C NMR of aglycone and **2:** see in Table 1. – HREIMS (70 eV) (aglycone **2**) m/z (%) = 518.0123 (2.9), 270.0313 (60.8), 248.3261 (79.3), 203.0318 (100), 188.0119 (28.0), 145.4639 (18.3).

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