Saponins of *Allium elburzense*

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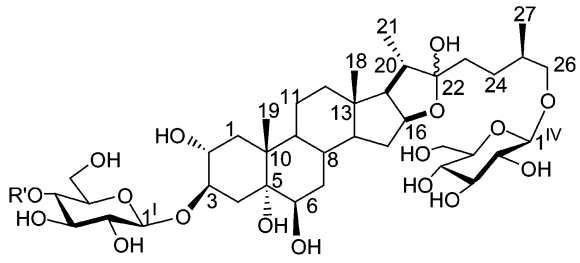
A phytochemical investigation of the bulbs of *Allium elburzense* has been undertaken, leading to the isolation of 13 furostanol and spirostanol saponins, eight of which are new, namely, elburzensosides A1/A2 (**1a/1b**), B1/B2 (**2a/2b**), C1/C2 (**3a/3b**), and D1/D2 (**4a/4b**). On the basis of spectroscopic analysis, mainly 2D NMR and mass spectrometry, and chemical methods, the structures of the new compounds were determined as furost-2α,3*β*,5α,6*β*,22α-pentol 3-*O*-*β*-D-glucopyranosyl 26-*O*-*β*-D-glucopyranoside (**1a**), furost-2α,3*β*,5α,6*β*,22α-pentol 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl] 26-*O*-*β*-D-glucopyranoside (**2a**), furost-2α,3*β*,5α,22α-tetrol 3-*O*-*β*-D-glucopyranosyl 26-*O*-*β*-D-glucopyranoside (**3a**), and furost-2α,3*β*,5α,22α-tetrol 3-*O*-[*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranosyl] 26-*O*-*β*-D-glucopyranoside (**4a**), and the corresponding epimers at position 22 (**1b**−**4b**). Along with these compounds we have isolated the corresponding 22-*O*-methyl derivatives that we consider extraction artifacts. All the new elburzensosides A1/A2−D1/D2 possess as a common structural feature an OH-5α that is rare among furostanol saponins. The reported compounds have been isolated in large amounts, and this makes *A. elburzense* a prolific producer of saponins of the furostanol and spirostanol types.

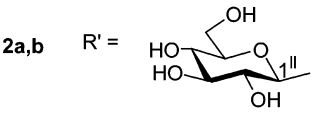
Garlic and onions are among the oldest cultivated herbs, with their origin dating back to ancient Egypt (3200−2800 B.C.).[1] Cloves of garlic have been found in the tomb of Tutankhamen and in the sacred underground temple of the bulls of Saqqara. Egyptians thought garlic and onions aided endurance and consumed large quantities of these. Raw plants were routinely given to asthmatics and to those suffering from bronchial-pulmonary complaints. The Persians constituted a link for the transfer of these plants from Egypt to the Greeks and Romans, who used these plants as important healing agents, just as they are still used by many people in the Mediterranean area.[2] In fact, *Allium* species are a rich source of phytonutrients, potentially useful for the treatment or prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataracts, and disturbances of the gastrointestinal tract (e.g., colic pain, flatulent colic, and dyspepsia).[3−8]

In the course of our ongoing investigation on the chemistry of the genus *Allium*,[8−10] we have investigated *A. elburzense* Wendelbo (Alliaceae), which has not been studied before phytochemically. This species is endemic in Iran and has been found only on the Elburz mountain range (3300 m), from where the plant takes its name. Commonly known by the local name of "Walak", it is a perennial bulbous plant that grows 5 to 16 cm in height. The plant produces pink flowers that bloom from April to May.[11] Leaves and bulbs of this plant are used as food, due to its sweet taste, typical of the plant species belonging to the onion taxum. In Iranian folk medicine it has also been used as an antirheumatic, aphrodiasic, antiduretic, and anthelminthic herb.[12]

Examination of the MeOH extract of the bulbs of *A. elburzense* revealed the presence of high concentrations of saponins, and we have isolated eight new furostanol saponins as major metabolites, named elburzensosides A1/A2 (**1a/1b**), B1/B2 (**2a/2b**), C1/C2 (**3a/3b**), and D1/D2 (**4a/4b**), together with five known saponins of the spirostanol type (**5**−**9**). Here we report a detailed description of the new compounds, which possess in common the rare OH-5α group.



1a,b R' = H



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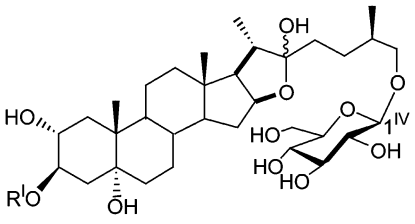
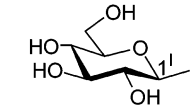
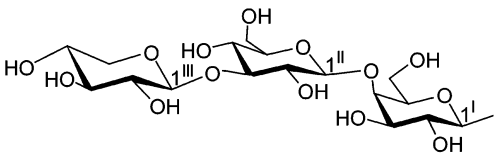




3a,b R' =



4a,b R' =

a and b, respectively, for OH-22α and OH-22β

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Table 1. ^{1}H NMR Data in CD_3OD of the Aglycon Portions of Elburzensosides A1 (**1a**), B1 (**2a**), C1 (**3a**), and D1 (**4a**)

position	**1a** δ_H (int., mult., *J* in Hz)	**2a** δ_H (int., mult., *J* in Hz)	**3a** δ_H (int., mult., *J* in Hz)	**4a** δ_H (int., mult., *J* in Hz)
1a	2.26 (1H,dd,11.5,7.5)	2.25[a]	2.28 (1H,dd,11.5,7.5)	2.16[a]
1b	1.48 (1H,dd,11.5,4.0)	1.48[a]	1.36 (1H,dd,11.5,4.0)	1.36[a]
2	3.59 (1H,ddd,10.0,7.5,4.0)	3.59[a]	3.60 (1H,ddd,10.0,7.5,4.0)	3.60[a]
3	3.73 (1H,ddd,10.0,7.5,4.0)	3.74[a]	3.73 (1H,ddd,10.0,7.5,4.0)	3.73[a]
4a	1.53 (1H,dd,11.5,7.5)	1.53 (1H,dd,11.5,7.3)	1.41 (1H,dd,11.5,7.5)	1.52 (1H,dd,11.5,7.3)
4b	1.35 (1H,dd,11.5,4.0)	1.38 (1H,dd,11.5,3.5)	1.32 (1H,dd,11.5,4.0)	1.35 (1H,dd,11.5,3.5)
6a	3.91 (1H,bd,4.0)	3.90 (1H,bd, 4.0)	1.60 (1H,bdd, 10.5, 4.0)	1.58 (1H,bdd,10.5,4.0)
6b			1.56 (1H,ddd,10.5,7.5,4.0)	1.55 (1H,ddd,10.5,7.5,4.0)
7a	1.77[a]	1.75[a]	1.77[a]	1.76[a]
7b	1.61 (1H,bd,10)	1.64 (1H,bd,10)	1.62 (1H,bd,10)	1.60 (1H,bd,10)
8	1.26 (1H, m)	1.28 (1H, m)	1.26 (1H,m)	1.26 (1H,m)
9	1.45 (1H,dt,10.0,10.0,4.0)	1.46 (1H,dt,10.0,10.0,4.0)	1.36 (1H,dt,10.0,10.0,4.0)	1.45 (1H,dt,10.0,10.0,4.0)
11a	1.37[a]	1.38[a]	1.38[a]	1.37[a]
11b	1.31[a]	1.30[a]	1.29[a]	1.30[a]
12a	1.76[a]	1.74[a]	1.75[a]	1.74[a]
12b	1.15[a]	1.20[a]	1.16[a]	1.15[a]
14	1.22 (1H,m)	1.22 (1H,m)	1.21 (1H,m)	1.19 (1H,m)
15a	1.97[a]	1.98[a]	1.98[a]	1.93[a]
15b	1.21[a]	1.22[a]	1.23[a]	1.21[a]
16	4.58 (1H,td,6.0,6.0,7.5)	4.62 (1H,td,6.0,6.0,7.5)	4.62 (1H,td,6.0,6.0,7.5))	4.63 (1H,td,6.0,6.0,7.5)
17	1.76[a]	1.73[a]	1.74[a]	1.72[a]
18	0.82 (3H,s)	0.80 (3H,s)	0.83 (3H,s)	0.80 (3H,s)
19	1.10 (3H,s)	1.00 (3H,s)	0.90 (3H,s)	0.89 (3H,s)
20	2.08 (dq,6.5,5.5)	2.08 (dq,6.5,5.5)	2.11 (dq,6.5,5.5)	2.00 (dq,6.5,5.5)
21	1.05 (3H,d,6.5)	1.10 (3H,d,6.5)	1.01 (3H,d,6.5)	1.00 (3H,d,6.5)
23a	1.70[a]	1.70[a]	1.70[a]	1.70[a]
23b	1.68[a]	1.68[a]	1.68[a]	1.68[a]
24a	1.63[a]	1.62[a]	1.62[a]	1.63[a]
24b	1.22[a]	1.23[a]	1.19[a]	1.17[a]
25	1.73 (1H,m)	1.73 (1H,m)	1.71 (1H,m)	1.73 (1H,m)
26a	3.87 (1H,dd,9.5,3.9)	3.86 (1H,dd,9.5,3.9)	3.85 (1H,dd,9.5,3.9)	3.88 (1H,dd,9.5,3.9)
26b	3.20 (dd,9.0,6.0)	3.20 (dd,9.0,6.0)	3.19 (dd,9.0,6.0)	3.18 (dd,9.0,6.0)
27	0.96 (3H,d,6.5)	0.96 (3H,d,6.5)	0.96 (3H,d,6.5)	0.96 (3H,d,6.5)

[a] Overlapped with other signals.

Results and Discussion

Bulbs of *A. elburzense* were air-dried and exhaustively extracted at room temperature with hexane, $CHCl_3$, $CHCl_3$–MeOH (9:1), and MeOH (see Experimental Section). The MeOH extracts were partitioned between butanol and water, and the butanol-soluble portion was separated by sequential chromatographic techniques, affording, as major metabolites, furostanol saponins (total saponin content 1367.0 mg/kg).

Elburzensoside A1 (**1a**) was isolated as an amorphous solid in high yield with a molecular formula of $C_{39}H_{66}O_{17}$, deduced by a HRFABMS measurement. Preliminary ^{1}H NMR analysis of **1a** (CD_3OD, Tables 1 and 3) indicated a glycoterpenoid nature of the compound. The ^{1}H NMR spectra showed four methyls, two tertiary (δ 0.82 and 1.10) and two secondary (δ 0.96, 1.05), and two anomeric (δ 4.25 and 4.34) protons. The ^{13}C NMR spectrum (Tables 2 and 3) showed 39 resonance lines (supporting the molecular formula deduced above), with 27 attributed to the aglycon part and 12 to two monosaccharides. In particular, the two anomeric carbons resonated at δ 103.3 and 104.6. In this spectrum, a diagnostic signal was observed at δ 111.9, indicating the presence of a hemiacetal carbon and suggesting a furostane nature for the steroidal aglycon of **1a**.[9]

Combined analysis of the 2D COSY and HOHAHA spectra of **1a** allowed the detection of five spin systems, with three belonging to the aglycon moiety and the other two to the two monosaccharides. Each proton was related to the directly bonded carbon through a HSQC spectrum. For the aglycon, the first spin system included the ring A protons (C-1 to C-4), the second started from the oxygenated C-6 and extended to the rings B–E proton to C-21, while the third included the side-chain protons (C-23 to C-27). Then, the HMBC spectrum allowed these partial substructures to be interconnected (Figure 1). The following cross-peaks were diagnostic: H_3-19 with C-1, C-5, and C-9; H-3, H_2-4, and H-6 with C-5; H_3-18 with C-12, C-13, and C-14; H_3-21 with C-17; and H_2-23 with C-20, C-22, and C-24. Concerning the stereochemistry of the aglycon, the

Table 2. ^{13}C NMR Data in CD_3OD of the Aglycon Portions of **1a**, **2a**, **3a**, and **4a**

position	**1a** δ_C (mult.)	**2a** δ_C (mult.)	**3a** δ_C (mult.)	**4a** δ_C (mult.)
1	37.4 (CH_2)	37.4 (CH_2)	39.3 (CH_2)	37.9 (CH_2)
2	71.6 (CH_2)	71.6 (CH_2)	71.6 (CH_2)	81.0 (CH_2)
3	83.1 (CH)	83.1 (CH)	82.7 (CH)	84.6 (CH)
4	35.8 (CH_2)	35.8 (CH_2)	35.1 (CH_2)	35.8 (CH_2)
5	75.2 (C)	74.9 (C)	75.2 (C)	75.2 (C)
6	76.0 (CH)	76.1 (CH)	31.0 (CH_2)	30.8 (CH_2)
7	38.0 (CH_2)	38.0 (CH_2)	35.1 (CH_2)	35.0 (CH_2)
8	32.8 (CH)	32.5 (CH)	32.8 (CH)	32.8 (CH)
9	45.9 (CH)	45.8 (CH)	44.2 (CH)	45.8 (CH)
10	42.2 (C)	41.8 (C)	42.1 (C)	42.1 (C)
11	22.3 (CH_2)	22.3 (CH_2)	22.2 (CH_2)	22.3 (CH_2)
12	41.1 (CH_2)	41.1 (CH_2)	41.2 (CH_2)	41.0 (CH_2)
13	42.3 (C)	42.3 (C)	42.6 (C)	43.9 (C)
14	56.9 (CH)	57.1 (CH)	57.2 (CH)	57.3 (CH)
15	34.5 (CH_2)	34.6 (CH_2)	35.0 (CH_2)	35.0 (CH_2)
16	82.2 (CH)	81.8 (CH)	82.1 (CH)	82.2 (CH)
17	64.0 (CH)	65.9 (CH)	64.1 (CH)	65.1 (CH)
18	17.0 (CH_3)	14.7 (CH_3)	17.0 (CH_3)	17.0 (CH_3)
19	17.1 (CH_3)	17.0 (CH_3)	14.6 (CH_3)	13.7 (CH_3)
20	40.8 (CH)	40.7 (CH)	40.9 (CH)	41.0 (CH)
21	15.9 (CH_3)	13.7 (CH_3)	15.8 (CH_3)	15.8 (CH_3)
22	111.9 (C)	112.7 (C)	111.9 (C)	107.8 (C)
23	34.9 (CH_2)	34.6 (CH_2)	35.0 (CH_2)	34.0 (CH_2)
24	28.6 (CH_2)	30.7 (CH_2)	30.7 (CH_2)	29.0 (CH_2)
25	36.9 (CH)	37.4 (CH)	37.0 (CH)	37.1 (CH)
26	71.7 (CH_2)	71.8 (CH_2)	71.8 (CH_2)	71.4 (CH_2)
27	17.3 (CH_3)	17.1 (CH_3)	17.3 (CH_3)	17.3 (CH_3)

Table 3. ^{1}H and ^{13}C NMR Data of the Sugar Portions of Compounds **1a/1b/3a/3b** (data extracted from **1a**), **2a/2b** (data extracted from **2a**), and **4a/4b** (data extracted from **4a**)

	1a		**2a**		**4a**	
position	δ_H (int., mult., *J* in Hz) (mult.)	δ_C	δ_H (int., mult., *J* in Hz) (mult.)	δ_C	δ_H (int., mult., *J* in Hz) (mult.)	δ_C
	Glc		Glc		Gal	
1^I	4.34 (1H,d,7.5)	104.6 (CH)	4.47 (1H,d,7.5)	104.7 (CH)	4.36 (1H,d,7.4)	102.8 (CH)
2^I	3.40 (1H,t,7.5)	78.1 (CH)	3.40 (1H,t,7.5)	78.2 (CH)	3.68 (1H,dd,7.4,8.1)	76.0 (CH)
3^I	3.30[a]	77.9 (CH)	3.30[a]	74.9 (CH)	3.53[a]	78.3(CH)
4^I	3.61 (1H,dd,6.8,6.5)	77.1 (CH)	3.69 (1H,dd,6.8,6.5)	81.8 (CH)	4.04 (1H,bd,2.2)	79.8 (CH)
5^I	3.40 (1H,m)	74.9 (CH)	3.40 (1H,m)	74.9 (CH)	3.90[a]	67.2 (CH)
6^I a	3.65 (1H,bd,11.5)	62.8 (CH_2)	3.65 (1H,bd,11.5)	62.7 (CH_2)	3.59[a]	62.9 (CH_2)
6^I b					3.67[a]	
			Glc		Glc	
1^{II}			4.68 (1H,d,7.5)	104.2 (CH)	4.64 (1H,d,7.3)	102.9 (CH)
2^{II}			3.37 (1H,t,7.5)	77.9 (CH)	3.78 (1H,dd,7.3,8.1)	78.1 (CH)
3^{II}			3.20[a]	77.7 (CH)	3.75 (1H,dd,7.3,8.1)	87.8 (CH)
4^{II}			3.23 (1H,dd,6.8,6.5)	71.5 (CH)	3.32[a]	75.3 (CH)
5^{II}			3.27 (1H,m)	78.2 (CH)	3.68[a]	65.1 (CH)
6^{II} a			3.65 (1H,bd,11.5)	63.0 (CH_2)	3.80[a]	63.1 (CH_2)
6^{II} b			3.84 (1H,bd,11.5)		3.55[a]	
					Xyl	
1^{III}					4.96 (1H,d,7.4)	104.3 (CH)
2^{III}					3.28[a]	75.7 (CH)
3^{III}					3.55[a]	78.3 (CH)
4^{III}					3.61 (1H,ddd,2.1,7.8,8.8)	72.8 (CH)
5^{III}					3.30[a]	71.0 (CH_2)
6^{III} a					3.92[a]	
6^{III} b						
	Glc		Glc		Glc	
1^{IV}	4.25 (1H,d,7.5)	103.3 (CH)	4.34 (1H,d,7.5)	103.3 (CH)	4.24 (1H,d,7.5)	104.9 (CH)
2^{IV}	3.38 (1H,t,7.5)	78.1 (CH)	3.38 (1H,t,7.5)	78.4 (CH)	3.40 (1H,t,7.5)	78.1 (CH)
3^{IV}	3.20[a]	77.9 (CH)	3.20[a]	77.8 (CH)	3.30[a]	77.5 (CH)
4^{IV}	3.23 (1H,dd,6.8,6.5)	77.1 (CH)	3.23 (1H,dd,6.8,6.5)	77.1 (CH)	3.61 (1H,dd,6.8,6.5)	77.9 (CH)
5^{IV}	3.25 (1H,m)	74.9 (CH)	3.25 (1H,m)	71.5 (CH)	3.40 (1H,m)	71.7 (CH)
6^{IV} a	3.66 (1H,bd,11.5)	62.6 (CH_2)	3.66 (1H,bd,11.5)	62.7 (CH_2)	3.68 (1H,bd,11.5)	62.9 (CH_2)
6^{IV} b	3.84 (1H,bd,11.5)		3.84 (1H,bd,11.5)		3.84 (1H,bd,11.5)	

[a] Overlapped with other signals.

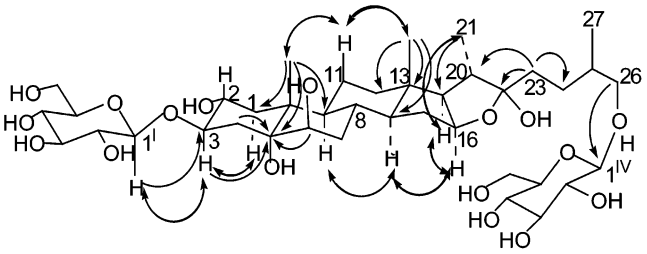

Figure 1. Selected HMBC (H→C) and ROESY (H↔H) NMR correlations observed for compound **1a**.

diaxial coupling between H-3 and H-1α, detected in a ROESY spectrum (Figure 1), indicated a *cis* orientation among these protons and OH-5α. This last was evidenced by the downfield shifted H-1α signal in the ^{1}H NMR spectrum (Table 1). The *β*-orientation of OH-6 was determined by the small coupling constants found for the H-6 signal (δ 3.91, bd, J = 4.0 Hz), indicating its equatorial position. The 25*R* stereochemistry of the side chain was deduced by the resonances of protons and carbons at C-25, C-26, and C-27 and by the vicinal couplings between H-25 and the two H-26, in comparison with literature data.[13] ROESY correlations (Figure 1) between H-11/H_3-19, H-11/H_3-18, H-9/H-14, H-14/H-16, H-16/H-17, and H-17/H_3-21 completed the relative stereochemistry of **1a** with the usual B/C *trans*, C/D *trans*, D/E *cis*, and C-20α stereochemistry.[8,14] On the basis of these data, the stereochemistry of the aglycon chiral centers has been assigned as shown. Finally, the stereochemistry at C-22 was assigned as α by accurate analysis of NMR data, as described above and by comparison with other compounds previously described.[8,9]

The first step in the analysis of the saccharide part of **1a** was the association of the two anomeric carbons (δ 103.3 and 104.6) with the relevant anomeric proton signals (δ 4.25 and 4.34, respectively), through the HSQC experiment. Their nature was determined by combined analysis of the 2D COSY, HOHAHA, and HSQC spectral data. Then, the HMBC and ROESY spectra gave key information on the glycosidic linkages. The sugar unit, for which the anomeric proton resonated at lower field, was placed at C-3 of **1a** by interpretation of the key HMBC correlation peak (Figure 1) between H-1^I (δ 4.34) and C-3 (δ 83.1), with this junction being also confirmed by the strong ROESY peak (Figure 1) between H-1^I and H-3 (δ 3.73). The second sugar had to be linked at position 26 on the furostanol structure of the aglycon. As expected, a HMBC cross-peak (Figure 1) was observed between C-26 (δ 71.7) and H-1^{IV} (δ 4.25).

The nature of the monosaccharides was determined by analysis of their 2D COSY, HOHAHA, HSQC, and HMBC spectra. Starting from the anomeric proton at δ 4.34 (H-1^I), we identified the sequence of a hexopyranose unit. The large coupling constants, observed in the 2D HOHAHA subspectra (Table 3) for all the proton resonances, indicated its *β*-glucopyranose nature. With the same type of analysis the sugar linked at C-26 was identified as a further *β*-glucopyranose.

To confirm the nature of the sugar units and to determine their absolute configuration, **1a** was subjected to acid hydrolysis (1 N HCl), followed by trimethylsilylation and GC analysis on a chiral column in comparison with both series of glucose. By this procedure the sugars were identified as D-glucose. This procedure has been applied to all the new isolated compounds. All these data indicated the structure of **1a** as furost-2α,3*β*,5α,6*β*,22α-pentol 3-*O*-*β*-D-glucopyranosyl 26-*O*-*β*-D-glucopyranoside.

High-resolution FABMS indicated that elburzensoside A2 (**1b**) was isomeric to elburzensoside A1. The ^{1}H and ^{13}C

NMR spectra of **1b** (Tables 1−3 and Experimental Section) were almost identical with that of **1a**, differing only in the resonances of the atoms located near C-22. This suggested that the two compounds are epimers having opposite configuration at the hemiacetal carbon (C-22). This was confirmed by the observation that both **1a** and **1b**, after being kept overnight in aqueous solution at room temperature, gave rise to an equilibrium between the two forms (about 40% of **1a** and 60% of **1b**). In a manner described for other furostanol saponins,[8,9] we tentatively assigned the 22α orientation to **1a** and the 22*β* orientation to **1b**. This was based on the observation of the downfield shifts in the ^{1}H NMR resonances of H_3-21 (*δ* 1.05) and H-16 (*δ* 4.58) of **1a**, in comparison with those of **1b** (H_3-21 *δ* 1.02; H-16 *δ* 4.38), due to the deshielding effect of the *cis*-oriented OH-22 group. All the new compounds have been isolated in both the 22α and 22*β* forms. The structure elucidation of the 22α form is described in detail. Concerning the 22*β* form, the structure elucidation has been performed using the same arguments described for **1a/1b**.

Elburzensoside B1 (**2a**), $C_{45}H_{76}O_{22}$, deduced by HR-FABMS, was isolated as a minor constituent from more polar fractions. The presence of three sugars was apparent from the three anomeric proton signals (*δ* 4.34, 4.47, 4.68) associated with the relevant carbon atoms in the ^{13}C NMR spectrum (*δ* 103.3, 104.2, 104.7, respectively) through an HSQC spectrum. Comparison of the molecular formula of **2a** with the corresponding data for **1a** provided evidence for the presence of an additional hexose monosaccharide. In addition, while the resonance for the aglycon moiety and for the glucose linked to C-26 appeared almost identical, some slight modifications were detected among the resonances of the glucose that was linked to C-3. In particular, signals of C-4 (*δ* 77.1) and of H-4 (*δ* 3.61) of this residue were downfield shifted (+4.7 and +0.09 ppm, respectively), accompanied by a slight upfield shift of C-3 (*δ* 74.9 in **2a** vs 77.9 in **1a**), as compared with those of **1a**. These data suggested that C-4 of the innermost glucose was the glycosylated position to which the additional sugar is linked. This was also confirmed by HMBC ($H\text{-}1^{II}/C\text{-}4^{I}$) and ROESY ($H\text{-}1^{II}/H\text{-}4^{I}$) correlations. The H-1 proton of the additional glucose was used as a starting point to deduce by combined analysis of 2D COSY and HOHAHA spectra the nature of the additional residue, which pointed to a *β*-glucopyranose residue. Analysis of all the available data suggested the structure of **2a** to be furost-2α,3*β*,5α,6*β*,22α-pentol 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl] 26-*O*-*β*-D-glucopyranoside.

Elburzensoside C1 (**3a**), $C_{39}H_{66}O_{16}$ by HRFABMS, was isolated in low yeld. It was found to be the 6-dehydroxy analogue of **1a**. From the FABMS data, the molecular ion of elburzensoside C1 was 16 amu less when compared to **1a**. Indeed, inspection of the 1D and 2D NMR data revealed that these two molecules differed only in the chemical shifts of the C/H atoms around C−6 (see Tables 1 and 2). In particular, an unoxygenated methylene carbon atom (*δ* 31.0) was apparent and the lack of oxygenation was also confirmed by the upfield shift observed for H_3-19 as compared to the corresponding resonance of **1a** (*δ* 0.90 in **3a** vs *δ* 1.10 in **1a**). This evidence defined the structure of **3a** as furost-2α,3*β*,5α,22α-tetrol 3-*O*-*β*-D-glucopyranosyl 26-*O*-*β*-D-glucopyranoside.

Elburzensoside D1 (**4a**), $C_{50}H_{84}O_{25}$ by HRFABMS, was isolated in comparatively large amounts (309.0 mg). It differed from **3a** in having two additional sugar residues. This was suggested by MS data (see Experimental Section) and confirmed by the ^{1}H and ^{13}C NMR spectra of **4a** (Tables 1 and 2). The four anomeric proton signals (*δ* 4.24, 4.36, 4.64, and 4.96) correlated with the corresponding carbons in the HSQC spectrum (*δ* 104.9, 102.8, 102.9, and 104.3, respectively). Analysis of the NMR data also revealed the same furostanol aglycon for both compounds, including the presence of a *β*-glucopyranose unit at C-26.

In addition, a sugar with an anomeric proton resonating at *δ* 4.36 was identified as a hexose in the pyranose form. The large coupling constants observed in 2D HOHAHA subspectra for $H\text{-}1^{I}/H\text{-}2^{I}$ and $H\text{-}2^{I}/H\text{-}3^{I}$, and the relatively small coupling constants of $H\text{-}3^{I}/H\text{-}4^{I}$ and $H\text{-}4^{I}/H\text{-}5^{I}$, indicated the presence of *β*-galactose. This monosaccharide was placed at C-3 by interpretation of the key HMBC correlation peak between $H\text{-}1^{I}$ (*δ* 4.36) and C-3 (*δ* 84.6), with this junction also confirmed by the strong ROESY peak between $H\text{-}1^{I}$ and H-3 (*δ* 3.73). The sugar with an anomeric proton resonating at *δ* 4.64 was also identified as a hexopyranose. The large couplings observed for all the protons, indicating their axial−axial relationships, led to its identification as a *β*-glucopyranose. The HMBC cross-peak between $C\text{-}4^{I}$ (*δ* 79.8) and $H\text{-}1^{II}$ (*δ* 4.64) and the ROESY correlations between $H\text{-}4^{I}$ (*δ* 4.04) and $H\text{-}1^{II}$ (*δ* 4.64) defined position 4 of the galactose as a linkage site of this additional glucose unit. In an analogous manner, the last residue, with the anomeric proton at *δ* 4.96, has been elucidated as a pentose in the pyranose form. The axial−axial relationships found for the vicinal protons by the observation of large coupling constants determined its *β*-xylopyranose nature. The HMBC cross-peaks between $C\text{-}3^{II}$ (*δ* 87.8) and $H\text{-}1^{III}$ (*δ* 4.96) and the ROESY correlations between $H\text{-}3^{II}$ (*δ* 3.75) and $H\text{-}1^{III}$ (*δ* 4.96) indicated position 3 of glc^{II} as the linkage site of the fourth monosaccharide unit.

To confirm the nature of the sugars and to determine their absolute configuration, **1a** was subjected to acid hydrolysis (1 N HCl), followed by trimethylsilylation and GC analysis on a chiral column, in comparison with both series of glucose, galactose, and xylose. Using this procedure, the sugars were identified as belonging to the common D-series. Accordingly, **4a** was identified as furost-2α,3*β*,5α,22α-tetrol 3-*O*-[*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranosyl] 26-*O*-*β*-D-glucopyranoside.

The corresponding 22-*O*-methyl derivatives of the new elburzensosides have also been isolated, but we consider them as secondary products formed from the corresponding 22-hydroxyfuranosides during the extraction of the plant material in methanol[9] and the successive purification steps. The ^{1}H and ^{13}C NMR resonances of the 22-*O*-methyl derivatives (present as α and *β* epimers) were superimposable to those obtained for the corresponding 22-α (**1a**−**4a**, Table 1) and 22-*β* (**1b**−**4b**, Table 1) hydroxy compounds, with the exception of an additional methoxy group signal in each spectrum [^{1}H NMR *δ* 3.12 (3H, s); ^{13}C NMR *δ* 47.2]. However, their isolation was used to confirm the stereochemistry of the corresponding 22-hydroxy compounds. In fact, the ROESY spectrum of the 22-α-*O*-methyl derivatives showed key correlations of the methoxy group with H-16 and H_3-21, thus confirming a spatial proximity among these protons and their *cis* relationship.

Along with these compounds we have isolated two sapogenins, alliogenin[15] and agapanthagenin,[16] their 3-*O*-glucopyranosides,[17,18] and a gitogenin bidesmoside (gitogenin 3-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-glucopyranoside).[19]

The saponins from *A. elburzense* add to the growing array of saponins isolated from *Allium* species.[20] The new

elburzensosides possess as a common feature a rare OH-5α functionality for saponins of the furostanol type, being found previously only in saponins from *A. karataviense*[21] and *A. giganteum.*[22] Furthermore, the high concentrations of saponins found in our samples make *A. elburzense* a prolific producer of saponins of the furostanol and spirostanol types. Finally, the endemism of this species should help to provide an incentive to studying the biodiversity conservation and further chemical prospecting in those areas still rich in biodiversity.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and 10 cm microcell. FTIR spectra were run on a Bruker IFS-48 spectrometer in KBr. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Varian Unity-500 spectrometer. Chemical shifts were referred to the residual solvent signal (CD_3OD: δ_H 3.31, δ_C 49.0). The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. ^{1}H connectivities were determined using COSY and HOHAHA experiments; the 2D HOHAHA experiments were performed in the phase-sensitive mode (TPPI) using the MLEV-17 (mixing time 125 ms) sequence. One-bond heteronuclear ^{1}H−^{13}C connectivities were determined with a 2D HSQC[19] pulse sequence with an interpulse delay set for $^1J_{CH}$ of 130 Hz. Two- and three-bond heteronuclear ^{1}H−^{13}C connectivities were determined with 2D HMBC experiments, optimized for $^{2-3}J_{CH}$ of 8 Hz. Nuclear Overhauser effect (NOE) measurements were performed by 2D ROESY experiments. High-resolution FABMS (glycerol matrix) were measured on a Prospect Fisons mass spectrometer. GC/MS analyses were performed on a Carlo Erba instrument. Medium-pressure liquid chromatography (MPLC) was performed on a Büchi 861 apparatus using LiChroprep RP-18 (40−63 μm) columns. HPLC in isocratic mode was performed on a Varian apparatus equipped with an RI-3 refractive index detector using Waters columns [semipreparative μ-Bondapack C_{18} column (7.8 mm × 300 mm, i.d.) and analytical μ-Bondapack C_{18} column (3.9 mm × 300 mm, i.d.)].

Plant Material. Wild samples of *Allium elburzenze* Wendelbo were collected on Elburz mountain range (3300 m), near Tehran, Iran, in May 2003 and identified by Mr. Iraj Mehregan (Medical University of Isfahan). A reference specimen (No. 1145) has been deposited at the Department of Pharmacognosy, Isfahan University of Medical Sciences.

Extraction and Isolation. The bulbs (2.0 kg dry weight) were air-dried under a controlled temperature (22 °C), without exposure to light. They were powdered and then exhaustively extracted at room temperature with the following solvents: hexane, $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH. Each solvent extraction stage was conducted for 1 day and four times using 4 L of solvent, under stirring.

The $CHCl_3$−MeOH (9:1) extract was concentrated in vacuo, affording a crude extract (26.0 g), which was chromatographed by MPLC on a RP-18 column using a linear gradient solvent system from H_2O to MeOH. Preliminary NMR analysis on the eluates led to three fractions containing saponins: fraction A eluted with MeOH−H_2O (8:2) (651.3 mg), fraction B eluted with MeOH−H_2O (9:1) (1.999 g), and fraction C eluted with MeOH (5.974 g). The three fractions also showed the same NMR profile and thus were pooled and purified by HPLC [on a semipreparative C_{18} column with a mobile phase MeOH−H_2O (7:3)], finally affording the known compounds alliogenin (163.3 mg), agapanthagenin (686.8 mg), alliogenin 3-*O*-β-D-glucopyranoside (12.4 mg), agapanthagenin 3-*O*-β-D-glucopyranoside (92.2 mg), and gitogenin 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranoside (79.8 mg).

The MeOH extract (102.3 g) was partitioned between butanol and water. The butanolic phase was filtered and then concentrated in vacuo, giving a crude extract (45 g), which was chromatographed by MPLC on a RP-18 column using a linear gradient solvent system from H_2O to MeOH. Preliminary NMR study of the eluates allowed us to select the following fractions containing saponins. A fraction eluted with MeOH−H_2O (1:1) (176.5 mg) was chromatographed by HPLC on a analytical C_{18} column with the mobile phase MeOH−H_2O (3:7) to give the pure compounds **2a** (8.6 mg, t_R = 40.8 min) and **2b** (5.7 mg, t_R = 43.7 min). A fraction eluted with MeOH−H_2O (6:4) (1019.7 mg) was purified by HPLC on a semipreparative C_{18} column with MeOH−H_2O (6:4) to give the pure compounds **1a** (211.3 mg, t_R = 36.6 min) and **1b** (169.5 mg, t_R = 39.6 min). A fraction eluted with MeOH−H_2O (7:3) (486.4 mg) was chromatographed by HPLC on a semipreparative C_{18} column with MeOH−H_2O (45:55) to give the pure compounds **3a** (19.4 mg, t_R = 19.8 min) and **3b** (10.6 mg, t_R = 22.7 min). A fraction eluted with MeOH−H_2O (8:2) (1360.1 mg) was purified by HPLC on a semipreparative C_{18} column with MeOH−H_2O (6:4) to afford the pure compounds **4a** (309.0 mg, t_R = 9.0 min) and **4b** (195.2 mg, t_R = 12.0 min).

Elburzensoside A1 (furost-2α,3β,5α,6β,22α-pentol 3-*O*-β-D-glucopyranosyl 26-*O*-β-D-glucopyranoside, 1a): $[\alpha]_D^{25}$ −41.67° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3400, 2934, 1159, 1050 cm^{-1}; ^{1}H NMR data, see Tables 1 and 3; ^{13}C NMR data, see Tables 2 and 3; HRFABMS (negative ion) of the equilibrated mixture, *m/z* found 806.9276 [M − H]$^-$ (calcd for $C_{39}H_{66}O_{17}$, 806.9285).

Elburzensoside A2 (furost-2α,3β,5α,6β,22β-pentol 3-*O*-β-D-glucopyranosyl 26-*O*-β-D-glucopyranoside, 1b): $[\alpha]_D^{25}$ −41.65° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3400, 2934, 1159, 1050 cm^{-1}; ^{1}H and ^{13}C NMR data are the same as those reported for **1a**, except for the resonances δ 1.02 (3H, d, *J* = 6.5 Hz, H_3-21), 1.71 (1H, overlapped, H-17), 4.38 (1H, td, *J* = 6.0, 6.0, 7.5 Hz, H-16), 16.1 (C-21), 34.7 (C-23), 65.1 (C-17); HRFABMS (negative ion) of the equilibrated mixture, *m/z* found 806.9278 [M − H]$^-$ (calcd for $C_{39}H_{66}O_{17}$, 806.9285).

Elburzensoside B1 (furost-2α,3β,5α,6β,22α-pentol 3-*O*-[β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl] 26-*O*-β-D-glucopyranoside, 2a): $[\alpha]_D^{25}$ −43.59° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3410, 2933, 1157, 1050 cm^{-1}; ^{1}H NMR data, see Tables 1 and 3; ^{13}C NMR data, see Tables 2 and 3; HRFABMS (negative ion) found *m/z* 969.0675 [M − H]$^-$ (calcd for $C_{45}H_{76}O_{22}$, 969.087).

Elburzensoside B2 (furost-2α,3β,5α,6β,22β−pentol 3-*O*-[β-D-glucopyranosyl−(1→4)-*O*-β-D-glucopyranosyl] 26-*O*-β-D-glucopyranoside, 2b): $[\alpha]_D^{25}$ −43.61° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3410, 2933, 1157, 1050 cm^{-1}; ^{1}H and ^{13}C NMR data are the same as those reported for **2a**, except for the resonances δ 1.08 (3H, d, *J* = 6.5 Hz, H_3-21), 1.68 (1H, overlapped, H-17), 4.42 (1H, td, *J* = 6.0, 6.0, 7.5 Hz, H-16), 13.9 (C-21), 34.4 (C-23), 66.9 (C-17); HRFABMS (negative ion) found *m/z* 969.0679 [M − H]$^-$ (calcd for $C_{45}H_{76}O_{22}$, 969.087).

Elburzensoside C1 (furost-2α,3β,5α,22α-tetrol 3-*O*-β-D-glucopyranosyl 26-*O*-β-D-glucopyranoside, 3a): $[\alpha]_D^{25}$ −13.72° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3414, 2936, 1155, 1045 cm^{-1}; ^{1}H NMR data, see Tables 1 and 3; ^{13}C NMR data, see Tables 2 and 3; HRFABMS (negative ion) of the equilibrated mixture, found *m/z* 790.9285 [M − H]$^-$ (calcd for $C_{39}H_{66}O_{16}$, 790.9291).

Elburzensoside C2 (furost-2α,3β,5α,22β-tetrol 3-*O*-β-D-glucopyranosyl 26-*O*-β-D-glucopyranoside, 3b): $[\alpha]_D^{25}$ −13.70° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3414, 2936, 1155, 1045 cm^{-1}; ^{1}H and ^{13}C NMR data are the same as those reported for **3a**, except for the resonances δ 0.99 (3H, d, *J* = 6.5 Hz, H_3-21), 1.69 (1-H, overlapped, H-17), 4.45 (1H, td, *J* = 6.0, 6.0, 7.5 Hz, H-16), 16.0 (C-21), 34.8 (C-23), 65.2 (C-17); HRFABMS (negative ion) of the equilibrated mixture, found *m/z* 790.9280 [M − H]$^-$ (calcd for $C_{39}H_{66}O_{16}$, 790.9291).

Elburzensoside D1 (furost-2α,3β,5α,22α-tetrol 3-*O*-[β-D-xylopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-galactopyranosyl] 26-*O*-β-D-glucopyranoside, 4a): $[\alpha]_D^{25}$ −23.75° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3412, 2930, 1150, 1048 cm^{-1}; ^{1}H NMR data, see Tables 2 and 3; ^{13}C NMR data, see Tables 2 and 3; HRFABMS (negative ion) of the equilibrated mixture, found *m/z* 1084.9477 [M − H]$^-$ (calcd for $C_{50}H_{84}O_{25}$, 1084.9486).

Elburzensoside D2 (furost-2α,3β,5α,22β-tetrol 3-*O*-[β-D-xylopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-galactopyranosyl] 26-*O*-β-D-glucopyranoside, 4b): $[\alpha]_D^{25}$ −23.65° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3412, 2930, 1150, 1048 cm^{-1}; ^1H and ^{13}C NMR data are the same as those reported for **4a**, except for the resonances δ 0.98 (3H, d, J = 6.5 Hz, H_3-21), 1.68 (1H, overlapped, H-17), 4.44 (1H, td, J = 6.0, 6.0, 7.5 Hz, H-16), 16.0 (C-21), 33.9 (C-23), 66.2 (C-17); HRFABMS (negative ion) of the equilibrated mixture, found *m/z* 1084.9480 $[M - H]^-$ (calcd for $C_{50}H_{84}O_{25}$, 1084.9486).

Determination of the Absolute Configuration of Sugars. A solution of each isolated compound (1 mg) in 1 N HCl (0.25 mL) was stirred at 80 °C for 4 h. On cooling, the solution was concentrated in a stream of N_2. The residue was dissolved in 1-(trimethyl silyl)imidazole (Trisil-Z) and pyridine (0.1 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution with a stream of N_2, the residue was partitioned between water and CH_2Cl_2 (1 mL, 1:1). The CH_2Cl_2 layer was analyzed by GC (Alltech l-Chirasil-Val column, 0.32 mm × 25 m; temperatures for injector and detector, 200 °C; temperature gradient system for the oven, 100 °C for 1 min and then raised to 180 °C; rate 5 °C/min). In the hydrolysate of **1a/1b**, **2a/2b**, and **3a/3b** one peak at 14.66 min was detected. Peaks of the hydrolysate of **4a/4b** were detected at 10.98, 13.98, and 14.66 min in the ratio 1:1:2. Retention times for authentic samples after being treated simultaneously with Trisil-Z were 10.98 (D-xylose) and 11.05 (L-xylose), 13.98 (D-galactose) and 13.75 (L-galactose), 14.66 (D-glucose) and 14.73 min (L-glucose). Co-injection of each hydrolysate with standard D-xylose, D-galactose, and D-glucose gave single peaks.

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