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Chemical Composition of Shallot (*Allium ascalonicum* Hort.)†

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An extensive phytochemical analysis of the polar extracts from bulbs of shallot, *Allium ascalonicum* Hort., led to the isolation of two new furostanol saponins, named ascalonicoside A1/A2 (**1a**/**1b**) and ascalonicoside B (**4**), respectively, along with compounds **2a** and **2b**, most likely extraction artifacts. On the basis of 2D NMR and mass spectrometry data, the structures of the novel compounds were elucidated as furost-5(6)-en-3β,22α-diol 1β-*O*-β-D-galactopyranosyl 26-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside] (**1a**), its epimer at position 22 (**1b**), and furost-5(6),20(22)-dien-3β-ol 1β-*O*-β-D-galactopyranosyl 26-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside] (**4**). This is the first report of furostanol saponins in *A. ascalonicum*. High concentrations of quercetin, isorhamnetin, and their glycosides were also isolated and described.

KEYWORDS: Shallot; *Allium ascalonicum* Hort.; Liliaceae; furostanol saponins; flavonol glycosides; NMR spectroscopy

INTRODUCTION

The use of Liliaceae bulbs as food flavoring is known worldwide and, particularly, garlic and onion have been used in folk medicine since ancient times. (*1*) The oldest citation of shallot, *Allium ascalonicum* Hort. (*2*), a cultivated variety derived from *A. cepa* L., is found in the work of Pliny the Elder who described six different types of onions with their therapeutic uses, indicating shallot as the most important one. Again, in the XVII century doctor, Pisanelli describes shallot as "a delicious food that stimulate the appetite when it is hot and make tasty to drink".

The name *Allium* is known to derive from the Celtic word "all" (pungent), whereas the name *ascalonicum* could either derive from its original site of cultivation, Ascalon, an old Palestinian town, or from the French name Echalogne (*3*). This variety of onion differs from the common variety (*A. cepa* L.) because it forms smaller bulbs shaped like large cloves of garlic, and has a milder flavor. The French have particularly developed the use of this species both as a cooking ingredient or to prepare a sauce, obtained by boiling the minced bulbs in white wine (*4*).

As part of our ongoing investigation of the chemistry of *Allium* species, (*5*, *6*) we have now analyzed the chemical composition of *A. ascalonicum*. Block et al. reported the composition of the volatile sulfur compounds of shallot (*7*, *8*) but no data are found in the literature on the polar compounds. Analysis of the MeOH extracts from the bulbs of shallot revealed the presence of saponins and flavonoids in high concentrations, and, as major metabolites, we have isolated two new furostanol saponins, named ascalonicoside A1/A2 (**1a** and **1b**) and ascalonicoside B (**4**), along with two *O*-methyl artifacts (**2a** and **2b**) (**Figure 1**). This is the first report of furostane saponins in *A. ascalonicum*. High concentrations of quercetin (**5**) and isorhamnetin (**6**), and of their glycosides (**7**−**10**) were also found (**Figure 2**).

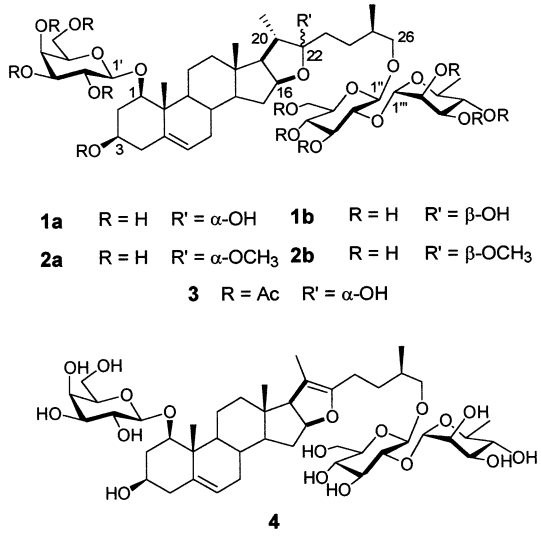

Figure 1. Saponins isolated from *A. ascalonicum*.

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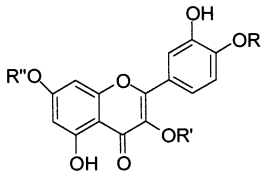
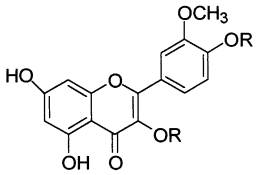


5 R = R' = R" = H

7 R = β-Glu R' = R" = H

8 R = R' = H R" = β-Glu

9 R = R' = β-Glu R" = H



6 R = H

10 R = β-Glu

Figure 2. Flavonoids of *A. ascalonicum*.

MATERIALS AND METHODS

General Experimental Procedures. FABMS (recorded in a glycerol matrix) were measured on a Prospec Fisons mass spectrometer. Optical rotations were determined on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and 10-cm microcell. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AMX-500 spectrometer. Chemical shifts were referred to the residual solvent signal (CD_3OD: δH 3.34, δC 49.0, $CDCl_3$: δH 7.26, δC 77.0). The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. ^{1}H connectivities were determined by 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 for mixing. One-bond heteronuclear ^{1}H−^{13}C connectivities were determined with 2D HMQC experiments, with a BIRD pulse 0.5 s before each scan to suppress the signal from protons not directly bonded to ^{13}C. The interpulse delays were adjusted for an average ^1J CH of 125 Hz. Two and three bond heteronuclear ^{1}H−^{13}C connectivities were determined with 2D HMBC experiments, optimized for ^{2-3}JCH of 8 Hz. Nuclear Overhauser effect (nOe) measurements were performed by 2D ROESY experiments. 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.

Plant Material. Bulbs of shallot, *Allium ascalonicum* Hort., available in the market, coming from the Bretagne (France), were identified (and reference specimens deposited) at the Dipartimento di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche, Campobasso.

Extraction and Isolation. The bulbs (293 g, dry weight) were air-dried under controlled temperature (22 °C) and without exposure of light. They were chopped and then exhaustively extracted at room temperature with the following solvents in the order listed: *n*-hexane, $CHCl_3$, $CHCl_3$/MeOH (9:1), and MeOH. Each solvent extraction takes a day and was repeated four times using 500 mL of solvent, under stirring. The MeOH extract (74 g) was partitioned between *n*-BuOH and water, and the organic layer was then filtered and concentrated in vacuo to afford a crude extract (35 g), which was chromatographed by MPLC on RP-18 column using a linear gradient solvent system from H_2O to MeOH.

Fractions eluted with H_2O/MeOH 1:9 (441 mg) were rechromatographed on RP-18 column using a linear gradient system from H_2O/MeOH (1:1) to MeOH to give six fractions, A1−A6. Fraction A3 (218 mg), eluted with H_2O/MeOH (3:7), was purified by HPLC on a 300 × 7.8 mm i.d μ-Bondapack C-18 column with a mobile phase of H_2O/MeOH (1:9), affording crude saponins and pure quercetin (**5**, 150 mg). Saponins were further purified by HPLC on a 300 × 3.9 mm i.d μ-Bondapack C-18 column with a mobile phase of H_2O/MeOH (1:1) yielding pure ascalonicoside A1 (**1a**, 15 mg), A2 (**1b**, 20 mg), compound **2a** (11 mg), compound **2b** (4 mg), and ascalonicoside B (**4**, 13 mg).

Fractions eluted with MeOH (1260 mg), were rechromatographed on RP-18 column using a linear gradient system from H_2O/MeOH (1:1) to MeOH, yielding fractions B1−B6. Fraction B5, eluted with H_2O/MeOH (1:9) was composed by pure isorhamnetin (**6**, 30 mg). Fraction B4 (452 mg), eluted with H_2O/MeOH (2:8), was further purified by HPLC on a 300 × 3.9 mm i.d μ-Bondapack C-18 column with a mobile phase of H_2O/MeOH (1:1) to give pure quercetin 4^l-glucoside (**7**, 34 mg) and quercetin 7-glucoside (**8**, 25 mg). Fraction B2 (189 mg), eluted with H_2O/MeOH (4:6), was purified by HPLC on a 300 × 3.9 mm i.d μ-Bondapack C-18 column with a mobile phase of H_2O/MeOH (6:4) to give pure isorhamnetin 4′,3-diglucoside (**10**, 20 mg). Fraction B1 (190 mg), eluted with H_2O/MeOH (1:1), was further purified by HPLC on a 300 × 3.9 mm i.d μ-Bondapack C-18 column with a mobile phase of H_2O/MeOH (7:3) to give pure quercetin 4′,3-diglucoside (**9**, 15 mg).

Table 1. ^{1}H NMR Data of the Aglycon Portion of Ascalonicoside A1 (**1a**), A2 (**1b**), and B (**4**) (Spectra Were Measured in CD_3OD)

	δ_H (int., mult., *J* in Hz)		
pos.	**1a**	**1b**	**4**
1	3.36[a]	3.36[a]	3.36[a]
2a	2.12[a]	2.10[a]	2.12[a]
2b	1.70[a]	1.70[a]	1.70[a]
3	3.31[a]	3.30[a]	3.31[a]
4a	2.28 (1H, dd, 11.5, 7.3)	2.27 (1H, dd, 11.5, 7.3)	2.30[a]
4b	2.22 (1H, dd, 11.5, 3.5)	2.22 (1H, dd, 11.5, 3.5)	2.22[a]
6	5.59 (1H, bd, 3.2)	5.59 (1H, bd, 3.2)	5.55 (1H, bd, 3.2)
7a	1.98[a]	1.98[a]	1.98[a]
7b	1.96[a]	1.96[a]	1.96[a]
8	1,50 (1H, m)	1,50 (1H, m)	1,50[a]
9	1.28[a]	1.28[a]	1.28[a]
11a	2.57 (1H, dd, 10.5, 2.5)	2.56 (1H, dd, 10.5, 2.5)	2.57 (1H, dd, 10.5, 2.5)
11b	1.45 (1H, m)	1.46 (1H, m)	1.45 (1H, m)
12a	1.70[a]	1.70[a]	1.70[a]
12b	1.22[a]	1.23[a]	1.22[a]
14	1.18 (1H, m)	1.18 (1H, m)	1.20 (1H, m)
15a	1.99[a]	1.99[a]	2.02[a]
15b	1.30[a]	1.30[a]	1.30[a]
16	4.59 (1H, q, 5.5)	4.39 (1H, q, 5.5)	4.70 (1H, q, 5.5)
17	1.78[a]	1.73[a]	2.45 (1H, d, 5.5)
18	0.87 (3H, s)	0.87 (3H, s)	0.70 (3H, s)
19	1.13 (3H, s)	1.13 (3H, s)	1.09 (3H, s)
20	2.13[a]	2.15[a]	
21	1.04 (3H, d, 6.6)	1.01 (3H, d, 6.6)	1.89 (3H, s)
23a	1.72[a]	1.75[a]	2.20[a]
23b	1.63[a]	1.61[a]	2.15[a]
24a	1.38[a]	1.34[a]	1.65 (1H, m)
24b	1.30[a]	1.31[a]	1.55[a]
25	1.78 (1H, m)	1.78 (1H, m)	1.78 (1H, m)
26a	3.82 (1H, dd, 8.5, 6.9)	3.82 (1H, dd, 8.5, 6.9)	3.80 (1H, dd, 8.5, 6.9)
26b	3.29[a]	3.29[a]	3.30[a]
27	0.99 (3H, d, 6.6)	0.99 (3H, d, 6.6)	0.97 (3H, d, 6.6)

[a] Overlapped with other signals.

Ascalonicoside A1 (1a). Furost-5(6)-en-3β,22α-diol 1β-*O*-β-D-galactopyranosyl 26-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside]: $[\alpha]_D^{25}$ −60 (*c* = 0.1 MeOH). HRFABMS (negative ion): found *m*/*z* 917.4763 [M − H]$^-$; calculated for $C_{45}H_{73}O_{19}$ *m*/*z* 917.4744. ^{1}H NMR data: **Tables 1** and **3**. ^{13}C NMR data: **Tables 2** and **3**.

Acetylation of Ascalonicoside A1. Ascalonicoside A1 (**1a**, 3 mg) was dissolved in dry pyridine (100 μL) and treated with Ac_2O (100 μL). After keeping overnight at room temperature, the reaction was worked up by addition of a few drops of methanol, water, and EtOAc. The organic phase afforded 3.9 mg of the undecaacetyl derivative **3**: $[\alpha]_D^{25}$ −58 (*c* = 0.01 $CHCl_3$). FABMS (negative ion) *m*/*z* 1379 [M − H]$^-$. ^{1}H NMR ($CDCl_3$): δ 5.62 (H-6, d, *J* = 3.2 Hz), 5.22 (H-4″, dd, *J* = 6.5, 4.5 Hz), 5.12 (H-4′, bs), 5.10 (H-3′, overlapped), 5.09 (H-1‴, bs), 5.08 (H-3‴, overlapped), 5.06 (H-4‴, overlapped), 5.05 (H-2′, t, *J* = 7.5 Hz), 5.03 (H-2‴, bs), 4.92 (H-3″, dd, *J* = 6.5, 6.0 Hz), 4.70 (H-16, q, *J* = 5.5 Hz), 4.49 (H-3, m), 4.48 (H-1′, d, *J* = 7.5 Hz), 4.39 (H-1″, d, *J* = 7.5 Hz), 4.28 (H-5‴, overlapped), 4.27 (H-6′a, overlapped), 4.12 (H-6′b, d, *J* = 11.5 Hz), 3.98 (H-5″, overlapped), 3.97 (H-6″a, overlapped), 3.88 (H-2″, t, *J* = 7.5 Hz), 3.83 (H-26a, dd, *J* = 8.5, 6.9 Hz), 3.69 (H-5^l, m), 3.57 (H-6″b, d, *J* = 11.5 Hz), 3.48 (H-1, dd, *J* = 7.2, 2.5 Hz), 3.19 (H-26b, dd, *J* = 6.9, 6.5 Hz), 2.20 (H_2-4, overlapped), 2.18 (H-2a, overlapped), 2.12 (H-17, d, *J* = 5.5 Hz), 2.10−1.95 (11 CH_3CO, H-15a, H-25, H_2-7), 1.90−1.60 (H-2b,

Table 2. ^{13}C NMR Data of the Aglycon Portion of **1a**, **1b**, and **4** (Spectra Were Measured in CD_3OD)

pos.	δ_C (mult.) **1a**	**1b**	**4**
1	84.2 (CH)	84.2 (CH)	84.2 (CH)
2	37.1 (CH_2)	37.1 (CH_2)	37.1 (CH_2)
3	68.9 (CH)	68.9 (CH)	69.2 (CH)
4	43.2 (CH_2)	43.2 (CH_2)	43.1 (CH_2)
5	141.1 (C)	141.1 (C)	141.1 (C)
6	126.2 (CH)	126.2 (CH)	126.2 (CH)
7	32.5 (CH_2)	32.3 (CH_2)	32.5 (CH_2)
8	33.5 (CH)	33.5 (CH)	33.5 (CH)
9	51.5 (CH)	51.5 (CH)	51.9 (CH)
10	43.0 (C)	43.0 (C)	43.0 (C)
11	24.9 (CH_2)	24.9 (CH_2)	25.0 (CH_2)
12	40.8 (CH_2)	40.7 (CH_2)	41.0 (CH_2)
13	41.0 (C)	41.0 (C)	43.2 (C)
14	57.7 (CH)	57.7 (CH)	56.3 (CH)
15	33.0 (CH_2)	33.0 (CH_2)	31.5 (CH_2)
16	82.2 (CH)	82.2 (CH)	84.6 (CH)
17	65.3 (CH)	65.2 (CH)	65.3 (CH)
18	17.1 (CH_3)	17.0 (CH_3)	15.0 (CH_3)
19	15.5 (CH_3)	15.3 (CH_3)	15.5 (CH_3)
20	40.5 (CH)	40.2 (CH)	104.2 (C)
21	16.0 (CH_3)	16.3 (CH_3)	12.3 (CH_3)
22	112.0 (C)	115.5 (C)	153.0 (C)
23	36.5 (CH_2)	36.2 (CH_2)	34.2 (CH_2)
24	31.7 (CH_2)	31.6 (CH_2)	25.3 (CH_2)
25	35.1 (CH)	35.0 (CH)	34.5 (CH)
26	75.8 (CH_2)	76.0 (CH_2)	76.0 (CH_2)
27	17.5 (CH_3)	17.5 (CH_3)	17.5 (CH_3)

Table 3. ^1H and ^{13}C NMR Data of the Sugar Portion of **1a/1b/4** (Spectra Were Measured in CD_3OD)

pos.	δ_H (int., mult., *J* in Hz)	δ_C (mult.)
1′	4.30 (1H, d, 7.5)	101.2 (CH)
2′	3.71[a]	75.1 (CH)
3′	3.68 (1H, dd, 6.8, 2.5)	76.3 (CH)
4′	3.87 (1H, dd, 3.2, 2.5)	78.2 (CH)
5′	3.45[a]	74.4 (CH)
6′a	3.47[a]	62.5 (CH_2)
b	3.65[a]	
1″	4.26 (1H, d, 7.5)	104.3 (CH)
2″	3.21 (1H, t, 7.5)	76.1 (CH)
3″	3.38[a]	74.1 (CH)
4″	3.30 (1H, dd, 7.0, 6.4)	71.9 (CH)
5″	3.27 (1H, m)	77.0 (CH)
6″a	3.85 (1H, bd, 11.5)	66.0 (CH_2)
b	3.52 (1H, bd, 11.5)	
1‴	5.32 (1H, bs)	102.5 (CH)
2‴	3.91 (1H, bs)	72.5 (CH)
3‴	3.72 (1H, d, 6.5)	70.5 (CH)
4‴	3.40 (1H, dd, 6.5, 6.0)	72.0 (CH)
5‴	4.11 (1H, dq, 6.6, 6.0)	68.8 (CH)
6‴	1.28 (3H, d, 6.6)	18.2 (CH_3)

[a] Overlapped with other signals.

H-8, H-11a, H-12a, H-15b, H-20, H_2-23, H_2-24 not assigned), 1.50 (H-11b, m), 1.30 (H-9, overlapped), 1.25 (H-12b, overlapped), 1.20 (H_3-6‴, d, J = 6.6 Hz), 1.20 (H-14, overlapped), 1.08 (H_3-19, s), 0.89 (H_3-21, d, J = 6.6 Hz), 0.90 (H_3-27, d, J = 6.6 Hz), 0.69 (H_3-18, s).

Ascalonicoside A2 (1b). Furost-5(6)-en-3β,22β-diol 1β-*O*-β-D-galactopyranosyl 26-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside]: $[\alpha]_D^{25}$ −55 (c = 0.1 MeOH). HRFABMS (negative ion): found *m/z* 917.4722 $[M - H]^-$; calculated for $C_{45}H_{73}O_{19}$ *m/z* 917.4744. ^1H NMR data: **Tables 1** and **3**. ^{13}C NMR data: **Tables 2** and **3**.

Ascalonicoside B (4). Furost-5(6),20(22)-dien-3β-ol 1β-*O*-β-D-galactopyranosyl 26-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside]: $[\alpha]_D^{25}$ − 8 (c = 0.1 MeOH); HR−FABMS (negative ion): found *m/z* 899.4652 $[M - H]^-$; calculated for $C_{45}H_{71}O_{18}$ *m/z* 899.4639; ^1H NMR data: **Tables 1** and **3**. ^{13}C NMR data: **Tables 2** and **3**.

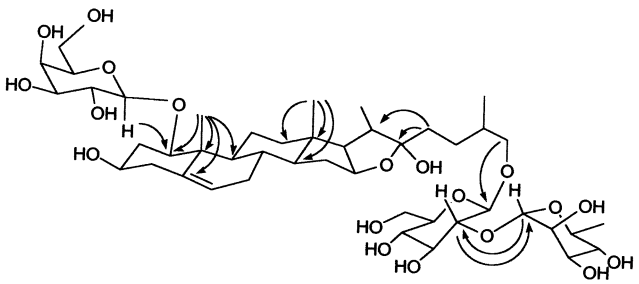


Figure 3. Selected HMBC correlations exhibited by compound **1a**.

RESULTS AND DISCUSSION

Bulbs of French shallot, *Allium ascalonicum* Hort. were briefly air-dried and exhaustively extracted with *n*-hexane, $CHCl_3$, $CHCl_3$/MeOH (9:1), and MeOH. The MeOH extracts were partitioned between *n*-butanol and water, and the organic layer was fractionated by sequential chromatography, by using column under medium pressure and HPLC techniques affording, as major components, furostanol saponins (total saponin concentration 200 mg/kg) and flavonoids (total flavonoid concentration 940 mg/kg).

The FAB mass spectrum of ascalonicoside A1 (**1a**, **Figure 1**) exhibited a quasi-molecular ion peak at *m/z* 917 $[M - H]^-$, and high-resolution measurements indicated the molecular formula $C_{45}H_{74}O_{19}$, in accordance with ^{13}C NMR data. ^1H NMR spectrum of **1a** (CD_3OD, **Tables 1** and **3**) exhibited the signals of five distinct methyl groups (two singlets and three doublets), some overlapping signals from δ 1.1 to 2.6, and a number of signals between δ 3.2 and 5.4, attributable to protons on oxygen-bearing carbons. A further signal at δ 5.59 (broad doublet) was ascribable to a proton on sp^2 carbon. This pattern of proton chemical shifts led us to initially suppose the glycoterpene nature of compound **1a**. This was further corroborated by the ^{13}C NMR spectrum of **1a** (CD_3OD, **Tables 2** and **3**) which showed the resonances of three anomeric carbons (δ 101.2, 102.5, and 104.3) indicating the trisaccharide nature of the sugar portion. Furthermore, the presence of the semiketal carbon signal at δ 112.0 (singlet) suggested the furostanol nature of the aglycon of **1a**. All the above proton resonances of **1a** were unambiguously associated with the relevant carbon atoms by using the HMQC spectrum.

Inspection of HOmonuclear HArtmann HAhn (HOHAHA) spectrum of **1a** allowed us to detect six distinct spin systems, three of them belonging to the aglycon moiety and the remaining three belonging to the three monosaccharides. The proton sequence within each spin system was elucidated by following the series of cross-peaks of the COSY spectrum, while data arising from the HMBC experiment were used to interconnect the partial structures.

Concerning the aglycon moiety, the first spin system connects the oxygen-bearing carbon C-1 (δ_H 3.36; δ_C 84.2) with C-4, while the second one, starting from the sp^2 C-6 (δ_H 5.59; δ_C 126.2) and encompassing all the protonated carbons of rings B, C, and D runs out with C-21. The last spin system of the aglycon of **1a** is constituted by protons of the side chain, from C-23 to the oxygen-bearing C-26 (δ_H 3.29 and 3.82, δ_C 75.8). The following HMBC cross-peaks (**Figure 3**) were particularly diagnostic to assemble the above substructures: H_3-19 (δ 1.13) with C-10 (δ 37.1), C-1, C-5 (δ 141.1), and C-9 (δ 51.5); H_3-18 (δ 0.87) with C-13 (δ 41.0), C-12 (δ 40.8), and C-14 (57.7); H_2-23 (δ 1.72 and 1.63) with C-20 (δ 40.5) and the semiketal carbon C-22 (δ 112.0). The total of this evidence allowed us to identify the aglycon moiety of **1a** as a Δ^5-furostan-1,3,22,26-tetraol.

With respect to the stereochemistry of the aglycon, in the ^{1}H NMR spectrum of **1a**, signals of H-1 and H-3 were overlapped with some signals of the sugar portion and partially observed by the solvent signal. However, inspection of the 1D subspectra of the 2D HOHAHA experiment relative to the first spin system allowed a better evaluation of the coupling constants of these protons indicating the relative stereochemistry of the chiral carbons C-1 and C-3 of the furostanol moiety. In particular, H-1 appeared as a double doublet (J = 7.5 and 2.5 Hz), whereas H-3 appeared as a multiplet with two large (axial−axial) and two small (axial−equatorial) coupling constants. These data pointed to the axial position of both H-1 and H-3. The 25*R* stereochemistry was inferred by the resonances of protons and carbons at position 25, 26, and 27 and by the $^3J_{HH}$ values between H-25 and H-26 in comparison with literature data (*9*). On the basis of the data described above, and assuming that the aglycon moiety possesses the same absolute configuration as found in all the furostane derivatives isolated to date, the stereochemistry of the chiral centers of the aglycon (except C-22, see below) can be assigned as reported in **Figure 3**.

Although the mid-field region of the ^{1}H NMR spectrum of **1a** contained several overlapping signals, analysis of homonuclear COSY and HOHAHA (2D and 1D subspectra) experiments allowed the assignments of all the proton resonances of the sugar moieties, whereas evaluation of the coupling constants was used to elucidate the monosaccharide relative stereochemistry. Hence, when the anomeric proton at δ 4.30 (H-1′) was used as a starting point, a sequence of four oxymethines and one oxymethylene (**Table 3**) was identified from the above spectra. The large coupling constants observed for H-1′/H-2′ and H-2′/H-3′ vicinal couplings, and the relatively small coupling constants of H-3′/H-4′ and H-4′/H-5′, indicated the β-galactopyranose nature of this sugar. The pattern of ^{13}C NMR resonances (**Table 3**) confirmed this assignment. The galactopyranose residue should be linked to the C-1 of the aglycon, as indicated by the HMBC correlation peak (**Figure 3**) between the anomeric proton H-1′ and the downfield shifted C-1 (δ 84.2).

On the other hand, the HMBC cross-peak of H_2-26 with the anomeric carbon at δ 104.3 (C-1″) (**Figure 3**) allowed us to identify C-26 as a further glycosidic linkage site. Starting from H-1″ (δ 4.26), we identified a sequence of four oxymethynes and one oxymethylene and observed large coupling constants between all the oxymethine protons, in agreement with axial−axial relationships. These data led to the assignment of this sugar as a β-glucopyranoside. The spin system starting with the last anomeric proton (H-1‴, δ 5.32) extended to four oxymethine and one methyl group. This sugar moiety was identified as a rhamnopyranose because of the axial−axial couplings H-3‴/H-4‴ and H-4‴/H-5‴ and the axial−equatorial relationship between H-2‴ and H-3‴. The chemical shift of C-3‴ (δ 70.5) and C-5‴ (δ 68.8) indicated the α-anomeric configuration of this sugar, in accordance with data reported in the literature (*10*). The linkage of this monosaccharide at C-2″ of the glucose was inferred on the basis of the HMBC cross-peaks H-1‴/C-2″ and H-2″/C-1‴ (**Figure 3**). Therefore, if we assume that these monosaccharides belong to the most commonly found stereochemical series (D for glucose and galactose and L for rhamnose) the sugar moiety of ascalonicoside A1 (**1a**) is completely defined.

To further prove the stereochemical assignments, we submitted compound **1a** to an acetylation reaction under standard conditions (Ac_2O/py, room temperature). The undecacetyl derivative **3**, thus obtained, exhibited a good dispersion of the signals in the mid-field region of ^{1}H NMR spectrum ($CDCl_3$),

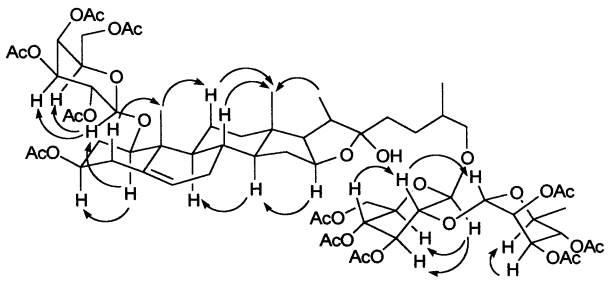


Figure 4. Selected ROESY correlations exhibited by compound **3**.

allowing an easy inspection of COSY and HOHAHA experiments. All the ^{1}H NMR signals are assigned and reported in Material and Methods; evaluation of $^3J_{HH}$ values, along with some diagnostic spatial couplings evidenced through the ROESY spectrum of **3** (see **Figure 4**), fully confirmed the stereostructure of ascalonicoside A1 (**1a**) as furost-5(6)-en-3β,22-diol 1β-*O*-β-D-galactopyranosyl 26-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside].

Mass spectrometric analysis of ascalonicoside A2 (**1b**) indicated the same molecular formula as that of compound **1a**. Analogously, as a result of inspection of ^{1}H and ^{13}C NMR spectra of **1b (Tables 1−3)** through 2D NMR experiments, the same gross structure of ascalonicoside A1 (**1a**) was found. Therefore, we argued that these molecules should differ only in the stereochemistry of one (or more) of the chiral carbons. This was readily identified as the hemiketal carbon C-22, considering that both ascalonicoside A1 (**1a**) and A2 (**1b**), after being kept one night in aqueous solution at room temperature, gave the same mixture constituted approximately of 40% of compound **1a** and 60% of compound **1b**. Accordingly, ^{1}H and ^{13}C NMR spectra of ascalonicoside A1 and A2 appeared to differ considerably only in the resonances of atoms located close to C-22. We have tentatively assigned the 22α orientation to ascalonicoside A1 and the 22β orientation to ascalonicoside A2 on the basis of the ^{1}H NMR resonances of H_3-21 and H-16 slightly downfield shifted (H_3-21 at δ 1.04 instead of 1.01; H-16 at δ 4.59 instead of 4.39) in ascalonicoside A1 (**1a**), suggesting that, most likely, they are deshielded by the *cis*-oriented OH-22 group.

The corresponding 22*O*-methyl derivatives **2a** and **2b** have been isolated along with ascalonicoside A1 (**1a**) and A2 (**1b**). ^{1}H and ^{13}C NMR resonances of these compounds appeared quite similar to those obtained for **1a** and **1b**, respectively, with the exception of additional methoxy group signals at δ 3.12 (3H, s) and at δ 47.2 in the ^{1}H and ^{13}C NMR spectra, respectively. Having repeatedly carried out the bulb extraction in methanol, compounds **2a** and **2b** are almost certainly extraction artifacts. Therefore, we have not characterized them further.

The structure of another saponin, named ascalonicoside B (**4**), was readily elucidated on the basis of its considerable similarities with ascalonicoside A1/A2. The HR−FABMS (negative ion) indicated its molecular formula as $C_{45}H_{72}O_{18}$, which differs from that of ascalonicoside A1/A2 only in having one water molecule less. The ^{1}H and ^{13}C NMR profiles obtained for **4** (**Tables 1−3**) showed strict resemblances with corresponding spectra of **1a−b**. In particular, the ^{1}H NMR spectrum of **4** differed from those of **1a−b** only by the following: (i) lack of the signal at δ 2.13−2.15 attributed to H-20; (ii) a low-field shift of H_3-21 (δ 1.89 instead of δ 1.04), H-17 (δ 2.45 instead of δ 1.78), H-16 (δ 4.70 instead of δ 4.59−4.39), and of H_2-23 (δ 2.20 and 2.15 instead of δ 1.72 and 1.63). On the other hand, the midfield region of the ^{1}H NMR spectrum of **4** appeared superimposable on that of ascalonicoside A1/A2,

suggesting that these saponins must possess the same sugar portion. Accordingly, the ^{13}C NMR resonances (**Tables 1** and **2**) of **4** appeared identical to those of **1a**−**b**, with only two exceptions, i.e., the signals of C-20 (δ 40.5) and C-22 (δ 102.0) were replaced by two sp^2 signals at δ 104.2 and 153.0, respectively. All these data led us to propose the structure **4** for ascalonicoside B, which corresponds to furost-5(6),20(22)-dien-3β-ol 1β-*O*-β-D-galactopyranosyl 26-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside].

Isolation of ascalonicosides A−B represents the first finding of furostane saponins in *A. ascalonicum*, a cultivated variety of *A. cepa*. Different furostanol saponins have been also isolated from other *Allium* species (*11*, *12*).

Generally onions are characterized by higher concentrations of flavonoids compared to those of garlic and leek (*6*, *13*). Leighton et al. reported that shallot shows the highest concentration of total flavonols among the onion varieties (*14*); however, the detailed composition was not reported. Therefore, we have also accurately analyzed the flavonoid fraction of shallot and found that it is composed of high amounts of free quercetin (**5**) and isorhamnetin (**6**), and by their glycosides: quercetin 4′-glucoside (**7**), quercetin 7-glucoside (**8**), quercetin 4′,3-diglucoside (**9**), isorhamnetin 4′,3-diglucoside (**10**) (**Figure 2**). These molecules have been identified by comparison of their spectroscopic data (UV, NMR) with those reported in the literature (*15*, *16*).

In vitro and in vivo pharmacological tests have shown that flavonoids, and in particular quercetin, exhibit the following variety of actions: (i) antioxidative (DNA protective) (*17*); (ii) reduction of cardiovascular disease (*18*); (iii) reduction of carcinogenic activity (*19*); (iv) inhibition of the growth of transformed-tumorigenic cells (ras/3T3 and H359) and prevention of the neoplastic transformation of NIH/3T3 cells with the oncogene H-ras (*14*). The finding of high concentrations of quercetin, isorhamnetin, and their glycosides in bulbs of shallot make this a proper food for a healthy diet.

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