The Sapogenin Atroviolacegenin and Its Diglycoside Atroviolaceoside from *Allium atroviolaceum*

Behzad Zolfaghari,[†] Elisa Barile,[‡] Raffaele Capasso,[§] Angelo A. Izzo,[§] S. Ebrahim Sajjadi,[†] and Virginia Lanzotti*[,‡]

Department of Pharmacognosy, Isfahan University of Medical Sciences, Hezar Jerib Avenue, 73461 Isfahan, Iran, Dipartimento di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche, Università del Molise, Via F. De Sanctis, 86100 Campobasso, Italy, and Dipartimento di Farmacologia Sperimentale, Università di Napoli Federico II, Napoli, Italy

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A phytochemical analysis of broadleaf wild leek, *Allium atroviolaceum*, has led to the isolation of a new sapogenin, named atroviolacegenin (**1**, Chart 1), and its diglycoside derivative, named atroviolaceoside (**2**), both possessing a hydroxyl group at C-27, a rare feature among sapogenins and saponins. On the basis of chemical and spectroscopic analyses, including 2D NMR spectroscopy and mass spectrometry, the structures of the new compounds were elucidated as (25*R*)-5α-spirostan-2α,3*β*,6*β*,27-tetrol (**1**) and (25*R*)-5α-spirostan-2α,3*β*,6*β*,27-tetrol 3-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside (**2**). These compounds are accompanied by three known spirostanol and furostanol saponins. In addition, 4,4′-dihydroxy-3-methoxychalcone, *p*-coumaroyl-*N*-tyrosine, and *p*-feruloyl-*N*-tyrosine have been found in the flowers and bulbs. Atroviolacegenin and atroviolaceoside were assayed to evaluate their antispasmodic activity in the guinea-pig isolated ileum and the data compared to those previously found for tropeosides (**3a/3b** and **4a/4b**) from Tropea red onion bulbs. The absence of activity for both atroviolacegenin and atroviolaceoside highlighted the key role of the furostanol-type aglycone moiety for antispasmodic activity.

Since ancient times medicinal plants have been used in all cultures as a source of remedies and for healthcare preparations. In fact, for centuries the word medicine has indicated herbal remedies. Only after the 1950s has medicine been based on the use of synthetic drugs, which became so dominant as to totally eclipse herbal cures. However, recently a renewed interest in medicinal plants re-emerged for restoring and maintaining health. In fact, today more than 40% of prescription drugs contain at least one natural element.[1]

Plants of the genus *Allium* have been used as a folk medicine since Egyptian times. Interestingly, epidemiological studies have suggested that *Allium* consumption may protect against several diseases (e.g., carcinogenesis, hypercholesterolemia, type 2 diabetes, hypertension, cataracts, and disturbances of the gastrointestinal tract).[2−6]

In our ongoing investigation on the chemistry of the genus *Allium*,[7−11] we have now analyzed phytochemically an *Allium ampeloprasum* variety, *Allium atroviolaceum* Boiss.,[12] commonly named broadleaf wild leek. This species has been collected in Iran, where it is known under the local name "Sir-Deng" and largely used in cooking and for treatment of arthritis and rheumatism. It can also be found in other parts of Asia, Europe, and North America. Analysis of a flower extract has led to the isolation of a new sapogenin, named atroviolacegenin (**1**, Chart 1), and its diglycoside derivative, named atroviolaceoside (**2**), both possessing a hydroxyl group at C-27, a rare feature among sapogenins[13] and saponins.[14,15]

Results and Discussion

The new compounds have been tested to evaluate their ability to act as antispasmodic agents, and the data obtained were compared with those of tropeosides (**3a/3b** and **4a/4b**), previously found in Tropea red onion.[11] The absence of activity observed for atroviolacegenin and atroviolaceoside highlighted the key role of the furostanol-type aglycone moiety in the antispasmodic activity.

Flowers of *A. atroviolaceum* Boiss. were exhaustively extracted with $CHCl_3$/MeOH (9:1) and MeOH. The MeOH extracts were partitioned between n-BuOH and H_2O, and the n-BuOH-soluble portions were separated by sequential chromatographic techniques, affording, as major metabolites, sapogenins and saponins (total content, 4986.3 mg/kg). Among these metabolites were the new sapogenin, named atroviolacegenin (**1**), and its diglycoside derivative, named atroviolaceoside (**2**, Chart 1). (25*R*)-5α-Spirostan-2α,3*β*,6*β*-triol 3-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside,[16] furost-2α,3*β*,6*β*,22α-tetrol 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranosyl] 26-*O*-*β*-D-glucopyranoside,[17] furost-2α,3*β*,6*β*,22α-tetrol 3-*O*-[*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranosyl] 26-*O*-*β*-D-glucopyranoside,[17] 4,4′-dihydroxy-3-methoxychalcone,[18] *p*-coumaroyl-*N*-tyrosine,[19] and *p*-feruloyl-N-tyrosine[20,21] were also identified. In particular, *p*-coumaroyl-*N*-tyrosine, recently characterized from cacao, *Theobroma cacao*, exhibited antioxidant properties, while *p*-feruloyl-*N*-tyrosine, recently characterized by some of us in bulbs and roots of garlic and leek, further showed antidiabetic activity.[21]

Atroviolacegenin (**1**) showed in the HRFABMS spectrum a pseudomolecular ion peak at *m*/*z* 463.6357 $[M - H]^-$, which indicated the molecular formula $C_{27}H_{44}O_6$, also confirmed by ^{13}C NMR data (Table 1). Characteristic resonances in the ^1H NMR spectrum of **1** (CD_3OD, Table 1) were those attributed to one secondary (*δ* 0.96) and two tertiary methyls (*δ* 0.82 and 1.06), four oxymethines (*δ* 3.37, 3.50, 3.78, 4.38), and two oxymethylenes (*δ* 3.34, 3.47 and *δ* 3.40, 3.70). The ^{13}C NMR spectrum showed in addition a characteristic acetal signal (*δ* 110.8), thus suggesting a hydroxylated spirostane skeleton. Analysis of 2D COSY and HOHAHA spectra of **1** permitted recognition of two spin systems. The first was a large segment starting from ring A and extending up to ring E, while the second segment included the F-ring protons. Inspection of the HSQC spectrum permitted correlation of all the proton signals to the relevant carbon atoms (Table 1), while examination of the HMBC spectrum (Figure 1) gave useful information for assigning all the quaternary carbons and for connecting the two substructures as depicted. Further analysis of ^1H and ^{13}C chemical shifts of the first segment of **1** indicate three hydroxyl groups C-2, C-3, and C-6. Moreover, the H-2 and H-3 couplings (Table 1) established their axial orientation, while the small couplings observed for H-6 defined its equatorial nature. In addition, a NOE correlation between H-3 and H-5 defined their axial orientation. With respect to the F-ring segment, the key correlations of H_2-23 with C-22 and C-20 (Figure 1) connected the two spin systems, building up the full structure. In particular,

* To whom correspondence should be addressed. Tel: 39-874-404649. Fax: 39-874-404652. E-mail: lanzotti@unimol.it.
[†] Isfahan University of Medical Sciences.
[‡] Università del Molise.
[§] Università di Napoli Federico II.

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Chart 1. Structures of Atroviolacegenin (**1**), Atroviolaceoside (**2**), Tropeosides A1/A2 (**3a/3b**) and B1/B2 (**4a/4b**), and Ascalonicosides A1/A2 (**5a/5b**)

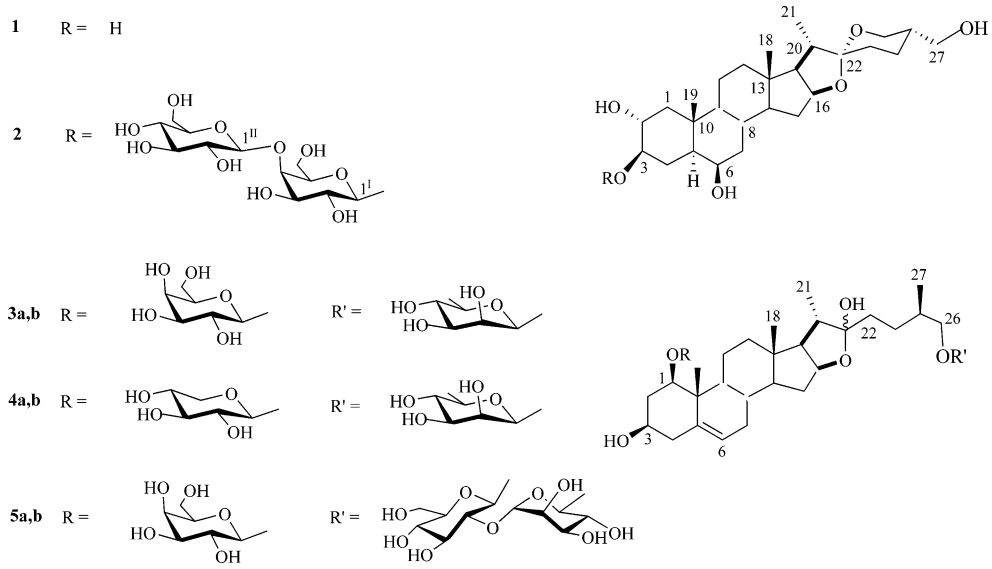

Table 1. ^{1}H and ^{13}C NMR Data of Atroviolacegenin (**1**) and of the Aglycone Portion of Atroviolaceoside (**2**)[a]

	1		**2**	
position	δ_H (int., mult., *J* in Hz)	δ_C (mult.)	δ_H (int., mult., *J* in Hz)	δ_C (mult.)
1a	0.91[b]	48.1 (CH_2)	0.91[b]	47.2 (CH_2)
1b	1.88[b]		1.89[b]	
2	3.50 (1H, ddd, 4.7, 8.8, 11.6)	73.4 (CH)	3.57 (1H, ddd, 4.7, 8.8, 11.6)	73.0 (CH)
3	3.37[b]	77.4 (CH)	3.50[b]	85.5 (CH)
4a	1.73[b]	34.5 (CH_2)	1.70[b]	37.8 (CH_2)
4b	1.89[b]		1.83[b]	
5	1.20 (1H, td, 2.8, 2.8, 12.0)	48.8 (CH)	1.20 (1H, td, 2.8, 2.8, 12.0)	48.8 (CH)
6	3.78 (1H, td, 2.5, 2.5, 5.3)	71.6 (CH)	3.79 (1H, td, 2.5, 2.5, 5.3)	71.5 (CH)
7a	1.27[b]	40.8 (CH_2)	1.28[b]	39.3 (CH_2)
7b	1.82 (1H, td, 1.4, 1.4, 11.6)		1.82 (1H, td, 1.4, 1.4, 11.6)	
8	1.97[b]	30.7 (CH)	1.96[b]	30.7 (CH)
9	0.80 (1H, dt, 4.2, 4.2, 11.9)	55.6 (CH)	0.78 (1H, dt, 4.2, 4.2, 11.9)	55.6 (CH)
10		38.2 (C)		38.2 (C)
11a	1.58[b]	22.1 (CH_2)	1.58[b]	22.1 (CH_2)
11b	1.45[b]		1.46[b]	
12a	1.22[b]	41.0 (CH_2)	1.25[b]	41.0 (CH_2)
12b	1.76[b]		1.77[b]	
13		41.8 (C)		41.8 (C)
14	1.21[b]	57.2 (CH)	1.20[b]	57.1 (CH)
15a	2.00[b]	32.7 (CH_2)	1.99[b]	32.7 (CH_2)
15b	1.33[b]		1.33[b]	
16	4.38 (1H, td, 6.3, 6.3, 7.8)	82.2 (CH)	4.36 (1H, td, 6.3, 6.3, 7.8)	82.2 (CH)
17	1.79 [b]	63.9 (CH)	1.79 [b]	63.8 (CH)
18	0.82 (3H, s)	17.0 (CH_3)	0.82 (3H, s)	17.0 (CH_3)
19	1.06 (3H, s)	17.2 (CH_3)	1.06 (3H, s)	17.2 (CH_3)
20	1.92[b]	43.0 (CH)	1.92[b]	43.0 (CH)
21	0.96 (3H, d, 7.0)	14.9 (CH_3)	0.96 (3H, d, 7.0)	14.9 (CH_3)
22		110.8 (C)		110.8 (C)
23a	1.59[b]	39.3 (CH_2)	1.57[b]	39.3 (CH_2)
23b	1.74 (1H, dt, 4.6, 13.3, 13.3)		1.74 (1H, dt, 4.6, 13.3, 13.3)	
24a	1.44[b]	30.7 (CH_2)	1.45[b]	30.7 (CH_2)
24b	1.67[b]		1.67[b]	
25	1.61[b]	32.0 (CH)	1.61[b]	31.9 (CH)
26a	3.34[b]	65.1 (CH_2)	3.34[b]	65.1 (CH_2)
26b	3.47[b]		3.47[b]	
27a	3.40[b]	64.4 (CH_2)	3.40[b]	64.4 (CH_2)
27b	3.70[b]		3.70[b]	

[a] The spectra were measured in CD_3OD. [b] Overlapped with other signals.

both the presence of a further oxymethylene signal (^{1}H: δ 3.40, δ 3.70; ^{13}C: δ 64.4) and the absence of the characteristic Me-27 doublet strongly indicated an oxidation at C-27. This has been confirmed by the HMBC correlations of H_2-27 with C-24, C-25, and C-26 (Figure 1).

The 25*R* configuration 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 C-26 protons, derived from a subspectra of the 2D HOHAHA experiment (J_{25-26a} = 11 Hz and J_{25-26b} = 3.5 Hz) in comparison with literature data.[7−11,22] Finally, the

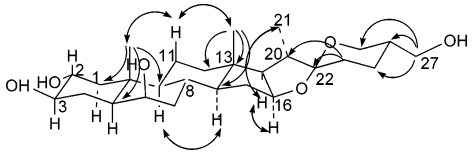

Figure 1. Selected HMBC (H→C) and ROESY (H↔H) correlations exhibited by compound **1**.

ROESY correlations (Figure 1) H-11/H_3-19, H-11/H_3-18, H-9/H-14, H-16/H-17, and H-17/H_3-21 completed the assignment of the relative configuration of **1** with the usual A/B, B/C, and C/D *trans*, D/E *cis*, and C-20α configurations.[23]

High-resolution FABMS of atroviolaceoside (**2**) indicated its molecular formula as $C_{39}H_{64}O_{16}$. Preliminary NMR data of **2** revealed a saponin structure for this compound that differed from **1** by the presence of signals attributed to two sugar residues. This was first suggested by MS data (the psedomolecular ion of **2** being 324 amu more that those of **1**, see Experimental Section) and confirmed by the ^{1}H and ^{13}C NMR spectra of **2** (Table 1). Also for this compound the characteristic singlet at δ 110.8 was indicative of an acetal function. Although the high-field region of the ^{1}H NMR spectrum contained some overlapped signals, analysis of COSY and HOHAHA experiments permitted assignment of all the proton resonances of the aglycone moiety. Among these signals the presence of three methyl protons (δ 0.82, 0.96, and 1.06), four oxymethines (δ 3.50, 3.57, 3.79, 4.36), and two oxymethylenes (δ 3.34, 3.47 and δ 3.40, 3.70) were evident. All these resonances were associated with those of directly attached carbon signals in the ^{13}C NMR spectrum by using a HSQC experiment, and the analysis of all the data was in agreement with a 25*R* atroviolacegenin structure.

The analysis of the saccharide part started with the association of the two anomeric carbons (δ 103.1 and 106.2) with the relevant anomeric proton signals (δ 4.37 and 4.51, respectively), through the HSQC experiment. The nature of the monosaccharides and their connectivity were determined by combined analysis of COSY, HOHAHA, HMQC, and HMBC spectra.

Starting from the anomeric proton at δ 4.37 (H-1^I), the sequence of a hexopyranose unit could be identified. The large coupling constants observed in a 2D HOHAHA subspectra for H-1^I/H-2^I and H-2^I /H-3^I, and the relatively small coupling constants of H-3^I / H-4^I and H-4^I /H-5^I, indicated its β-galactopyranose nature. With the same type of analysis the other sugar was identified as a β-glucopyranose on the basis of the large value of the coupling constants found throughout the spin system.

The β-galactose was placed at C-3 of atroviolaceoside by interpretation of the key HMBC correlation peaks between H-1 Gal (δ 4.37) and C-3 (δ 85.5) and between H-3 (δ 3.50) and C-1 Gal (δ 103.1). The linkage of the glucose at position 4 on the galactose was suggested by considering the low-field value found for the characteristic broad singlet attributed to H-4 Gal (δ 4.05). This interglycosidic linkage has been confirmed by the HMBC cross-peaks of H-1 Glc (δ 4.51) with C-4 Gal (δ 79.4).

To confirm the nature of the sugar units and to determine their absolute configuration, compound **2** 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 and galactose. By this procedure both sugars were identified to belong to the common D-series.

Recently, we have reported from red onion, *A. cepa* var. Tropea, the isolation and structure elucidation of four new disaccharide furostanol saponins, tropeosides A1/A2 (**3a/3b**) and B1/B2 (**4a/4b**), and their ability to act as antispasmodic agents in reducing the contraction evoked by histamine and acetylcholine on the guinea-pig isolated ileum.[11] Furthermore, we have also found that the trisaccharide furostanol saponins, ascalonicosides A1/A2 (**5a/5b**), based on the same aglycone, and differing from tropeosides in having a further glucose unit at C-3, were totally inactive.[11] These data indicated that an increase of sugar residues on the furostanol aglycone leads to a decrease of the antispasmodic activity.

Atroviolacegenin and atroviolaceoside were subjected to antispasmodic tests, by monitoring the contractions evoked by histamine on the same animal intestinal tract. The data showed the absence of pharmacological activity for both compounds. Further comparison of the chemical structures of the disaccharide spirostanol saponin atroviolaceoside (**2**) with the spirostanol aglycone moiety, and tropeosides (**3a/3b** and **4a/4b**) with furostanol aglycone moieties, in terms of bioactivity (0% inhibition vs 50% inhibition, respectively) highlighted the importance of the furostanol-type aglycone moiety for the bioactivity. In fact, atroviolaceoside, which lacks this structural feature, being based on a spirostanol-type aglycone, does not act as an antispasmodic agent. These data are confirmed by the observation that also its corresponding sapogenin, atroviolacegenin, is totally inactive.

In conclusion, pharmacological tests performed on atroviolacegenin and atroviolaceoside, in comparison with those of tropeosides,[11] showed that a furostanol-type aglycone unit is key for the antispasmodic properties of saponins.

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 on a Varian Unity-500 spectrometer. Chemical shifts were referenced 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 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.[24] One-bond heteronuclear ^{1}H−^{13}C connectivities were determined with a 2D HSQC[25] pulse sequence with an interpulse delay set for $^1J_{CH}$ = 130 Hz. Two- and three-bond heteronuclear ^{1}H−^{13}C connectivities were determined with 2D HMBC experiments,[26] optimized for $^{2,3}J_{CH}$ = 8 Hz. NOE measurements were performed by 2D ROESY experiments.[27] Low- and high-resolution FAB mass spectra (glycerol matrix) were measured on a Prospect Fisons mass spectrometer. 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 a RI-3 refractive index detector using semipreparative μ-Bondapack C_{18} columns (7.8 mm × 300 mm, i.d.) with a flow rate of 2.5 mL/min.

Plant Material. Wild samples of bulbs and flowers of *A. atroviolaceum* were collected in the Semirum (2300 m), Isfhan Provinces, Iran, in June 2002 and 2003, respectively, and identified by Mr. Iraj Mehregan, Isfahan Medical Sciences and Health Services University. A voucher specimen (No. 1143) has been deposited at the Department of Pharmacognosy, Isfahan University of Medical Sciences. A taxonomic revision of the Iranian flora by Prof. Shahin Zarre, Department of Biology, University of Teheran, changed the name of this species from *A. atroviolaceum* Boiss. to *Allium iranicum* Wendelbo.[28]

Extraction and Isolation. The flowers and bulbs were air-dried immediately after collection, under controlled temperature (22 °C) and without exposure to light, giving a dry weight of 1.5 and 2 kg, respectively. They were chopped and then submitted to the same extraction procedure with the following solvents: hexane, $CHCl_3$, $CHCl_3$/MeOH (9:1), and MeOH. Each solvent extraction stage was conducted for 1 day and was repeated four times using 4 L of solvent, under stirring.

The $CHCl_3$/MeOH (9:1) extract from flowers was concentrated in vacuo, affording a crude organic extract (55.0 g), which was chromatographed by MPLC on an RP-18 column using a linear gradient solvent system from H_2O to MeOH. Preliminary NMR analysis on the eluates led to selection of fractions that eluted with H_2O/MeOH (4:6) (1.21 g) and H_2O/MeOH (3:7) (7.09 g). The first fraction was chromatographed by HPLC on a semipreparative C_{18} column with the mobile phase H_2O/MeOH (1:1) to give the pure sapogenin **1** (53.8 mg,

Table 2. ^{1}H and ^{13}C NMR Data of the Sugar Portion of Atroviolaceoside (**2**)[a]

	δ_H (mult., *J* in Hz)	δ_C (mult.)
Gal I		
1	4.37 (1H, d, 7.4)	103.1 (CH)
2	3.43 (1H, dd, 7.4,8.1)	75.7 (CH)
3	3.34[b]	78.3 (CH)
4	4.05 (1H, bd, 2.5)	79.4 (CH)
5	3.22[b]	68.0 (CH)
6a	3.53[b]	62.9 (CH_2)
6b	3.58[b]	
Glc II		
1	4.51 (1H, d, 7.3)	106.2 (CH)
2	3.78[b]	78.1 (CH)
3	3.20[b]	75.1 (CH)
4	3.32[b]	71.9 (CH)
5	3.26 (1H, m)	75.7 (CH)
6a	3.65[b]	64.0 (CH_2)
6b	3.84[b]	

[a] The spectra were measured in CD_3OD. [b] Overlapped with other signals.

t_R = 22.5 min) as an amorphous solid. The second fraction afforded the known saponin (25*R*)-5α-spirostan-2α,3*β*,6*β*-triol 3-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside.[16]

The MeOH extract (102.3 g) of the flowers was partitioned between n-BuOH and H_2O. The n-BuOH-soluble fraction was filtered and then concentrated in vacuo, giving a crude extract (78.8 g), which was chromatographed by MPLC on an RP-18 column using a linear gradient solvent system from H_2O to MeOH. Preliminary NMR study of the eluates led to selection of one fraction containing saponin compounds (see below), together with three fractions containing phenolic compounds. The latter, eluted with H_2O/MeOH (6:4, 1:1, and 4:6), were further purified by HPLC on a semipreparative C_{18} column with the mobile phase H_2O/MeOH (75:25, 65:35, and 55:45, respectively) to afford 4,4′-dihydroxy-3-methoxychalcone (13.2 mg),[18] *p*-coumaroyl-*N*-tyrosine (68.7 mg),[19] and *p*-feruloyl-*N*-tyrosine (105.2 mg),[20,21] respectively. The fraction containing saponins, eluted with H_2O/MeOH (3:7), was chromatographed by HPLC on a semipreparative C_{18} column with the mobile phase H_2O/MeOH (45:55) to give the known compound furost-2α,3*β*,6*β*,22α-tetrol 3-*O*-[*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranosyl] 26-*O*-*β*-D-glucopyranoside (96.3 mg)[17] and four peaks, which were pooled and further purified by HPLC on a semipreparative C_{18} column with the mobile phase H_2O/MeOH (1:1), thus affording the known saponin furost-2α,3*β*,6*β*,22α-tetrol 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranosyl] 26-*O*-*β*-D-glucopyranoside (85.6 mg)[17] and pure saponin **2** (153.8 mg, t_R = 22.2 min), isolated as an amorphous solid.

The $CHCl_3$/MeOH (9:1) extract and the butanol-soluble fraction of MeOH extract from bulbs were both purified by MPLC on a RP-18 column, using a linear gradient solvent system from 100% H_2O to 100% MeOH. Successive NMR analyses on the eluted fractions did not show sapogenin and saponin compounds.

Atroviolacegenin [(25*R*)-5α-spirostan-2α,3*β*,6*β*,27-tetrol, 1]: $[\alpha]_D^{25}$ −28.7 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3410, 2933, 1157, 1050 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS (negative ion) found *m*/*z* 463.6357 [M − H]$^-$ (calcd for $C_{27}H_{44}O_6$ *m*/*z* 464.6446).

Atroviolaceoside [(25*R*)-5α-spirostan-2α,3*β*,6*β*,27-tetrol 3-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside]: $[\alpha]_D^{25}$ −25.7 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3412, 2928, 1152, 1048 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS (negative ion) found *m*/*z* 787.9248 [M − H]$^-$ (calcd for $C_{39}H_{64}O_{16}$ *m*/*z* 788.9346).

Determination of the Absolute Configuration of Sugars. A solution of atroviolaceoside (1 mg) in 1 N HCl (0.25 mL) was stirred at 80 °C for 4 h. After cooling, the solution was concentrated in a stream of N_2. The residue was dissolved in 1-(trimethylsilyl)imidazole (Trisil-Z) and pyridine (0.1 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution in a stream of N_2, the residue was partitioned between H_2O and CH_2Cl_2 (1 mL, v/v 1:1). The CH_2Cl_2 layer was analyzed by GC using an L-Chirasil-Val column (0.32 mm × 25 m). Temperatures of both injector and detector were 200 °C. A temperature gradient system was used for the oven; the initial temperature was maintained at 100 °C for 1 min and then raised to 180 °C at the rate of 5 °C/min.

Peaks of the hydrolysate of **2** were detected at 13.98 min (D-galactose) and 14.66 min (D-glucose) in the ratio of 1:1. Retention times for authentic samples after being treated simultaneously with Trisil-Z were 13.98 min (D-galactose) and 13.75 min (L-galactose), 14.66 min (D-glucose) and 14.73 min (L-glucose). Co-injection of the hydrolysate of **2** with standards D-galactose and d-glucose gave single peaks.

Biological Assay. Male guinea-pigs (250−350 g, Harlan Italy) were killed by asphyxiation with CO_2, and segments (1−1.5 cm) of ileum were removed, flushed of luminal contents, and placed in Krebs solution (mM: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, $MgSO_4$ 1.5, and glucose 11). The segments were set up as previously described.[29] In brief, the segments were set up (in such a way as to record contractions mainly from the longitudinal axis) in an organ bath, containing 20 mL of Krebs equilibrated with 95% O_2 and 5% CO_2 at 37 °C. The tissues were connected to an isotonic transducer (load 0.5 g), connected to a Gemini recording apparatus (Ugo Basile, Comerio Italy). Ileal segments were allowed to equilibrate 60 min before experiments. To evaluate the antispasmodic activity of the tested compounds, contractions were elicited by histamine (10^{-7}). After stable control contractions evoked by histamine had been recorded, the responses were observed in the presence of increasing concentrations (10^{-8}−10^{-5} M) of the test compounds (or papaverine used as a reference compound). The contact time for each concentration was 30 min. Atroviolacegenin and its diglycoside derivative, atroviolaceoside, were dissolved in DMSO. DMSO (less than 0.01%) did not modify histamine-induced contractions. Results are expressed as mean ± SEM. Nonlinear regression analysis for all concentration response curves was performed. Data were analyzed by ANOVA. The results were considered significant if the probability of error was <5.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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