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Antispasmodic Saponins from Bulbs of Red Onion, *Allium cepa* L. Var. Tropea

GABRIELLA COREA,[†] ERNESTO FATTORUSSO,[†] VIRGINIA LANZOTTI,*[,‡] RAFFAELE CAPASSO,[§] AND ANGELO A. IZZO[§]

Dipartimento di Chimica delle Sostanze Naturali and Dipartimento di Farmacologia Sperimentale, Università di Napoli "Federico II", Via D. Montesano 49, I-80131 Napoli, Italy, and Dipartimento di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, Via F. De Sanctis, I-86100 Campobasso, Italy

A phytochemical analysis of the polar extract from the red bulbs of *Allium cepa* L. var. Tropea, typical of Calabria, a southern region of Italy, was performed extensively for the first time, leading to the isolation of four new furostanol saponins, named tropeoside A1/A2 (**1a**/**1b**) and tropeoside B1/B2 (**3a**/**3b**), along with the respective 22-*O*-methyl derivatives (**2a**/**2b** and **4a**/**4b**), almost certainly extraction artifacts. High concentrations of ascalonicoside A1/A2 (**5a**/**5b**) and ascalonicoside B (**6**), previously isolated from *Allium ascalonicum* Hort., were also found. This is the first report of furostanol saponins in this *A. cepa* variety. The chemical structures of the new compounds were established through a combination of extensive nuclear magnetic resonance, mass spectrometry and chemical analyses. High concentrations of quercetin, quercetin 4[I]-glucoside, taxifolin, taxifolin 7-glucoside, and phenylalanine were also isolated. The new saponins were found to possess antispasmodic activity in the guinea pig isolated ileum; such an effect might contribute to explaining the traditional use of onion in the treatment of disturbances of the gastrointestinal tract.

KEYWORDS: Alliaceae; red onion; saponins; tropeosides A and B; flavonoids; antispasmodic activity

INTRODUCTION

Onion (*Allium cepa* L.) is one of the oldest cultivated plants, and it is now used both as a food and for medical purposes. In fact, onion is a rich source of a number of phytonutrients, which make it an important element of the Mediterranean diet. It is also useful for the treatment or prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, type 2 diabetes, hypertension, cataract, and disturbances of the gastrointestinal tract (e.g., colic pain, flatulent colic, and dyspepsia) (*1*).

The history of the onion is well documented and can be traced to its origin in a wide area from India to Israel, where its production started in 3000 B.C., and then it was introduced in Europe by the Phoenicians around 2000 years ago. In ancient Egypt, the onion was believed to be a sacred food, and it was also widely consumed by the Romans and Greeks, who liked its taste and knew of its curative properties (*2*). Accordingly, Pliny the Elder, in his *Naturalis historia*, lists 30 ailments that can be treated by onions and pointed out that any dishes containing the onions are, to a certain extent, curative as well as more nourishing and tasty. Epidemiological studies have consistently shown an inverse association between consumption of vegetables (onion included) and the risk of human diseases. Notably, a population-based case-control study showed that the consumption of *Allium* vegetables was associated with a reduced risk of prostate cancer (*3*). These observations prompted us to continue the phytochemical investigation into this prolific source of interesting compounds.

The red onion, *A. cepa* L. var. Tropea, is a typical Italian variety, cultivated in some areas of southern Italy (Calabria region). This variety, characterized by both white and purple flowers, is known for its distinctive bulb, lengthened or oval, red, and sweet. Analysis of the MeOH extract from the bulbs of Tropea onions revealed the presence of high concentrations of flavonoids and saponins. Among these, four saponins, named tropeosides A1/A2 (**1a**/**1b**) and B1/B2 (**3a**/**3b**), appeared to be new compounds.

Plant-derived saponins present a broad spectrum of biological uses, such as anti-cancer, anti-inflammatory, ion channel blocking, immune stimulating, antifungal, antithrombotic, and hypocholesterolemic properties (*4*, *5*). Due to the traditional use of onion in the treatment of gastrointestinal tract disturbances, the isolated saponins have been tested for the first time to evaluate an eventual antispasmodic activity on the guinea pig isolated ileum, and the results will be discussed herein.

* Author to whom correspondence should be addressed (telephone +39-0874-404649; fax +39-0874-404652; e-mail lanzotti@unimol.it).
[†] Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II.
[‡] Università degli Studi del Molise.
[§] Dipartimento di Farmacologia Sperimentale, Università di Napoli Federico II.

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MATERIALS AND METHODS

General Methods. Optical rotations were determined on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 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: 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 of 125 ms) sequence. One-bond heteronuclear ^{1}H−^{13}C connectivities were determined with a 2D HSQC 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 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 an RI-3 refractive index detector using a Waters semipreparative 300 × 7.5 mm i.d. μ-Bondapack C_{18} column (injection amount = 180 μL, flow rate = 2.5 mL/min) and an analytical 300 × 3.9 mm i.d. μ-Bondapack C_{18} column (injection amount = 100 μL, flow rate = 0.8 mL/min).

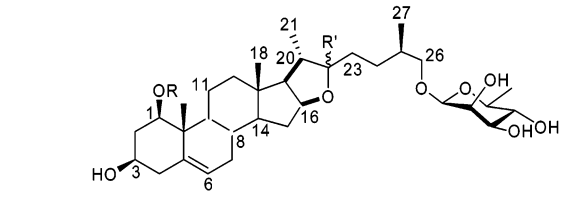
Plant Material, Extraction, and Isolation. Cultivated bulbs of *A. cepa* L. var. Tropea were collected in Tropea (Calabria, Italy) in April 2003. A voucher specimen (no. 2003ACT1) has been deposited at the Dipartimento di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche, Campobasso. Fresh bulbs (5.05 kg) were finely hand-cut and then exhaustively extracted, at room temperature, first with $CHCl_3$/MeOH (9:1) and then with pure MeOH. Each solvent extraction was conducted for several days and was repeated four times using 4 L of solvent, under stirring. The MeOH extract (241 g) was partitioned between water and BuOH to remove sugar compounds and water, and the organic layer was then filtered and concentrated in vacuo to afford a crude extract (44.7 g), which was chromatographed by MPLC on an RP-18 column, using a linear gradient from H_2O to MeOH. Preliminary NMR studies revealed that fractions eluted from H_2O/MeOH (7:3) to H_2O/MeOH (4:6) contained flavonoids in high concentrations and fractions eluted from H_2O/MeOH (3:7) to H_2O/MeOH (1:9) contained saponins as major metabolites.

Fractions eluted in H_2O/MeOH (7:3) (231.6 mg) and H_2O/MeOH (6:4) (551.3 mg) yielded the known taxifolin 7-glucoside (29.5 mg) and taxifolin (490.4 mg), respectively. This last fraction also contained the amino acid phenylalanine in relatively high amounts (50.1 mg). Fractions eluted in H_2O/MeOH (1:1) (215.3 mg) and H_2O/MeOH (4:6) (501.6 mg) yielded the known quercetin 4^l-glucoside (140.5 mg) and the flavonol quercetin (511.8 mg), respectively.

The fraction eluted in H_2O/MeOH (3:7) (271.2 mg) yielded the known saponin ascalonicoside A1/A2 (**5a/5b**, 235.9 mg) (**Figure 1**), whereas ascalonicoside B (**6**, 250.6 mg) was found in the fraction eluted in H_2O/MeOH (2:8) (435.1 mg) (*6*). The last fraction, H_2O/MeOH (1:9), 375.3 mg, was further purified by HPLC on a semipreparative C_{18} column with a mobile phase of H_2O/MeOH (3:7), affording the mixtures of new saponins tropeoside A1/A2 (**1a/1b**, 67.4 mg, t_R = 11.0 min) and tropeoside B1/B2 (**3a/3b**, 106.4 mg, t_R = 14.6 min). Each mixture was separately purified on an analytical C_{18} column, mobile phase H_2O/MeOH (35:65), affording pure tropeoside A1 (**1a**, 26.4 mg, t_R = 5.7 min), tropeoside A1 (**1b**, 38.0 mg, t_R = 8.0 min), tropeoside B1 (**3a**, 46.1 mg, t_R = 7.2 min), and tropeoside B2 (**3b**, 60.3 mg, t_R = 9.5 min).

*Tropeoside A1 (**1a**):* yield, 26.4 mg; colorless amorphous solid. Furost-5(6)-en-3β,22α-diol 1β-*O*-β-D-galactopyranosyl 26-*O*-α-L-rhamnopyranoside: $[\alpha]_D^{25}$ −44.3° (*c* 0.1, MeOH); HRFABMS (negative ion) of the equilibrated mixture (*m*/*z*) [M − H]$^-$ calcd for $C_{39}H_{64}O_{14}$, 756.4278; found, 755.4205; ^{1}H NMR data, see **Tables 1** and **3**; ^{13}C NMR data, see **Tables 2** and **3**.

*Tropeoside A2 (**1b**):* yield, 38.0 mg; colorless amorphous solid. Furost-5(6)-en-3β,22β-diol 1β-*O*-β-D-galactopyranosyl 26-*O*-α-L-rhamnopyranoside: $[\alpha]_D^{25}$ −44.3° (*c* 0.1, MeOH); HRFABMS (negative ion) of the equilibrated mixture (*m*/*z*) [M − H]$^-$ calcd for $C_{39}H_{64}O_{14}$, 756.4278; found, 755.4205; ^{1}H NMR, data see **Tables 1** and **3**; ^{13}C NMR data, see **Tables 2** and **3**.

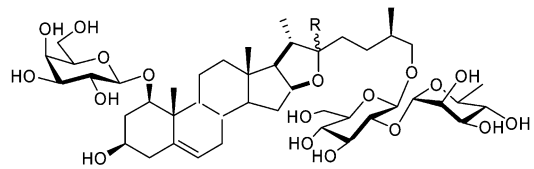


1a R = β-D-Gal R' = α-OH **1b** R =β-D-Gal R' =β-OH

2a R = β-D-Gal R' = α-OCH_3 **2b** R =β-D-Gal R' =β-OCH_3

3a R = β-D-Xyl R' = α-OH **3b** R = β-D-Xyl R' =β-OH

4a R = β-D-Xyl R' = α-OCH_3 **4b** R = β-D-Xyl R' =β-OCH_3



5a R = α-OH **5b** R = β-OH

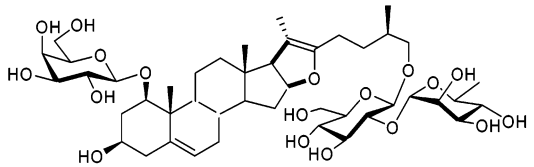


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Figure 1. Saponins of *A. cepa* L. var. Tropea.

*Tropeoside B1 (**3a**):* yield, 46.1 mg; colorless amorphous solid. Furost-5(6)-en-3β,22α-diol 1β-*O*-β-D-xylopyranosyl 26-*O*-α-L-rhamnopyranoside: $[\alpha]_D^{25}$ −43.2° (*c* 0.1, MeOH); HRFABMS (negative ion) of the equilibrated mixture (*m*/*z*) [M − H]$^-$ calcd for $C_{38}H_{62}O_{13}$, 726.4173; found, 725.4090; ^{1}H NMR data, see **Tables 1** and **3**; ^{13}C NMR data, see **Tables 2** and **3**.

*Tropeoside B2 (**3b**):* yield, 60.3 mg; colorless amorphous solid. Furost-5(6)-en-3β,22β-diol 1β-*O*-β-D-xylopyranosyl 26-*O*-α-L-rhamnopyranoside: $[\alpha]_D^{25}$ −43.2° (*c* 0.1, MeOH); HRFABMS (negative ion) of the equilibrated mixture (*m*/*z*) [M − H]$^-$ calcd for $C_{38}H_{62}O_{13}$, 726.4173; found, 725.4090; ^{1}H NMR data, see **Tables 1** and **3**; ^{13}C NMR data, see **Tables 2** and **3**.

Determination of Sugar Absolute Configurations. 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-(trimethylsilyl)imidazole (Trisil-Z) and pyridine (0.1 mL), and the solution was stirred at 60 °C for 5 min. After the solution had been dried with a stream of N_2, the residue was separated by water and CH_2Cl_2 (1 mL, 1:1). The CH_2Cl_2 layer was analyzed by GC on a 25 m × 0.32 mm × 25 m l-Chirasil-Val column (Alltech, Deerfield, IL). Temperatures for the injector and detector were 200 °C, and the temperature gradient system for the oven was as follows: 100 °C for 1 min, raised to 180 °C at 5 °C/min. Peaks of the hydrolysate of **1a**/**1b** were detected at 12.89 (L-rhamnose) and 13.77 min (D-galactose), in the ratio of 1:1. Peaks of the hydrolysate of **3a**/**3b** were detected at 10.97 (D-xylose) and 12.90 min (L-rhamnose), in the ratio of 1:1. Retention times for authentic samples after being treated simultaneously with Trisil-Z were 12.78 (D-rhamnose) and 12.89 min (L-rhamnose); 13.77 (D-galactose) and 13.84 min (L-galactose); and 10.97 (D-xylose) and 11.05 min (L-xylose). Co-injection of each hydrolysate with standard D-galactose, L-rhamnose, and D-xylose gave single peaks.

Table 1. ^{1}H NMR Data of the Aglycon Portion of Tropeosides A1 (**1a**), A2 (**1b**), B1 (**3a**), and B2 (**3b**) (Spectra Measured in CD_3OD at 500 MHz)

	δ_H (int, mult, *J* in Hz)			
position	**1a**	**1b**	**3a**	**3b**
1	3.36 (1H, dd, 7.2, 3.5)	3.36 (1H, dd, 7.2, 3.5)	3.36 (1H, dd, 7.2, 3.5)	3.36 (1H, dd, 7.2, 3.5)
2a	2.08[a]	2.06[a]	2.09[a]	2.07[a]
b	1.70[a]	1.70[a]	1.70[a]	1.70[a]
3	3.31[a]	3.30[a]	3.30[a]	3.29[a]
4a	2.24 (1H, dd, 11.5, 7.3)	2.23 (1H, dd, 11.5, 7.3)	2.27 (1H, dd, 11.5, 7.3)	2.26 (1H, dd, 11.5, 7.3)
b	2.20 (1H, dd, 11.5, 3.5)	2.20 (1H, dd, 11.5, 3.5)	2.21 (1H, dd, 11.5, 3.5)	2.21 (1H, dd, 11.5, 3.5)
6	5.55 (1H, dd, 5.5, 2.5)	5.55 (1H, dd, 5.5, 2.5)	5.56 (1H, dd, 5.5, 2.5)	5.56 (1H, dd, 5.5, 2.5)
7a	1.98[a]	1.98[a]	1.98[a]	1.98[a]
b	1.96[a]	1.96[a]	1.98[a]	1.98[a]
8	1.52 (1H, m)	1.52 (1H, m)	1.50 (1H, m)	1.50 (1H, m)
9	1.31[a]	1.31[a]	1.29[a]	1.29[a]
11a	2.56 (1H, dd, 10.5, 2.5)	2.55 (1H, dd, 10.5, 2.5)	2.55 (1H, dd, 10.5, 2.5)	2.54 (1H, dd, 10.5, 2.5)
b	1.41 (1H, m)	1.42 (1H, m)	1.43 (1H, m)	1.44 (1H, m)
12a	1.70[a]	1.70[a]	1.70[a]	1.70[a]
b	1.19[a]	1.20[a]	1.20[a]	1.21[a]
14	1.17[a]	1.17[a]	1.16[a]	1.16[a]
15a	1.98[a]	1.98[a]	1.98[a]	1.98[a]
b	1.31[a]	1.31[a]	1.31[a]	1.31[a]
16	4.60 (1H, q, 5.5)	4.38 (1H, q, 5.5)	4.60 (1H, q, 5.5)	4.39 (1H, q, 5.5)
17	1.72[a]	1.67[a]	1.72[a]	1.67[a]
18	0.80 (3H, s)	0.79 (3H, s)	0.80 (3H, s)	0.79 (3H, s)
19	1.09 (3H, s)	1.10 (3H, s)	1.09 (3H, s)	1.08 (3H, s)
20	2.07[a]	2.09[a]	2.08[a]	2.10[a]
21	0.99 (3H, d, 6.6)	0.96 (3H, d, 6.6)	0.99 (3H, d, 6.6)	0.96 (3H, d, 6.6)
23a	1.72 [a]	1.75 [a]	1.72[a]	1.75[a]
b	1.63[a]	1.61[a]	1.63[a]	1.61[a]
24a	1.40[a]	1.36[a]	1.40[a]	1.36[a]
b	1.31[a]	1.32[a]	1.31[a]	1.32[a]
25	1.71[a]	1.71[a]	1.71[a]	1.71[a]
26a	3.87 (1H, dd, 8.5, 6.9)	3.87 (1H, dd, 8.5, 6.9)	3.85 (1H, dd, 8.5, 6.9)	3.85 (1H, dd, 8.5, 6.9)
b	3.30[a]	3.30[a]	3.30[a]	3.30[a]
27	0.97 (3H, d, 6.6)	0.97 (3H, d, 6.6)	0.97 (3H, d, 6.6)	0.97 (3H, d, 6.6)

[a]Overlapped with other signals.

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 (119 mM NaCl, 4.75 mM KCl, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.5 mM $MgSO_4$, and 11 mM glucose). The segments were prepared as previously described (*7*). 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 for 60 min before experiments. To evaluate the antispasmodic activity of the tested compounds, contractions were elicited by acetylcholine (10^{-7} M) or histamine (10^{-7}). After stable control contractions evoked by acetylcholine (or histamine) had been recorded, the responses were observed in the presence of increasing concentrations (10^{-8}−10^{-5} M) of the tested compounds (or papaverine, used as a reference compound). The contact time for each concentration was 30 min. Ascalonicoside and tropeosides were dissolved in DMSO. DMSO (<0.01%) did not modify acetylcholine- or 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 to be significant if the probability of error was <5%.

RESULTS AND DISCUSSION

Bulbs of red onion, *A. cepa* L. var. Tropea, were exhaustively extracted with $CHCl_3$/MeOH, 9:1, and MeOH. The MeOH extracts were partitioned between butanol and water, and the butanol-soluble portions were separated by sequential chromatographic techniques, affording, as major metabolites, furostanol saponins (total saponin content = 130.75 mg/kg) and flavonoids (total flavonoid concentration = 232.12 mg/kg). As major saponin metabolites, we have isolated four new furostanol saponins (**Figure 1**), named tropeosides A1/A2 (**1a**/**1b**) and B1/B2 (**3a**/**3b**), the known ascalonicoside A1/A2 (**5a**/**5b**), and ascalonicoside B (**6**), previously isolated from *Allium ascalonicum* Hort. (*6*). Concerning the flavonoids, quercetin, quercetin 4^l-glucoside, both already described from *A. cepa* (*8*), taxifolin, and taxifolin 7-glucoside have been also found.

Tropeoside A1 (**1a**, **Figure 1**) was isolated as a white amorphous solid and showed in the negative FAB MS spectrum a pseudomolecular ion peak at *m/z* 755 $[M - H]^-$. High-resolution measurements on the pseudomolecular ion peak indicated the molecular formula $C_{39}H_{64}O_{14}$. Diagnostic resonances in the ^{1}H NMR spectrum of **1a** (CD_3OD, **Tables 1** and **3**) were those attributed to two tertiary methyls (δ 0.80 and 1.09), three secondary methyls (δ 0.97, 0.99, and 1.25), and one olefinic (δ 5.55), and two anomeric (δ 4.30 and 5.30) protons. The ^{13}C NMR spectrum (**Tables 2** and **3**) showed 39 resonance lines, supporting the molecular formula deduced from MS, 27 of them attributed to the aglycon and 12 to two monosaccharides. Analysis of 2D COSY, HOHAHA, HSQC, and HMBC spectra of **1a** allowed assignment of all the signals belonging to the aglycon moiety, which was identified as a trihydroxylated steroid of the furostanol type. In particular, ring A constitutes an isolated spin system possessing two hydroxyl groups at positions 1 and 3 (**Table 1**). The NOE effect between H-1 and H-3, observed in a ROESY spectrum (**Figure 2**), indicated the cis orientation of the two OH groups of ring A. The third hydroxyl group of the aglycon was easily located on the hemiacetal carbon at C-22 (δ 110.0). 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

Table 2. ^{13}C NMR Data of the Aglycon Portion of Tropeosides A1 (**1a**), A2 (**1b**), B1 (**3a**), and B2 (**3b**) (Spectra Measured in CD_3OD at 125 MHz)

	δ_C (mult)			
position	**1a**	**1b**	**3a**	**3b**
1	84.6 (CH)	84.6 (CH)	84.6 (CH)	84.6 (CH)
2	37.5 (CH_2)	37.5 (CH_2)	37.3 (CH_2)	37.3 (CH_2)
3	69.4 (CH)	69.4 (CH)	69.1 (CH)	69.1 (CH)
4	43.3 (CH_2)	43.3 (CH_2)	43.4 (CH_2)	43.4 (CH_2)
5	139.4 (C)	139.4 (C)	139.6 (C)	139.6 (C)
6	125.8 (CH)	125.8 (CH)	125.9 (CH)	125.9 (CH)
7	32.5 (CH_2)	32.3 (CH_2)	32.7 (CH_2)	32.5 (CH_2)
8	33.9 (CH)	33.9 (CH)	34.1 (CH)	34.1 (CH)
9	51.5 (CH)	51.5 (CH)	51.5 (CH)	51.5 (CH)
10	43.2 (C)	43.2 (C)	43.4 (C)	43.4 (C)
11	24.5 (CH_2)	24.5 (CH_2)	24.7 (CH_2)	24.7 (CH_2)
12	40.9 (CH_2)	40.9 (CH_2)	41.1 (CH_2)	41.0 (CH_2)
13	41.1 (C)	41.1 (C)	41.2 (C)	41.2 (C)
14	57.9 (CH)	57.9 (CH)	58.0 (CH)	58.0 (CH)
15	32.7 (CH_2)	32.7 (CH_2)	32.9 (CH_2)	32.9 (CH_2)
16	82.1 (CH)	82.1 (CH)	82.3 (CH)	82.3 (CH)
17	63.8 (CH)	63.7 (CH)	63.9 (CH)	63.8 (CH)
18	17.3 (CH_3)	17.2 (CH_3)	17.1 (CH_3)	17.0 (CH_3)
19	15.1 (CH_3)	15.0 (CH_3)	15.2 (CH_3)	15.1 (CH_3)
20	40.8 (CH)	40.6 (CH)	41.1 (CH)	40.8 (CH)
21	17.0 (CH_3)	17.3 (CH_3)	16.4 (CH_3)	16.6 (CH_3)
22	110.0 (C)	113.0 (C)	111.0 (C)	114.0 (C)
23	37.4 (CH_2)	37.2 (CH_2)	37.3 (CH_2)	37.0 (CH_2)
24	31.3 (CH_2)	31.1 (CH_2)	31.4 (CH_2)	31.3 (CH_2)
25	34.2 (CH)	34.2 (CH)	34.1 (CH)	34.1 (CH)
26	75.9 (CH_2)	76.0 (CH_2)	76.0 (CH_2)	76.2 (CH_2)
27	18.2 (CH_3)	18.2 (CH_3)	17.5 (CH_3)	17.5 (CH_3)

couplings between H-25 and the two H-26, in comparison with literature data (*9*). The ROESY correlations (**Figure 2**) H-11/H_3-19, H-11/H_3-18, H-9/H-14, H-14/H-16, H-12/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 stereochemistries (*10*, *11*). On the basis of those data, the stereochemistry of the aglycon chiral centers has been assigned as in **Figure 1**, except for the stereochemistry at C-22, which will be discussed below.

Concerning the sugar portions of the molecule, the first step of the analysis was the association of the two anomeric carbons (δ 100.7 and 101.3) with the relevant anomeric proton signals (δ 4.30 and 5.30, respectively), through the HSQC experiment.

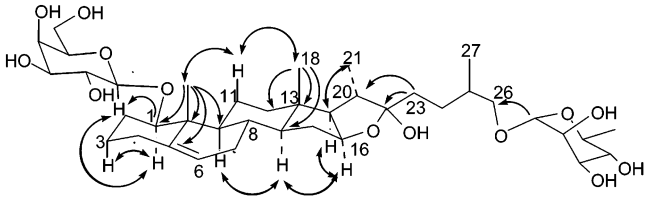

Figure 2. Selected HMBC (H→C) and ROESY (H↔H) correlations observed for compound **1a**.

The nature of the monosaccharides was determined by analysis of 2D COSY, HOHAHA, HSQC, and HMBC spectra. Starting from the anomeric proton at δ 4.30 (H-1 Gal), we identified the sequence of a hexopyranose unit. The large coupling constants observed in a 2D HOHAHA subspectrum for H-1 Gal/H-2 Gal and H-2 Gal/H-3 Gal, and the relatively small coupling constant of H-3 Gal/H-4 Gal, together with the ROESY correlation H-3 Gal/H-5 Gal, indicated its β-galactopyranose nature. With the same type of analysis the sugar linked at C-26 was identified as a 6-deoxyhexopyranose. Within its spin system, the axial−axial H-3 Rha/H-4 Rha and H-4 Rha/H-5 Rha couplings and the equatorial−axial H-2 Rha/H-3 Rha relationship led to the assignment of a rhamnose. Its α-anomeric configuration, suggested by the very small *J* value of H-1 Rha, resonating as a broad singlet, was confirmed by the chemical shift of C-3 Rha and C-5 Rha (**Table 3**), in accordance with data reported in the literature (*10*, *12*). The β-galactose was placed at C-1 of tropeoside A1 by interpretation of the key HMBC correlation peak (**Figure 2**) between H-1 Gal (δ 4.30) and C-1 (δ 84.6), this junction being also confirmed by the strong ROESY peak (**Figure 2**) between H-1 Gal and H-1 (δ 3.36). The α-rhamnose had to be linked at position 26 on the furostanol structure of the aglycon. In fact, we observed the HMBC cross-peak (**Figure 2**) between C-26 (δ 75.9) and H-1 Rha (δ 5.30).

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 galactose and rhamnose. By this procedure the sugars were identified as D-galactose and L-rhamnose. This procedure was applied to all of the new isolated compounds.

Table 3. ^1H and ^{13}C NMR Data of the Sugar Portion of **1a/1b** (Data Extracted from **1a**) and **3a/3b** (Data Extracted from **3a**) (Spectra Measured in CD_3OD at 500 and 125 MHz)

	1a			**3a**	
position	δ_H (int, mult, *J* in Hz)	δ_C (mult)	position	δ_H (int, mult, *J* in Hz)	δ_C (mult)
Gal			Xyl		
1	4.30 (1H, d, 7.8)	100.7 (CH)	1	4.27 (1H, d, 7.5)	101.0 (CH)
2	3.70 (1H, dd, 7.8, 6.8)	74.0 (CH)	2	3.68 (1H, dd, 7.5, 6.8)	74.1 (CH)
3	3.68 (1H, dd, 6.8, 2.5)	75.2 (CH)	3	3.88 (1H, dd, 7.0, 6.8)	75.6 (CH)
4	3. 87 (1H, dd, 3.2, 2.5)	76.6 (CH)	4	3.65 (1H, ddd, 2.0, 7.0, 8.5)	69.6 (CH)
5a	3.44[a]	74.0 (CH)	5a	3.50 (1H, dd, 2.0, 11.5)	66.0 (CH_2)
5b			5b	3.95 (1H, dd, 8.5, 11.5)	
6a	3.47[a]	62.2 (CH_2)			
6b	3.65[a]				
Rha			Rha		
1	5.30 (1H, bs)	101.3 (CH)	1	5.30 (1H, bs)	101.5 (CH)
2	3.89 (1H, bs)	72.2 (CH)	2	3.90 (1H, bs)	72.3 (CH)
3	3.72 (1H, d, 6.5)	70.6 (CH)	3	3.72 (1H, d, 6.5)	70.7 (CH)
4	3.40 (1H, dd, 6.5, 6.0)	72.0 (CH)	4	3.39 (1H, dd, 6.5, 6.0)	72.1 (CH)
5	4.10 (1H, dq, 6.6, 6.0)	69.0 (CH)	5	4.10 (1H, dq, 6.6, 6.0)	67.4 (CH)
6	1.25 (3H, d, 6.6)	18.2 (CH_3)	6	1.25 (3H, d, 6.6)	18.3 (CH_3)

[a] Overlapped with other signals.

High-resolution FABMS indicated that tropeoside A2 (**1b**) was isomeric with tropeoside A1. The ^{1}H and ^{13}C NMR spectra of **1b** (**Table**s **1**−**3**) were almost identical with that of **1a**, but slightly different in the resonances of the atoms located near C-22. This suggested that the two compounds were epimers having the opposite configuration at the hemiacetal carbon (C-22). This was confirmed by the observation that both **1a** and **1b**, after being kept one night in aqueous solution at room temperature, gave rise to an equilibrium between the two forms (about 40% of **1a** and 60% of **1b**). We have tentatively assigned the 22α orientation to tropeoside A1 and 22*β* orientation to tropeoside A2 on the basis of the downfield shift in the ^{1}H NMR resonances of H_3-21 and H-16 of **1a**, in comparison with those of **1b** (H_3-21 δ 0.99 instead of 0.96; H-16 δ 4.60 instead of 4.38), due to the deshielding effect of the cis-oriented OH-22 group (*13*).

The structure elucidation of tropeoside B1 (**3a**) was aided by comparison with the MS and NMR data obtained for **1a**. The high-resolution FABMS gave the molecular formula of **3a** as $C_{38}H_{62}O_{13}$, which differs from **1a** in being 30 amu less. The NMR profiles of **3a** (**Tables 1**−**3**) appeared to be superimposable with those of **1a** for the signals relative to the aglycon and to the α-rhamnose. Differences were related to the second sugar unit, which by MS and 2D NMR analyses appeared to be based on a pentopyranose structure. The large coupling constants observed in a 2D HOHAHA subspectrum for H-1 Xyl/H-2 Xyl, H-2 Xyl/H-3 Xyl, and H-3/H-4 Xyl indicated a *β*-xylopyranosyl nature. This was confirmed by acid hydrolysis, followed by silylation and analysis on a chiral column, which afforded D-xylose and L-rhamnose. Diagnostic HMBC correlations of H-1^{I} with C-1 and of H-1^{II} with C-26 located xylose and rhamnose at C-1 and C-26, respectively, thus fully defining the structure of **3a**.

Mass spectrometric analysis of tropeoside B2 (**3b**) showed the same molecular formula as that of **3a**. In analogy, the NMR profiles (**Table**s **1**−**3**) also revealed the same structure for both compounds, indicating that the difference had to be limited to the stereochemistry of a chiral carbon. As observed for **1a**/**1b**, the two compounds **3a**/**3b** were in equilibrium if left in solution overnight. We therefore determined the structure of **3b** as the epimer of **3a** at the hemiacetal carbon. The assignments of the ^{1}H and ^{13}C NMR spectra, obtained through 2D NMR spectra, are reported in **Table**s **1**−**3**. On the basis of the same arguments as for **1a**/**1b**, we tentatively assigned the configurations 22α to **3a** and 22*β* to **3b**.

Compounds **2a**/**2b** and **4a**/**4b** showed ^{1}H and ^{13}C NMR spectra superimposable with those of **1a**/**1b** and **3a**/**3b**, respectively. The only difference was the presence of an additional methoxy group signal in each spectrum of **2a**/**2b** and **4a**/**4b** [^{1}H NMR, δ 3.12 (3H, s); ^{13}C NMR, δ 47.2]. This allowed us to identify compounds **2a**/**2b** and **4a**/**4b** as the 22-*O*-methyl derivatives of **1a**/**1b** and **3a**/**3b**, respectively, from which they originate during the extraction and purification steps. This was confirmed by repeating the extraction procedure in acetone and observing the absence of **2a**/**2b** and **4a**/**4b** in the extract and their successive formation during the purification of tropeosides A1/A2 and B1/B2 by HPLC.

Finally, we have also isolated in high amounts the trisaccharide saponins ascalonicosides A1/A2 (**5a**/**5b**) and B (**6**), recently reported by some of the authors from shallot (*6*).

Because one of the remarkable traditional uses of onion is the treatment of intestinal spasm, in the present study we have investigated the possible antispasmodic effect of the isolated saponins on the contractions elicited by acetylcholine and histamine in the isolated guinea pig ileum. The pharmacological results are shown in **Figure 3**. Tropeosides A1/A2 and B1/B2 reduced, in a concentration-dependent manner, the contractions evoked by both acetylcholine (**Figure 3A**) and histamine (**Figure 3B**). The effect was significant at the concentration of 10^{-5} M (∼50% inhibition). By contrast, ascalonicoside A1/A2 did not show any significant effect. Papaverine, used as a reference compound, inhibited both acetylcholine-induced (percent inhibition, 10^{-8} M, 4 ± 3; 10^{-7} M, 16 ± 4; 10^{-6} M, 32 ± 5; 10^{-5} M, 68 ± 5; 10^{-4} M, 89 ± 6, $n = 4$) and histamine-induced (percent inhibition, 10^{-8} M, 3 ± 4; 10^{-7} M, 14 ± 6; 10^{-6} M, 35 ± 5; 10^{-5} M, 71 ± 4; 10^{-4} M, 90 ± 4, $n = 4$) contractions.

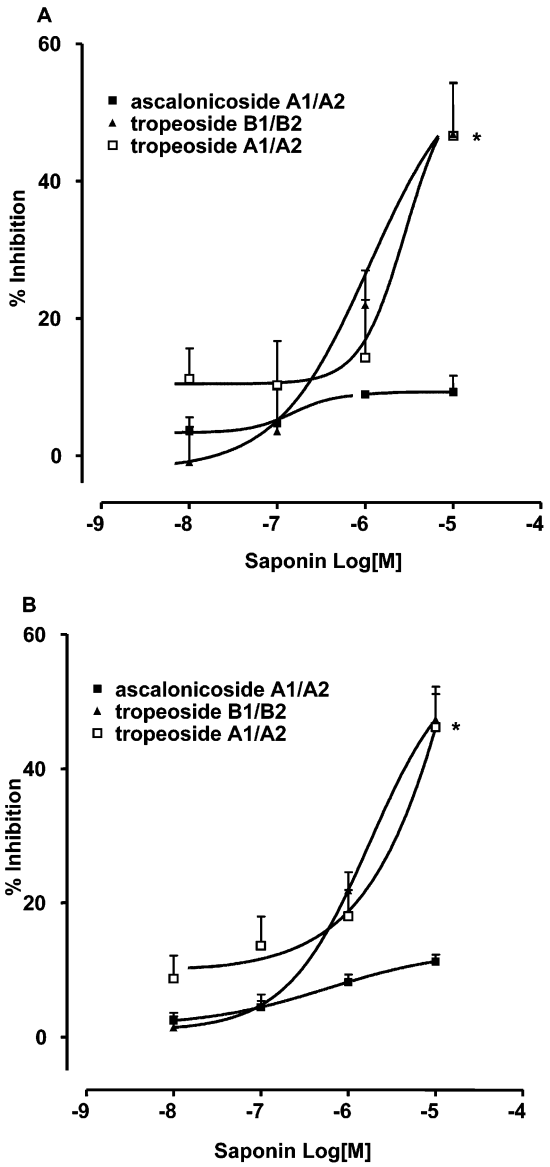

Figure 3. Effect of tropeoside A1/A2, tropeoside B1/B2, and ascalonicoside A1/A2 on the contractions evoked by acetylcholine (10^{-7} M) (**A**) or histamine (10^{-7} M) (**B**) in the guinea pig ileum. Each point represents the mean of four to five experiments. Vertical lines show SEM. *, $P < 0.05$ versus corresponding control.

Comparison of the chemical structures of the tested compounds in terms of bioactivity suggested that the presence of a further sugar in ascalonicoside A1/A2 results in the loss of the antispasmodic activity.

With regard to the flavonoids, the finding of high concentrations agree with the observation that onions are characterized

generally by a higher content of flavonoids if compared to garlic and leek (*14*, *15*). From the biological point of view, flavonoids are considered to be important factors of the overall antioxidant activity of dietary plants (*16*, *17*). In particular, quercetin has shown an anti-HIV property (*18*) and the ability to protect low-density lipoprotein (LDL) cholesterol from oxidation, thereby reducing the risk of cardiovascular (*19*) and neurodegenerative diseases, such as Alzheimer's and Parkinson's. In addition, flavonoids including quercetin and taxifolin also affect the gastrointestinal tract by possessing antiulcer, antispasmodic, and antidiarrheal activities (*20*). For all of these reasons, the finding of high concentrations of saponins and flavonols in bulbs of Tropea red onion makes it a healthy food in the Mediterranean diet. In addition, because these onions are often consumed uncooked, due to their crispness and sweetness, these phytonutrients are totally preserved.

In conclusion, the present study reports the isolation of high concentrations of flavonoids and saponins from a common food, the red onion. Among them, we have characterized two new saponins that exhibited antispasmodic activity. This result may be important from ethnopharmacological, pharmaceutical, or nutritional points of view. From an ethnopharmacological point of view, the antispasmodic activity of these compounds, together with the previously reported antispasmodic activity of flavonoids (*7*), might contribute to the justification of the traditional use of onion in the treatment of intestinal spasms. From a pharmaceutical point of view, the antispasmodic activity may open the way to study in vivo the effect of the isolated compounds in models of intestinal hyperactivity. Last but not least, from a nutritional point of view, the presence in onion of compounds with antispasmodic activity may provide input to perform epidemiological studies aiming at investigating the relationship between onion intake and chronic digestive illnesses, characterized by hypercontractility of intestinal smooth muscles (e.g., irritable bowel syndrome).

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