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Furostanol saponins in *Allium caepa* L. Var. *tropeana* seeds

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Abstract

An analysis of the polar extracts from seeds of *Allium caepa* L. var. *tropeana* led to the isolation of eight furostanol saponins, one of which was previously reported in the literature. On the basis of 1D, 2D NMR and mass spectrometry data, the structures of the compounds were elucidated as 1-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-1β,3β,22α,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-α-L-arabinopyranoside (**1a**), its epimer at position 22, 1-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-1β,3β,22β,26- tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-α-L-arabinopyranoside (**1b**), 1-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25R)-furost-5(6)-en-1β,3β,22ξ,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-α-L-arabinopyranoside (probably artefact) (**2**), 1-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-1β,3β,22β,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 6″)-*O*-β-D-galactopyranoside (**3**), 1-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25R)-furost-5(6)-en-1β,3β,22ξ,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 6″)-*O*-β-D-galactopyranoside (probably artefact) (**4**), 26-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-3β,22α,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside (**5a**) and its epimer at position 22,26-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-3β,22β,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside (**5b**) and the known compound 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25R)-furost-5(6)-en-3β,22ξ,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside (**6**) [Mimaki, Y., Satou, T., Kuroda, M., Sashida, Y., & Hatakeyama, Y. (1999). Steroidal saponins from the bulbs of *Lilium candidum*. *Phytochemistry, 51*, 567–573]. This is the first report on furostanol saponins in the seeds of *Allium caepa* L. var. *tropeana.*

Keywords: Red onion; *Allium caepa*; Liliaceae; Steroid saponins; NMR; Mass

1. Introduction

Red onion in Italy was cultivated along the line of coast between Capo Vaticano and Vibo Valentia, wherein is the town of Tropea, which names the plant. The Tropea red onion has always had a primary role in the Mediterranean cuisine as a constituent of the tastiest typical dishes. It represents one of the most precious economic resources of Calabria (Italian region). The history of the onion is well documented and can be traced back to its introduction by Phoenicians 2000 years ago. Since ancient times, people of widespread areas of the world have been using red onion as food, spice, and herbal remedy, especially in the northern hemisphere (Block, 1985). Pliny the Elder in his "Naturalis Historia" spoke about its many therapeutic qualities. Some of them are probably due to the presence of steroidal saponins, bioactive glycosides which typically occur in small quantities in the *Allium* genus. These constituents possess some biological properties, such as hypocholesterolemic, antidiabetic (Wang & Ng, 1999), antitumor and antitussive activities (Hiromichi, 2001). Analysis of the MeOH extracts from the seeds of *Allium caepa* L. var. *tropeana* revealed the presence of saponins in significant concentrations. Seven new furostanol saponins have been isolated.

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2. Materials and methods

2.1. General procedures

Electrospray ionization ESI-MS spectra were recorded in CH_3OH on an AB Applied Biosystems mass spectrometer API 2000. Optical rotations were determined on a Perkin–Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and 10 cm microcell. ^1H and ^{13}C NMR spectra were recorded at 500 MHz, on a Bruker AMX-500 spectrometer. Chemical shifts were referred to the residual solvent signal (CD_3OD: δ H 3.34, δ C 49.0). The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. ^1H 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 ^1H–^{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 ^1H–^{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. HPLC analysis in isocratic mode was performed on a Hewlett–Packard HP 1050 series pumping system apparatus with a Varian RI-4 refractive index detector, equipped with Waters μBondapack C_{18} column (7.8 × 300 mm). GC was run using a Hewlett–Packard 5890 gas chromatograph equipped with an HP-5 column (25 m × 0.2 mm i.d., 0.33 μm film).

2.2. Plant material

Red onion seeds, available in the market, originality from the Capo Vaticano (VV-Italy), were identified (and reference specimens deposited) at the Dipartimento di Chimica delle Sostanze Naturali, University of Federico II Napoli (Italy).

2.3. Extraction and isolation

The whole flour from the seeds (500 g) was extracted with MeOH. The MeOH extract (84.76 g) was defatted with petroleum ether and partitioned between *n*-BuOH and H_2O. The butanol extract (3.13 g) was evaporated and chromatographed on a Sephadex LH-20 column (100 × 5 cm), with MeOH as eluent. Fractions (9 ml) were collected and checked by TLC [Si gel plates in *n*-BuOH–HOAc–H_2O (60:15:25)]. Fractions 23–50 (1390 mg), containing the crude glycosidic mixture, were further purified by reversed-phase HPLC with MeOH:-H_2O/70:30 at a flow rate of 2.0 ml/min to yield eight pure compounds: **1a** (8.5 mg), **1b** (19.5 mg), **2** (26.3 mg), **3** (4.3 mg), **4** (1.7 mg), **5a** (50.0 mg), **5b** (12.0 mg), and **6** (9.0 mg).

Compound **1a** was 1-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-1β,3β,22α,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-α-L-arabinopyranoside: $[\alpha]_D^{20}$ −0.05 (c = 0.1 MeOH). ESI-MS (negative ion): *m/z* 887 $[M-H]^-$, *m/z* 741 $[887-146]^-$, *m/z* 609 $[887-132-146]^-$, *m/z* 447 $[887-132-146-162]^-$. ^1H NMR data: Tables 1 and 3. ^{13}C NMR data: Tables 2 and 3.

Compound **1b** was 1-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-1β,3β,22β,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-α-L-arabinopyranoside: $[\alpha]_D^{20}$ −0.04 (c = 0.1 MeOH). ESI-MS (negative ion): *m/z* 887 $[M-H]^-$, *m/z* 741 $[887-146]^-$, *m/z* 609 $[887-132-146]^-$, *m/z* 447 $[887-132-146-162]^-$. ^1H NMR data: Tables 1 and 3. ^{13}C NMR data: Tables 2 and 3.

Compound **2** was 1-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25R)-furost-5(6)-en-1β,3β,22ξ,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-α-L-arabinopyranoside: $[\alpha]_D^{20}$ −0.03 (c = 0.1 MeOH). ESI-MS (negative ion): *m/z* 901 $[M-H]^-$, *m/z* 755 $[901-146]^-$, *m/z* 623 $[901-132-146]^-$, *m/z* 461 $[901-132-146-162]^-$. ^1H NMR data: Tables 1 and 3. ^{13}C NMR data: Tables 2 and 3.

Compound **3** was 1-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-1β,3β,22β,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 6″)-*O*-β-D-galactopyranoside: $[\alpha]_D^{20}$ −0.04 (c = 0.1 MeOH). ESI-MS (negative ion): *m/z* 917 $[M-H]^-$, *m/z* 771 $[917-146]^-$, *m/z* 609 $[917-146-162]^-$. ^1H NMR and ^{13}C NMR data of aglycone moiety appeared similar to those obtained for **1b**: ^1H NMR (CD_3OD, 500 MHz) δ 5.56 (1H, bd, 3.2, H-6), δ 4.39 (1H, q, 5.2, H-16), δ 0.87 (3H, s, H-18), 1.13 (3H, s, H-19), δ 1.01 (3H, d, 6.2, H-21), 0.99 (3H, d,6.6, H-27), ^{13}C NMR (CD_3OD, 500 MHz) δ 84.6 (C-1), 37.5 (C-2), 69.7 (C-3), 43.4 (C-4), 139.6 (C-5), 125.9 (C-6), 32.7 (C-7), 32.9 (C-8), 51.3 (C-9), 43.7 (C-10), 24.6 (C-11), 41.6 (C-12), 41.3 (C-13), 57.9 (C-14), 32.9 (C-15), 82.4 (C-16), 65.2 (C-17), 17.0 (C-18), 15.6 (C-19), 40.2 (C-20), 16.2 (C-21), 114.0 (C-22), 30.7 (C-23), 29.0 (C-24), 35.1 (C-25), 75.3 (C-26), 17.4 (C-27), ^1H NMR and ^{13}C NMR data of sugars moieties are reported in Table 4.

Compound **4** was 1-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25R)-furost-5(6)-en-1β,3β,22ξ,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 6″)-*O*-β-D-galactopyranoside: $[\alpha]_D^{20}$ −0.03 (c = 0.1 MeOH). ESI-MS (negative ion): *m/z* 931 $[M-H]^-$, *m/z* 785 $[931-146]^-$, *m/z* 623 $[917-146-162]^-$. ^1H NMR and ^{13}C NMR data of aglycone moiety appeared similar to those obtained for **2**: ^1H NMR (CD_3OD, 500 MHz) δ 5.56 (1H, bd, 3.2, H-6), δ 4.39 (1H, q, 5.2, H-16), δ 0.87 (3H, s, H-18), 1.13 (3H, s, H-19), δ 1.01 (3H, d, 6.2, H-21), 0.99

Table 1
^{1}H NMR data of the aglycone moiety of compounds **1a/1b/2/5a/5b** spectra measured in CD_3OD

Position	δ_H (int., mult., *J* in Hz)					
	1a	**1b**	**2**	**5a**	**5b**	**6**
1a	3.36 (1H, dd, 7.5, 2.5)	3.36 (1H, dd, 7.5, 2.5)	3.36 (1H, dd, 7.5, 2.5)	1.89 (1H, dd, 7.5, 2.5)	1.89 (1H, dd, 7.5, 2.5)	1.89 (1H, dd, 7.5, 2.5)
1b				1.07	1.07	1.07
2a	2.12	2.10	2.10	1.30	1.30	1.30
2b	1.70	1.70	1.70	1.90	1.90	1.90
3	3.31 (1H, m, 7.5, 7.0, 2.5, 2.0)	3.30 (1H, m, 7.5, 7.0, 2.5, 2.0)	3.30 (1H, m, 7.5, 7.0, 2.5, 2.0)	3.26 (1H, m, 7.5, 7.0, 2.5, 2.0)	3.26 (1H, m, 7.5, 7.0, 2.5, 2.0)	3.26 (1H, m, 7.5, 7.0, 2.5, 2.0)
4a	2.28 (1H, dd, 11.5, 7.3)	2.27 (1H, dd, 11.5, 7.3)	2.27 (1H, dd, 11.5, 7.3)	2.28 (1H, dd, 11.5, 7.3)	2.28 (1H, dd, 11.5, 7.3)	2.28 (1H, dd, 11.5, 7.3)
4b	2.22 (1H, dd, 11.5, 3.5)	2.22 (1H, dd, 11.5, 3.5)	2.22 (1H, dd, 11.5, 3.5)	2.43 (1H, dd, 11.5, 3.5)	2.43 (1H, dd, 11.5, 3.5)	2.43 (1H, dd, 11.5, 3.5)
6	5.55 (1H, bd, 3.2)	5.55 (1H, bd, 3.2)	5.55 (1H, bd, 3.2)	5.37 (1H, bd, 3.2)	5.37 (1H, bd, 3.2)	5.37 (1H, bd, 3.2)
7a	1.98	1.98	1.98	2.0	2.0	2.0
7b	1.96	1.96	1.96	1.96	1.96	1.96
8	1.50 (1H, m)	1.50 (1H, m)	1.50 (1H, m)	1.58 (1H, m)	1.58 (1H, m)	1.58 (1H, m)
9	1.28	1.28	1.28	1.20	1.20	1.20
11a	2.57 (1H, dd, 10.5, 2.5)	2.56 (1H, dd, 10.5, 2.5)	2.56 (1H, dd, 10.5, 2.5)	1.59 (1H, dd, 10.5, 2.5)	1.59 (1H, dd, 10.5, 2.5)	1.59 (1H, dd, 10.5, 2.5)
11b	1.45 (1H, m)	1.46 (1H, m)	1.46 (1H, m)	1.46 (1H, m)	1.46 (1H, m)	1.46 (1H, m)
12a	1.70	1.70	1.70	1.92	1.92	1.92
12b	1.22	1.23	1.23	1.07	1.07	1.07
14	1.18 (1H, m)	1.18 (1H, m)	1.18 (1H, m)	1.16 (1H, m)	1.16 (1H, m)	1.16 (1H, m)
15a	1.99	1.99	1.99	1.65	1.65	1.65
15b	1.30	1.30	1.30	1.30	1.30	1.30
16	4.59 (1H, q, 5.5)	4.39 (1H, q, 5.5)	4.35 (1H, q, 5.5)	4.59 (1H, q, 5.5)	4.35 (1H, q, 5.5)	4.35 (1H, q, 5.5)
17	1.78	1.73	1.73	1.75	1.75	1.75
18	0.87 (3H, s)	0.87 (3H, s)	0.87 (3H, s)	0.84 (3H, s)	0.84 (3H, s)	0.84 (3H, s)
19	1.13 (3H, s)	1.13 (3H, s)	1.13 (3H, s)	1.05 (3H, s)	1.05 (3H, s)	1.05 (3H, s)
20	2.13	2.15	2.15	2.18	2.18	2.18
21	1.04 (3H, d, 6.6)	1.01 (3H, d, 6.6)	1.01 (3H, d, 6.6)	1.04 (3H, d, 6.6)	1.00 (3H, d, 6.6)	1.00 (3H, d, 6.6)
23a	1.72	1.75	1.75	1.82	1.82	1.82
23b	1.63	1.61	1.61	1.62	1.62	1.62
24a	1.38	1.34	1.34	1.48	1.48	1.48
24b	1.30	1.31	1.31	1.34	1.34	1.34
25	1.78 (1H, m)	1.78 (1H, m)	1.78 (1H, m)	1.75 (1H, m)	1.75 (1H, m)	1.75 (1H, m)
26a	3.82 (1H, dd, 8.5, 6.9)	3.82 (1H, dd, 8.5, 6.9)	3.82 (1H, dd, 8.5, 6.9)	3.52 (1H, dd, 8.5, 6.9)	3.52 (1H, dd, 8.5, 6.9)	3.52 (1H, dd, 8.5, 6.9)
26b	3.29	3.29	3.29	3.19	3.19	3.19
27	0.99 (3H, d, 6.6)	0.99 (3H, d, 6.6)	0.99 (3H, d, 6.6)	0.96 (3H, d, 6.6)	0.96 (3H, d, 6.6)	0.96 (3H, d, 6.6)
OCH_3			3.13 (3H, s)			3.13 (3H, s)

Table 2
^{13}C NMR data of the aglycone moiety of compounds **1a/1b/2/5a/5b** spectra measured in CD_3OD

Position	δ_C (mult.)				
	1a	**1b**	**2**	**5a**	**5b**
1	84.3 (CH)	84.5 (CH)	84.5 (CH)	38.0 (CH_2)	38.0 (CH_2)
2	37.2 (CH_2)	37.2 (CH_2)	37.2 (CH_2)	30.3 (CH_2)	30.3 (CH_2)
3	70.4 (CH)	69.7 (CH)	69.7 (CH)	78.3 (CH)	78.3 (CH)
4	43.4 (CH_2)	43.4 (CH_2)	43.4 (CH_2)	39.5 (CH_2)	39.5 (CH_2)
5	139.6 (C)	139.6 (C)	139.7(C)	141.9 (C)	141.9 (C)
6	125.7 (CH)	125.9 (CH)	126.0 (CH)	122.6 (CH)	122.6 (CH)
7	32.7 (CH_2)	32.7 (CH_2)	32.7 (CH_2)	32.7 (CH_2)	32.7 (CH_2)
8	32.9 (CH)	32.9 (CH)	32.9 (CH)	33.0 (CH)	33.0 (CH)
9	51.5 (CH)	51.5 (CH)	51.5 (CH)	51.5 (CH)	51.5 (CH)
10	43.4 (C)	43.4 (C)	43.4 (C)	37.3 (C)	34.3 (C)
11	24.7 (CH_2)	24.7 (CH_2)	24.7 (CH_2)	21.9 (CH_2)	21.9 (CH_2)
12	41.2 (CH_2)	41.2 (CH_2)	41.2 (CH_2)	38.9 (CH_2)	38.9 (CH_2)
13	41.5 (C)	41.3 (C)	41.3 (C)	40.8 (C)	40.8 (C)
14	57.7 (CH)	57.9 (CH)	57.9 (CH)	57.6 (CH)	57.6 (CH)
15	33.0 (CH_2)	32.9 (CH_2)	32.8 (CH_2)	33.2 (CH_2)	33.2 (CH_2)
16	82.7 (CH)	82.4 (CH)	82.5 (CH)	82.9 (CH)	82.9 (CH)
17	65.3 (CH)	65.3 (CH)	65.3 (CH)	65.5 (CH)	65.5 (CH)
18	17.0 (CH_3)	17.0 (CH_3)	17.0 (CH_3)	16.9 (CH_3)	16.9 (CH_3)
19	15.4 (CH_3)	15.3 (CH_3)	15.3 (CH_3)	20.0 (CH_3)	20.0 (CH_3)
20	40.5 (CH)	40.2 (CH)	40.8 (CH)	41.3 (CH)	41.3 (CH)
21	16.2 (CH_3)	16.2 (CH_3)	16.2 (CH_3)	16.2 (CH_3)	16.2 (CH_3)
22	111.9 (C)	114.0 (C)	114.0 (C)	111.9 (C)	114.0 (C)
23	30.7 (CH_2)	30.7 (CH_2)	30.7 (CH_2)	31.4 (CH_2)	31.4 (CH_2)
24	29.0 (CH_2)	29.0 (CH_2)	29.0 (CH_2)	28.9 (CH_2)	28.9 (CH_2)
25	35.0 (CH)	35.1 (CH)	35.1 (CH)	35.0 (CH)	35.0 (CH)
26	75.2 (CH_2)	75.2 (CH_2)	75.2 (CH_2)	75.3 (CH_2)	75.3 (CH_2)
27	17.4 (CH_3)	17.4 (CH_3)	17.4 (CH_3)	17.2 (CH_3)	17.2 (CH_3)
OCH_3			47.6 (CH_3)		

Table 3
^{1}H and ^{13}C NMR data of the sugar moiety of **1a/1b/2** spectra measured in CD_3OD

	Position	δ_H (int., mult., *J* in Hz)	δ_C (mult.)
Glc	1′	4.25 (1H, d, 7.5)	104.7 (CH)
	2′	3.21 (1H, dd, 7.5, 9.5)	75.2 (CH)
	3′	3.38 (1H, dd, 9.5, 9.5)	78.2 (CH)
	4′	3.70 (1H, dd, 9.5, 9.5)	71.8 (CH)
	5′	3.27 (1H, m)	77.9 (CH)
	6′a	3.88 (1H, dd, 12, 3.5)	62.9 (CH_2)
	6′b	3.69[a]	
Ara	1″	4.26 (1H, d, 7.5)	101.6 (CH)
	2″	3.38 (1H, dd, 7.5, 9)	84.3 (CH)
	3″	3.70 (1H, dd, 9, 3.5)	75.2 (CH)
	4″	3.73 (1H, m)	70.8 (CH)
	5″a	3.28 (1H, dd, 11.5,1.5)	67.4 (CH_2)
	5″b	3.48 (1H, dd, 11.5,1.5)	
Rha	1‴	5.30 (1H, d, 1.5)	101.0 (CH)
	2‴	3.89 (1H, dd, 1.5, 6.5)	72.2 (CH)
	3‴	3.74 (1H, dd, 6.5, 6.5)	72.2 (CH)
	4‴	3.40 (1H, dd, 6.5, 6.0)	74.2 (CH)
	5‴	4.09 (1H, dq, 6.6, 6.0)	68.3 (CH)
	6‴	1.24 (3H, d, 6.6)	18.4 (CH_3)

[a] Overlapped with other signals.

Table 4
^{1}H and ^{13}C NMR data of the sugar moiety of **3/4** spectra measured in CD_3OD

	Position	δ_H (int., mult., *J* in Hz)	δ_C (mult.)
Glc	1′	4.25 (1H, d, 7.5)	104.7 (CH)
	2′	3.21 (1H, dd, 7.5, 9.5)	75.4 (CH)
	3′	3.38 (1H, dd, 9.5, 9.5)	78.2 (CH)
	4′	3.70 (1H, dd, 9.5, 9.5)	70.8 (CH)
	5′	3.27 (1H, m)	77.9 (CH)
	6′a	3.88 (1H, dd, 12, 3.5)	62.9 (CH_2)
	6′b	3.69[a]	
Gal	1″	4.26 (1H, d, 7.5)	101.6 (CH)
	2″	3.78 (1H, dd, 7.5, 9.5)	71.8 (CH)
	3″	3.72 (1H, dd, 9.5, 2.5)	74.3 (CH)
	4″	3.32 (1H, dd, 2.5, 3.2)	77.9 (CH)
	5″	3.27 (1H, m)	76.1 (CH)
	6″a	3.48 (1H, bd, 11.5)	67.4 (CH_2)
	6″b	3.83 (1H, bd, 11.5)	
Rha	1‴	5.30 (1H, d, 1.5)	101.0 (CH)
	2‴	3.89 (1H, dd, 1.5, 6.5)	72.2 (CH)
	3‴	3.74 (1H, dd, 6.5, 6.5)	72.4 (CH)
	4‴	3.40 (1H, dd, 6.5, 6.0)	74.2 (CH)
	5‴	4.09 (1H, dq, 6.6, 6.0)	69.2 (CH)
	6‴	1.24 (3H, d, 6.6)	18.4 (CH_3)

[a] Overlapped with other signals.

(3H, d,6.6, H-27), 3.13 (3H, s, OCH_3), ^{13}C NMR (CD_3OD, 500 MHz) δ 84.6 (C-1), 37.5 (C-2), 69.7 (C-3), 43.4 (C-4), 139.6 (C-5), 125.9 (C-6), 32.7 (C-7), 32.9 (C-8), 51.3 (C-9), 43.7 (C-10), 24.6 (C-11), 41.6 (C-12), 41.3 (C-13), 57.9 (C-14), 32.9 (C-15), 82.4 (C-16), 65.2 (C-17), 17.0 (C-18), 15.6 (C-19), 40.2 (C-20), 16.2 (C-

21), 114.0 (C-22), 30.7 (C-23), 29.0 (C-24), 35.1 (C-25), 75.3 (C-26), 17.4 (C-27), 47.6 (OCH_3). ^1H NMR and ^{13}C NMR data of sugar moieties are reported in Table 4.

Compound **5a** was 26-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-3β,22α,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside: $[\alpha]_D^{20}$ −0.02 (*c* = 0.1 MeOH). ESI-MS (negative ion): *m/z* 1063 $[M-H]^-$, *m/z* 917 [1063 − 146]$^-$, *m/z* 901[1063 − 162]$^-$, *m/z* 755 [1063 − 162 − 146]$^-$. ^1H NMR data: Tables 1 and 5. ^{13}C NMR data: Tables 2 and 5.

Compound **5b** was 26-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-3β,22β,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside: $[\alpha]_D^{20}$ −0.03 (*c* = 0.1 MeOH). ESI-MS (negative ion): *m/z* 1063 $[M-H]^-$, *m/z* 917 [1063 − 146]$^-$, *m/z* 901 [1063 − 162]$^-$, *m/z* 755 [1063 − 162 − 146]$^-$. ^1H NMR data: Tables 1 and 5. ^{13}C NMR data: Tables 2 and 5.

Compound **6** was 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25R)-furost-5(6)-en-3β,22ξ,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside: $[\alpha]_D^{20}$ −0.05 (*c* = 0.1 MeOH). ESI-MS (negative ion): *m/z* 1077 $[M-H]^-$, *m/z* 931 [1077 − 146]$^-$, *m/z* 915 [1077 − 162]$^-$, *m/z* 769 [1077 − 162 − 146]$^-$. ^1H NMR (CD_3OD, 500 MHz) δ 5.36 (1H, bd, 3.2, H-6),δ 4.36 (1H, q, 5.2, H-16), δ 0.87 (3H, s, H-18), δ 1.05 (3H, s, H-19), δ 1.01 (3H, d, 6.2, H-21), 0.97 (3H, d,6.6, H-27), 3.13 (3H, s, OCH_3), ^{13}C NMR (CD_3OD, 500 MHz) δ 38.0 (C-1), 30.3 (C-2), 78.3 (C-3), 39.5 (C-4), 141.9 (C-5), 122.6 (C-6), 32.7 (C-7), 33.0 (C-8), 51.5 (C-9), 34.3 (C-10), 21.9 (C-11), 38.9 (C-12), 40.8 (C-13), 57.6 (C-14), 33.2 (C-15), 82.9 (C-16), 65.5 (C-17), 16.9 (C-18), 20.0 (C-19), 41.3 (C-20), 16.2 (C-21), 114.0 (C-22), 31.4 (C-23), 28.9 (C-24), 35.1 (C-25), 75.3 (C-26), 17.4 (C-27), 47.6 (OCH_3). ^1H NMR and ^{13}C NMR data of sugars moieties are reported in Table 5 (Mimaki, Satou, Kuroda, Sashida, & Hatakeyama, 1999).

For acid hydrolysis of compounds **1**–**6**, a solution of each compound (1 mg) in 10% H_2SO_4:EtOH (1:1, 3.5 ml) was refluxed for 4 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O. The H_2O layer was neutralized with Amberlite MB-3 ion-exchange resin and evaporated to dryness. The residue was dissolved in 1-(trimethyl silyl)imidazole (Fluka) and pyridine (0.1 ml), and was analyzed by GC; temperatures for injector and detector were 200 °C; temperature gradient system for the oven was 100 °C per 1 min, and then raised to 180 °C at rate 5 °C/min. Retention times were identical to those of the authentic trimethylsilylated sugars.

Table 5
^1H and ^{13}C NMR data of the sugar moiety of **5a/5b/6** spectra measured in CD_3OD

	Position	δ_H (int., mult., *J* in Hz)	δ_C (mult.)
Glc	1′	4.27 (1H, d, 7.5)	100.8 (CH)
	2′	3.66[a]	77.6 (CH)
	3′	3.56[a]	79.3 (CH)
	4′	3.73[a]	71.7 (CH)
	5′	3.74[a]	76.4 (CH)
	6′a	3.92[a]	69.8 (CH_2)
	6′b	4.70[a]	
Glc	1‴	4.47 (1H, d, 7.5)	106.3 (CH)
	2‴	3.51[a]	75.6 (CH)
	3‴	3.34[a]	78.2 (CH)
	4‴	3.28[a]	71.7 (CH)
	5‴	3.34[a]	78.3 (CH)
	6‴a	3.59[a]	62.4 (CH_2)
	6‴b	3.90[a]	
Glc	1⁗	4.24 (1H, d, 7.5)	104.6 (CH)
	2⁗	3.44[a]	75.2(CH)
	3⁗	3.37[a]	78.2 (CH)
	4⁗	3.34[a]	70.8 (CH)
	5⁗	3.27[a]	77.9 (CH)
	6⁗a	3.55[a]	62.8 (CH_2)
	6⁗b	3.82[a]	
Rha	1″	5.30 (1H, d, 1.5)	101.6 (CH)
	2″	3.89 (1H, d)	72.2 (CH)
	3″	3.72 (1H, d, 6.5)	72.4 (CH)
	4″	3.42 (1H, dd, 6.5, 6.0)	74.1 (CH)
	5″	4.09 (1H, dq, 6.6, 6.0)	69.2 (CH)
	6″	1.24 (3H, d, 6.6)	18.2 (CH_3)

[a] Overlapped with other signals.

3. Results and discussion

Seeds of red onion were exhaustively extracted with MeOH. The MeOH extracts were defatted with petroleum ether and partitioned between *n*-butanol and water. The organic layer was subjected to Sephadex LH-20 chromatography, followed by reverse phase HPLC to give saponins. The ESI-MS mass spectrum of compound **1a** (Fig. 1) exhibited a quasi-molecular ion peak at *m/z* 887 $[M-H]^-$, indicating the molecular formula $C_{44}H_{72}O_{18}$, in accordance with ^{13}C NMR and ^{13}C DEPT NMR data. Moreover, the ESI-MS mass spectrum showed fragment ion peaks at *m/z* 741, *m/z* 609 and *m/z* 447, indicating the loss of one deoxyhexose, one pentose and one hexose, respectively. The ^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 1.18–3.82, and a number of signals between δ 3.21 and 5.30, as expected for protons on oxygen-bearing carbons. A further signal at δ 5.55 (broad doublet) was ascribable to a proton on a sp^2 carbon. This pattern of proton chemical shifts, and the ^{13}C NMR spectrum of **1a** (CD_3OD, Tables 2 and 3) are ascribable to a glycoterpene compound and the occurrence of three anomeric carbons (δ 101.4, 102.2, and 104.4) in the ^{13}C NMR

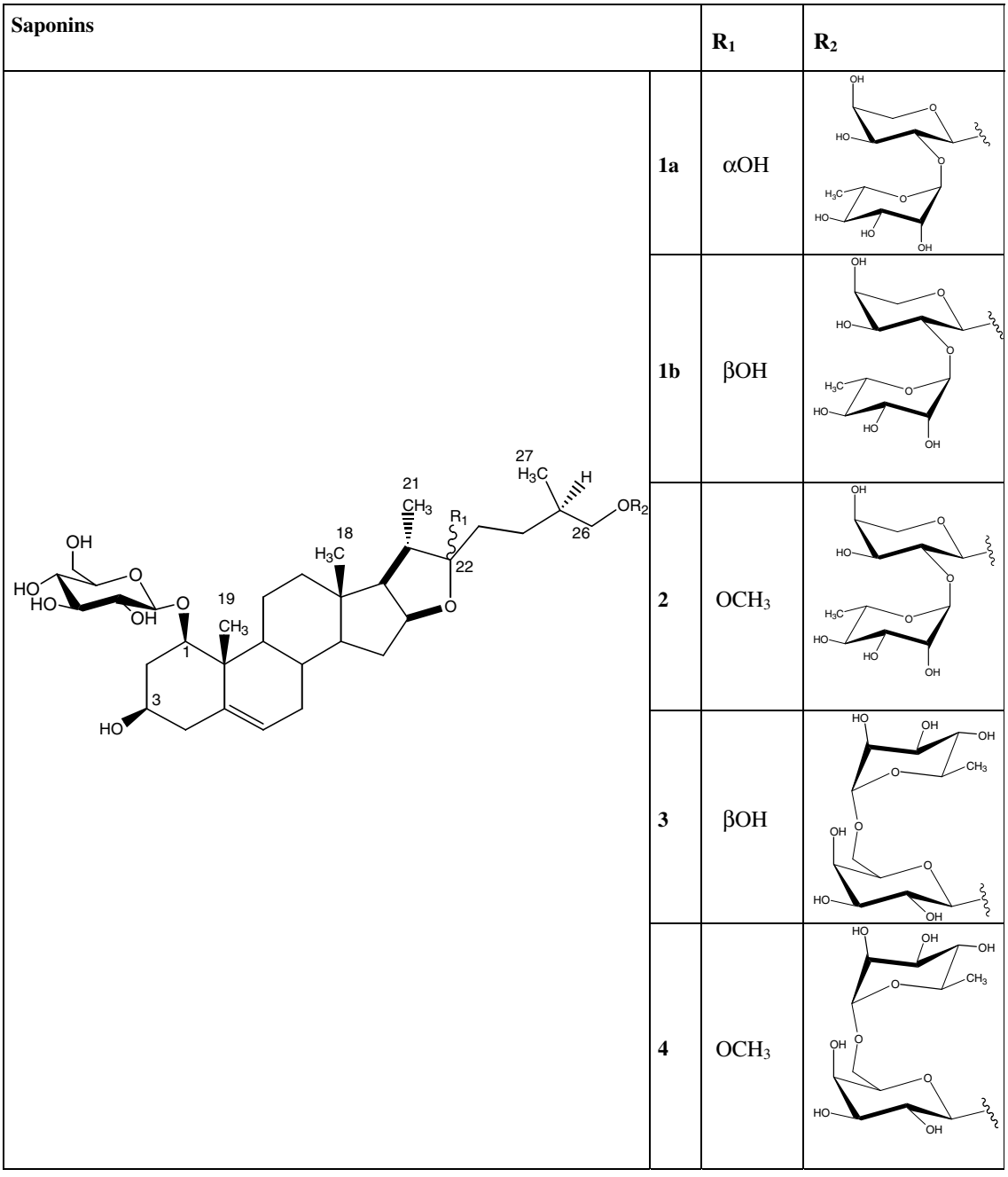

Fig. 1. Compounds **1a**, **1b**, **2**, **3** and **4** isolated from seeds of *Allium caepa* L. var. *tropeana*.

spectrum indicated the presence of three sugar units. Furthermore, the presence of the hemiacetal carbon signal at δ 111.9 (C-22) suggested the furostanol nature of the aglycone of **1a**. All the above proton resonances of **1a** were unambiguously associated with the relevant carbon atoms by using the HMQC spectrum. Inspection of the HOmonuclear HArtmann HAhn (HOHAHA) spectrum of **1a** allowed us to detect six distinct spin systems, three of them belonging to the aglycone 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. In the aglycone moiety, the first spin system connected the oxygen-bearing carbon C-1 (δ_H 3.36; δ_C 84.3) with C-4, while the second one, starting from the sp^2 C-6 (δ_H 5.55; δ_C 125.7), and encompassing all the protonated carbons of rings B, C, and D, ran out with C-21. The last spin system of the aglycone of **1a** was constituted of protons of the side chain, from C-23 to the oxygen-bearing C-26 (δ_H 3.29 and 3.82, δ_C 75.7).

The following HMBC cross-peaks were particularly diagnostic to assemble the above substructures: H_3-19 (δ 1.13) with C-10 (δ 43.4), C-1 (δ 84.3), C-5 (δ 139.6), and C-9 (δ 51.5); H_3-18 (δ 0.87) with C-13 (δ 41.5), C-12 (δ 41.2), and C-14 (57.7); H_2-23 (δ 1.72 and 1.63) with C-20 (δ 40.5) and the hemiacetal carbon C-22 (δ 111.9). The total of this evidence allowed us to identify the aglycone moiety of compound **1a** as a Δ^5-furostan-1,3,22,26-tetraol (Mimaki et al., 1998). With respect to the stereochemistry of the aglycone, in the ^{1}H NMR spectrum of **1a**, signals of H-1 and H-3 were overlapped with some signals of the sugar moiety 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 indicated the axial position of both H-1 and H-3. The 25*R* stereochemistry was deduced by the resonances of protons and carbons at positions C-25, C-26, and C-27 and by the $^3J_{HH}$ values between H-25 and H-26 (J 26ax, 25ax = 10.5 Hz). The equatorial orientation of the C-27 methyl was further verified by the axial–axial coupling of H-26 ax (δ 3.29) and H-25 ax (δ 1.78), in the ^{1}H NMR and the lower field resonance of C 27 (δ 17.4), in comparison with literature data (Agrawal, Jain, & Pathak, 1995; Dong, Feng, Wang, Wu, & Ikejima, 2001). ROESY correlations, of 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**, indicating the usual furostane ring junctions with rings B/C *trans*, C/D *trans*, D/E *cis*, and C-20 α (Agrawal, Guptq, & Thakur, 1985). On the basis of these data, and assuming that the aglycone possesses the same absolute configuration as found in all furostanes isolated to date (Pereira Da Silva, Bernardo, & Parente, 1999), the stereochemistry of the aglycone chiral centres (except for C-22, see below) could be assigned as in the structure shown for **1a**. 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 of the proton resonances of the sugar moieties, whereas evaluation of the coupling constants was used to elucidate the monosaccharide relative stereochemistry. The first step in the analysis of the saccharide part of the molecule of **1a** was the association of the anomeric carbons, resonating at δ_C 104.7, 101.6, and 101.0, with the relevant anomeric proton signals (δ_H 4.25, 4.26, and 5.30, respectively), through the HMQC experiment. The natures of each monosaccharide and their sequence were determined by combined analysis of the COSY, HOHAHA, ROESY, HMQC, and HMBC spectra. Starting from the anomeric proton of each sugar unit, all the proton signals within each spin system were delineated using the COSY spectrum, with the aid of a HOHAHA spectrum. Once the proton assignments had been made, each proton was correlated with the relative carbon through the cross-peaks observed in the HMQC spectrum. Hence, when the anomeric proton at δ 4.25 (H-1′) was used as a starting point, a sequence of three oxymethines and one oxymethylene (Table 3) was identified from the above-mentioned spectra. The large coupling constants, observed in the 2D HOHAHA subspectrum for all the oxymethines, indicated their axial–axial relationship and defined this sugar as β glucopyranose. The pattern of ^{13}C NMR resonances (Table 3) confirmed this assignment. This residue was linked to the C-1 of the aglycone, as indicated by the HMBC correlation peak between the anomeric proton H-1′ (δ_H 4.25) and the downfield shifted (δ 84.5) oxygen-bearing carbon C-1 signal. On the other hand, the HMBC cross-peak of H-26_a (δ_H 3.82) with the anomeric carbon at δ 101.6 (C-1″) allowed us to identify C-26 as a further glycosidic linkage site. Starting from H-1″ (δ 4.26), we identified a sequence of three oxymethines and one oxymethylene (Table 3). The large coupling constants observed for H-1″ indicated the α-arabinopyranose nature of this sugar (Rastrelli, De Simone, Schettino, & Dini, 1996). The spin system starting with the last anomeric proton (H-1‴, δ 5.30) extended to four oxymethines and one methyl group. This sugar moiety was identified as 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‴ in accordance with data reported in the literature (Harmatha, 2000; Sang, Lao, Wang, & Chen, 1999). The chemical shifts of C-3‴ (δ 72.2) and C-5‴ (δ 68.3) and the small coupling constants observed for H-1‴ indicated the α-anomeric configuration of this sugar, in accordance with data reported in the literature (Chen & Snyder, 1989). The ROESY correlation of H-1‴/H-2″, the HMBC correlation peak between the anomeric proton H-1‴ (δ_H 5.30) and the downfield shifted (δ 84.3) oxygen-bearing carbon C-2″ signal, indicated position 2 of the arabinose as the linkage site of the third monosaccharide unit. To confirm the nature of the sugar residues, acid hydrolysis with 1 N HCl was performed next on **1a**, followed by trimethylsilylation and GC analysis in comparison with standards. By this procedure, the sugar residues were identified as glucose, arabinose and rhamnose, in the molecular ratio 1:1:1, respectively. This same procedure was applied to all the new isolated compounds in the present investigation. Therefore, if we assume that these monosaccharides belong to the most commonly-found stereochemical series (D for glucose and L for arabinose and rhamnose), the sugar moiety of compound **1a** was completely defined. Furthermore,

the stereostructure of compound **1a** was 1-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-1β,3β,22α,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-α-L-arabinopyranoside (Fig. 1).

The ESI-MS mass spectrum of compound **1b** indicated the same molecular formula as that of compound **1a**. Analogously, as a result of inspection of the ^{1}H and ^{13}C NMR spectra of **1b** (Tables 1–3) through 2D NMR experiments, the same gross structure of **1a** was found. Therefore, we argue that these molecules should differ only in the stereochemistry of one (or more) of the chiral carbons. This was readily identified as the hemiacetal carbon C-22, considering that both **1a** and **1b**, after being kept for one night in aqueous solution at room temperature, gave the same mixture, constituted approximately of 30% of compound **1a** and 70% of compound **1b**. Accordingly, ^{1}H and ^{13}C NMR spectra of **1a** and **1b** appeared to differ considerably only in the resonances of atoms located close to C-22. We have tentatively assigned the 22α orientation to compound **1a** and the 22β orientation to **1b** on the basis of the ^{1}H NMR resonances of H-21 and H-16 slightly downfield shifted (H-21 at δ 1.04 instead of 1.01; H-16 at δ 4.59 instead of 4.39) in **1a**, suggesting that, most likely, they are deshielded by the *cis*-oriented OH-22 group. Therefore, compound **1b** is identified as: 1-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-1β,3β,22β,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-*O*-α-L-arabinopyranoside (Fig. 1).

The structure of compound **3** was readily elucidated on the basis of its considerable similarities to compound **1b**. The ESI-MS mass spectrum of compound **3** exhibited a quasi-molecular ion peak at *m/z* 917 $[M-H]^-$, indicating the molecular formula $C_{45}H_{74}O_{19}$, in accordance with ^{13}C and ^{13}C DEPT NMR data. We observed good coincidence in the chemical shifts of the aglycone moiety and glucose but some differences in the disaccharide moieties linked at C-26. On acidic hydrolysis, **3** afforded glucose, galactose and rhamnose, in the molecular ratio 1:1:1, as estimated by Glc analysis and by ^{1}H and ^{13}C NMR spectra (Table 3). Furthermore, the structures of the oligosaccharide moieties were deduced by COSY, HMQC and HMBC experiments. COSY experiments allowed the sequential assignments of all of the proton resonances to the individual monosaccharides, as shown in Table 4. The chemical shifts, the multiplicity of the signals, the absolute values of the coupling constants, and their magnitude in the ^{1}H NMR spectrum, as well as ^{13}C NMR data (Table 4) indicated the β-configuration at the anomeric position for the glucose ($J = 7.5$ Hz) and galactopyranoses ($J = 7.5$ Hz) (Mimaki, Nakamura, Sashida, Nikaido, & Ohmoto, 1995) and the α-configuration at the anomeric position for the rhamnopyranosyl unit ($J = 1.5$ Hz). HMQC experiments, which correlated all the proton resonances with those of each corresponding carbon, and HMBC diagnostic crosslink between the oxygen-bearing carbon C-1 (δ_C 84.5) and the anomeric proton H-1′ (δ_H 4.27); C-26 (δ 75.2) and the anomeric proton H-1″ (δ_H 4.26), and C-6″ (δ 67.4) and the anomeric proton H-1‴ (δ_H 5.30), allowed the assignments of the interglycosidic linkages of **3.** By comparison of the observed carbon chemical shifts with those of the corresponding free sugars the absence of any ^{13}C NMR glycosidation shift for the α-L-rhamnopyranosyl was clear, confirming that this sugar was a terminal unit. A glycosidation shift was observed for C-6″ (67.4 ppm) (Rastrelli, Pizza, Saturnino, Schettino, & Dini, 1995). On the basis of all of this evidence, compound **3** is identified as 1-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-1β,3β,22β,26-tetraol-26-*O*-α-L-rhamnopyranosyl-(1‴ → 6″)-*O*-β-D-galactopyranoside (Fig. 1).

The ESI-MS mass spectra of compounds **5a** and **5b** exhibited a quasi-molecular ion peak at *m/z* 1063 $[M-H]^-$, indicating the molecular formula $C_{51}H_{84}O_{23}$, in accordance with ^{13}C and ^{13}C DEPT NMR data (CD_3OD, Tables 2 and 5). ^{1}H NMR spectra of **5a** and **5b** appeared quite similar to those described in literature data for 26-*O*-β-D-glucopyranosyl-22- *O*-methyl-(25R)-furost-5(6)-en-3β,22ξ,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside (Mimaki et al., 1999) with the exception of absence of *O* -methyl group signals. On the basis of the data described previously, we could assign the stereochemistry of the C-22 chiral centre of the aglycone as α for **5a** and β for compound **5b**. Further, the structure of **5a** was 26-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-3β,22α,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside and the structure of **5b** was 26-*O*-β-D-glucopyranosyl-(25R)-furost-5(6)-en-3β,22β,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside (Fig. 2).

The ESI-MS mass spectrum of compound **6** exhibited a quasi-molecular ion peak at *m/z* 1077 $[M-H]^-$, and high-resolution measurements indicated the molecular formula $C_{52}H_{86}O_{23}$, in accordance with ^{13}C NMR and ^{13}C DEPT NMR (see before and Table 5) data reported in the literature for 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25R)-furost-5(6)-en-3β,22ξ,26-triol-3-*O*-α-L-rhamnopyranosyl-(1″ → 2′)-*O*-[β-D-glucopyranosyl-(1‴ → 6′)-*O*]-β-D-glucopyranoside (Mimaki et al., 1999) (Fig. 2).

The ^{1}H and ^{13}C NMR resonances of **2** and **4**, appeared to be superimposable upon those obtained for **1b** and **1a**, respectively, with the exception of an additional methoxy group signal in each spectrum [^{1}H NMR δ 3.13 (3H, s); ^{13}C NMR δ 47.6]. Compounds **2** and **4** were identified as the 22-*O*-methyl derivatives of **1b** and **1a**, respectively, and are considered as secondary products formed from the corresponding 22-hydroxyfurostanosides during the extraction of the plant in meth-

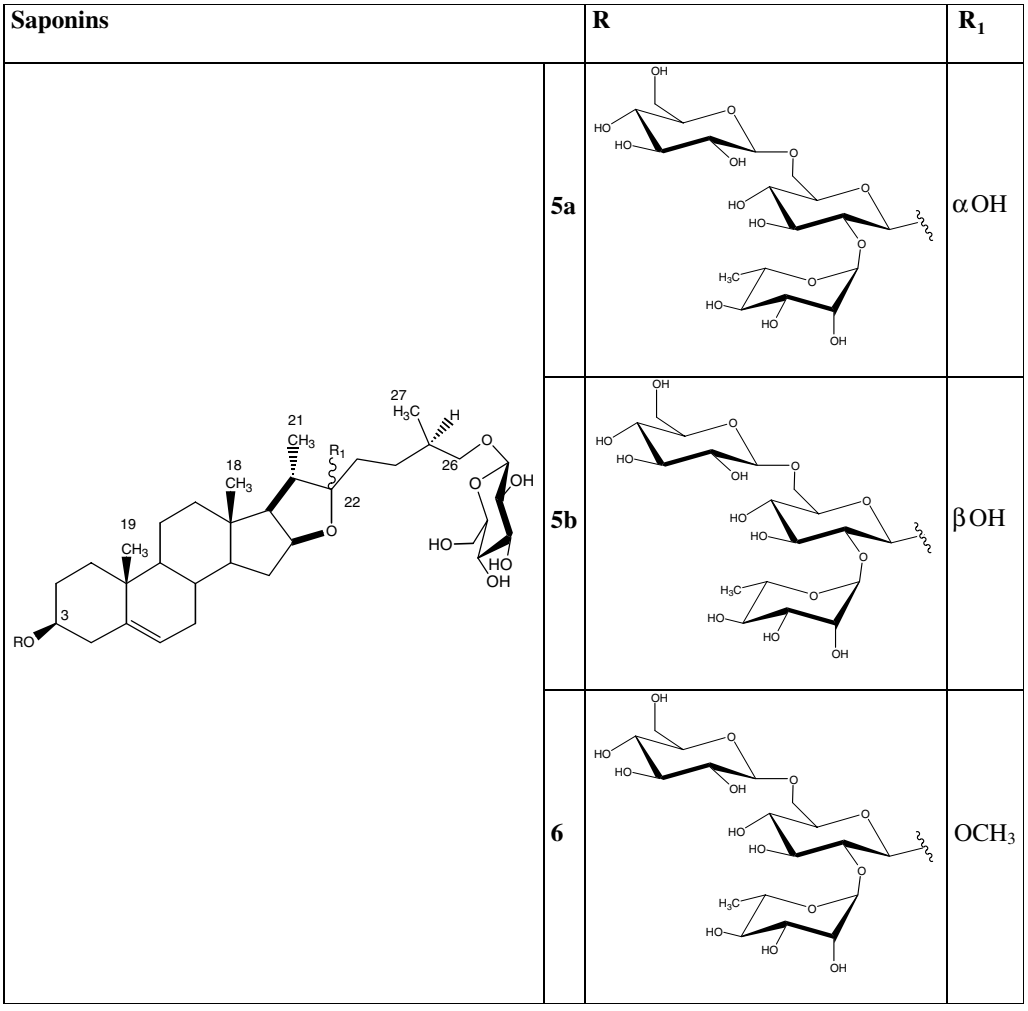

Fig. 2. Compounds **5a**, **5b** and **6** isolated from seeds of *Allium caepa* L. var. *tropeana*.

anol. This was confirmed by repeating the extraction procedure in acetone and observing the absence of **2** and **4** in the extract. Therefore, we have not characterized them further.

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