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Spirostanol saponins of *Allium porrum* L.*

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Abstract

An investigation of the extracts from bulbs of *Allium porrum* L. has led to the isolation of four spirostanol saponins. Two of them are new compounds and have been identified as: (25R)-5α-spirostan-3β,6β-diol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**3**) and (25*R*)-5α-spirostan-3β,6β-diol 3-*O*-{*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**4**). The isolated compounds were evaluated for their antifungal activity.

Keywords: Allium porrum L.; Liliaceae; Spirostanol saponins; Branched tetrasaccharides; Branched pentasaccharides; NMR; Antifungal activity

1. Introduction

Allium porrum L. is a bulbous perennial plant commonly called leek and widely cultivated as a vegetable throughout the world (Uphof, 1968; Stearn, 1980). Fresh juice of the plant is claimed to be bactericide; diuretic, hypotensive and digestive properties are also attributed to this plant (Schauenberg & Paris, 1977). A chemical study of *Allium porrum* L. was undertaken in the frame of a systematic survey on *Allium* species typical of Mediteranean area. This plant, similarly to other species belonging to the *Allium* genus, turned out to be a rich source of secondary metabolites, among which we have recently isolated eight new sapogenins based on spirostane structures (Carotenuto et al., 1997a, 1997b). Examination of the MeOH extract of the bulbs revealed the presence of four saponins (**1**–**4**), two of which (**3**, **4**) are new compounds. Compounds **1**–**4**, when tested against *Fusarium culmorum*, exhibited antifungal activity.

2. Results and discussion

Bulbs of *A. porrum* L. were collected and extracted with *n*-hexane followed by $CHCl_3$, $CHCl_3$/MeOH (9:1) and MeOH. Repeated column chromatographies of the MeOH extract yielded pure saponins **1**–**4**.

The structures of the known compounds (**1**–**2**) were assigned by comparison of their mass, ^1H and ^{13}C NMR spectral data with those reported in the literature (Kawasaki, Nishioka, Komori, Yamauchi, & Miyahara, 1965; Mimaki, Nakamura, Sashida, Nikaido, & Ohmoto, 1995).

Our initial study was focused on the structure of saponin **4** which was isolated in a relatively large amount and showed activity ($ED_{50}=30$ μg/ml) towards the fungus *Fusarium culmorum*. It showed a pseudomolecular ion peak at *m*/*z* 1211 $[M–H]^-$ in the negative ion FAB mass spectrum. The ^{13}C NMR spectrum (Table 1) contained characteristic resonances of a β-chlorogenin substituted at position 3 (Matsuura, Ushiroguchi, Itakura, Hayashi, & Fuwa, 1988). A number of oxymethylene and oxymethine signals in the ^1H and ^{13}C NMR spectra (Table 1) indicated the glycosidic nature of the compound. Signals attributable to five anomeric protons and to the relevant carbon atoms in the ^1H and ^{13}C NMR spectra, respectively (Table 1), were easily identified so determining the number of sugar residues.

To define the nature of the sugars, **4** was acetylated to give the exadecaacetate **4a** (see Section 3), whose ^1H NMR spectrum showed a better resolution of the oxymethine and oxymethylene proton resonances. An HMQC experiment allowed us to easily identify the ^1H and ^{13}C NMR resonances of the five anomeric functions [^1H: δ 4.47 (H-1^I), 4.44 (H-1^{II}), 4.79 (H-1^{III}), 4.77 (H-1^{IV}),

* Part 4 in the series "Chemistry of the genus *Allium*". For Part 3, see Carotenuto et al. [Carotenuto, A., Fattorusso, E., Lanzotti, V., Magno, S., De Feo, V., Carnuccio, R., & D'Acquisto, F. (1997). *J. Nat. Prod.*, *60*, 1003.]. For Part 1 and 2, see *Phytochemistry*, 1996, *41*, 531 and *Phytochemistry*, 1997, *44*, 949.

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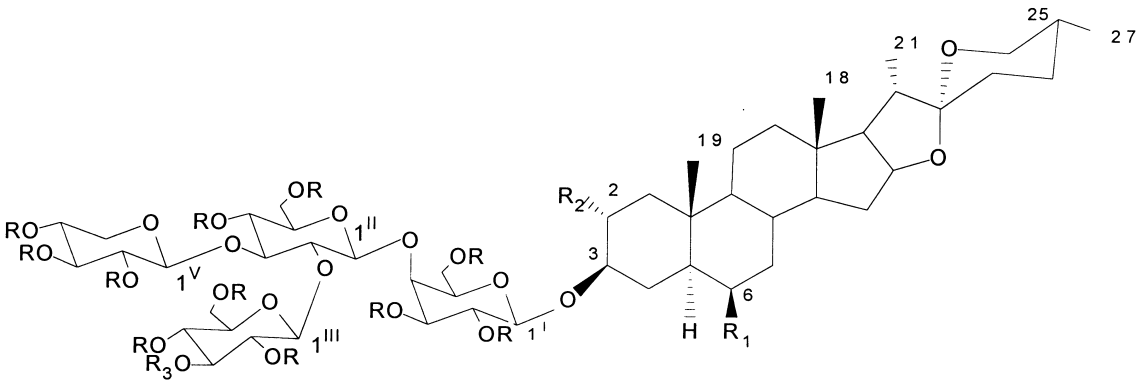

	R	R_1	R_2	R_3
1	H	H	OH	H
2	H	H	OH	β-Glc ^{IV}
3	H	OH	H	H
3a	Ac	OAc	H	Ac
4	H	OH	H	β-Glc^{IV}
4a	Ac	OAc	H	β-Glc^{IV}-2,3,4,6-OAc

Structure 1.

4.90 (H-1^{V}); ^{13}C: δ 98.1 (C-1^{I}), 109.3 (C-1^{II}), 103.5 (C-1^{III}), 96.2 (C-1^{IV}), 99.0 (C-1^{V})].

Using the anomeric proton at δ 4.77 (d, $J=8.2$ Hz, H-1^{IV}) as a starting point, analysis of COSY and HOHAHA experiments allowed the identification, in sequence, of four oxymethine and one oxymethylene groups (Table 2). All these protons, except H-5^{IV}, were linked to an acetoxylated carbon atom, as indicated by their low-field resonances, thus suggesting a terminal hexose unit in the pyranose form. The sugar moiety was identified as *β*-glucose on the basis of the large couplings observed for all the oxymethine protons, implying their axial position.

On the basis of the same arguments as those for the characterization of previous sugar unit, the anomeric protons at δ 4.44 (H-1^{II}) and δ 4.79 (H-1^{III}) were attributed to *β*-glucopyranose units (Table 2). Upfield shifts of H-2^{II} (δ 3.70) and H-3^{II} (δ 3.93) indicated a 2,3-disubstitution for the Glc II, while a similar upfield shift for H-3^{III} (δ 4.08) indicated a substitution at position 3 for the Glc III. Starting from the anomeric signal at δ 4.47 (H-1^{I}) we could identify, on the basis of COSY and HOHAHA data, another hexose unit sequence extending to an oxymethylene residue via four oxymethine protons. The relative high field resonances of H-5^{I} indicated its pyranose form. The relative stereochemistry of the monosaccharide unit was elucidated by the analysis of coupling constants in the ^{1}H NMR spectrum. When the measurement was not possible some crucial correlation peaks in the ROESY experiment gave us decisive indications. In particular the sugar unit was characterized by large axial-axial couplings between H-1^{I}/H-2^{I}, H-2^{I}/H-3^{I}, while the axial-equatorial relations H-3^{I}/H-4^{I} was deduced by the small coupling constant between these protons. The axial position of H-5^{I} is indicated by the strong ROESY correlation peaks with H-3^{I}. Because of these evidences this monosaccharide unit was identified as *β*-galactopyranose. In addition, the relatively high field chemical shift of H-4^{I} (δ 4.15) clearly prove the position 4 to be a glycosidic linkage site.

Finally, the last anomeric proton at δ 4.90 (H-1^{V}) by analysis of ^{1}H NMR experiments was proven to be a terminal pentose in the pyranose form (Table 2). Unfortunately, it was impossible to determine all the coupling constants between the protons belonging to this sugar residue because of their overlapping NMR resonances. However, the structure of the sugar moiety was clarified by chemical analysis. Thus **4** was submitted to acid methanolysis followed by silylation with trisil-Z. GC analysis of the released saccharides showed a sugar composition of glucose, galactose and xylose in a relative ratio 3:1:1,

Table 1
^{13}C and ^{1}H NMR data[a] of compounds **3** and **4**

	3		**4**	
Position	δ_C (mult.)	δ_H (mult., *J*, Hz)	δ_C (mult.)	δ_H (mult., *J*, Hz)
1ax	38.8 (CH_2)	0.76 (bdd, 12.7, 12.7)	38.7 (CH_2)	0.77 (bdd, 12.6, 12.6)
eq		1.44[b]		1.44[b]
2ax	29.9 (CH_2)	1.64 (bdd, 12.7, 12.7)	29.9 (CH_2)	1.63 (bdd, 12.6, 12.6)
eq		1.96[b]		1.97[b]
3	77.9 (CH)	3.96[b]	77.9 (CH)	3.96[b]
4ax	32.8 (CH_2)	2.13 (ddd, 12.4, 12.4, 12.4)	32.7 (CH_2)	2.12 (ddd, 12.4, 12.4, 12.4)
eq		1.95[b]		1.93[b]
5	47.9 (CH)	0.94 (bd, 12.4)	47.8 (CH)	0.94 (bd, 12.4)
6	70.7 (CH)	3.82 (bs)	70.7 (CH)	3.82 (bs)
7ax	40.8 (CH_2)	1.06[b]	40.8 (CH_2)	1.05[b]
eq		1.90 (bd, 13.7)		1.90 (bd, 13.8)
8	30.6 (CH)	2.09 (m)	30.6 (CH)	2.08 (m)
9	54.6 (CH)	0.54[b]	36.0 (CH)	0.56[b]
10	36.1 (C)	54.5 (C)		
11ax	21.2 (CH_2)	1.26 (13.9, 12.7)	21.1 (CH_2)	1.26 (13.9, 12.7)
eq		1.37 (bd, 13.9)		1.35 (bd, 13.9)
12ax	40.1 (CH_2)	0.99 (bdd, 12.7, 10.5)	40.1 (CH_2)	0.99 (bdd, 12.7, 10.5)
eq		1.59 (bd, 10.5)		1.58 (bd, 10.5)
13	40.8 (C)		40.8 (C)	
14	56.3 (CH)	1.02[b]	56.3 (CH))	1.01[b]
15a	32.2 (CH_2)	1.98[b]	32.2 (CH_2)	1.98[b]
b		1.31[b]		1.33[b]
16	81.0 (CH)	4.45 (ddd, 7.4, 7.4, 7.4)	81.0 (CH)	4.42 (ddd, 7.5, 7.5, 7.5)
17	63.0 (CH)	1.72 (dd, 7.4, 7.4)	63.0 (CH)	1.73 (dd, 7.5, 7.5)
18	16.5 (CH_3)	0.75 (s)	16.5 (CH_3)	0.75 (s)
19	15.9 (CH_3)	1.11 (s)	15.9 (CH_3)	1.12 (s)
20	42.0 (CH)	1.84 (dt, 6.9, 6.9)	41.9 (CH)	1.84 (dt, 6.6, 6.6)
21	14.9 (CH_3)	1.03 (d, 6.9)	15.0 (CH_3)	1.05 (d, 6.6)
22	109.1 (C)		109.1 (C)	
23a	31.7 (CH_2)	1.57[b]	31.7 (CH_2)	1.58[b]
b		1.49 (bd, 13.5)		1.49 (bd, 13.5)
24a,b	29.2 (CH_2)	1.44[b]	29.2 (CH_2)	1.44[b]
25	30.5 (CH)	1.46[b]	30.5 (CH)	1.46[b]
26ax	66.8 (CH_2)	3.38 (dd, 10.7, 10.7)	66.8 (CH_2)	3.39 (dd, 10.7, 10.7)
eq		3.46 (dd, 2.8, 10.7)		3.46 (dd, 2.8, 10.7)
27	17.2 (CH_3)	0.57 (d, 5.8)	17.2 (CH_3)	0.57 (d, 5.8)
Gal I				
1	102.3 (CH)	4.83 (d, 7.8)	102.1 (CH)	4.82 (d, 8.2)
2	73.1 (CH)	4.30[b]	73.1 (CH)	4.32[b]
3	75.7 (CH)	4.01[b]	75.7 (CH)	4.02[b]
4	79.9 (CH)	4.50 (bs)	79.9 (CH)	4.50 (bs)
5	75.9 (CH)	3.91[b]	75.9 (CH)	3.91[b]
6a,b	60.6 (CH_2)	4.59 (m), 4.13[b]	60.6 (CH_2)	4.59 (m), 4.12[b]
Glc II				
1	104.8 (CH)	5.08 (d, 7.8)	104.6 (CH)	5.07 (d, 7.8)
2	81.3 (CH)	4.32[b]	81.3 (CH)	4.30[b]
3	86.9 (CH)	4.05[b]	86.9 (CH)	4.04[b]
4	70.4 (CH)	3.71[b]	70.4 (CH)	3.71[b]
5	77.0 (CH)	3.75[b]	77.0 (CH)	3.75[b]
6a,b	62.9 (CH_2)	4.40[b], 4.24 (dd, 3.3, 12.3)	62.9 (CH_2)	4.39[b], 4.22[b]
Glc III				
1	105.2 (CH)	5.45 (d, 7.8)	105.3 (CH)	5.42 (d, 8.2)
2	76.2 (CH)	3.98[b]	75.2 (CH)	3.89[b]
3	77.5 (CH)	3.99[b]	87.5 (CH)	4.14[b]
4	71.0 (CH)	4.09[b]	70.0 (CH)	3.86[b]
5	78.6 (CH)	3.79[b]	78.6 (CH)	3.75[b]
6	62.5 (CH_2)	4.45[b], 4.25[b]	62.5 (CH_2)	4.37[b], 4.13[b]

Table continued on next page

Table 1—*continued.*

	3		**4**	
Position	δ_C (mult.)	δ_H (mult., *J*, Hz)	δ_C (mult.)	δ_H (mult., *J*, Hz)
Glc IV				
1			103.9 (CH)	4.99 (d, 8.2)
2			74.6 (CH)	3.83[b]
3			77.5 (CH)	3.93[b]
4			70.6 (CH)	4.02[b]
5			77.1 (CH)	3.95[b]
6			62.4 (CH_2)	4.35[b], 4.15[b]
Xyl V				
1	104.9 (CH)	5.12 (d, 7.0)	104.7 (CH)	5.04 (d, 7.0)
2	75.0 (CH)	3.85[b]	75.0 (CH)	3.83[b]
3	78.6 (CH)	3.97[b]	78.6 (CH)	3.95[b]
4	70.6 (CH)	4.00[b]	70.6 (CH)	4.02[b]
5eq	67.7 (CH_2)	4.10[b]	67.7 (CH_2)	4.12[b]
5ax		3.55 (dd, 4.1, 11.9)		3.58(dd, 6.7, 12.4)

[a] The spectra are in pyridine-d5 (Bruker AMX-500).
[b] Superimposed by other signals.

thus indicating the pentose to be xylose. The β-configuration at C-1^V was assigned on the basis of the vicinal coupling constant of 7.0 Hz observed in the ^{1}H NMR spectrum of **4** for the anomeric proton (δ 5.04, Table 1). Analysis of ROESY and HMBC correlations allowed us to link the sugar units. In particular, dipolar interactions H-1^{IV}/H-3^{III}, H-1^{III}/H-2^{II}, H-1^{III}/H-1^{II}, H-1^V/H-3^{II}, H-1^{II}/H-4^I, H-1^I/H-3 were diagnostic for the sugar moieties shown in the figure.

HMBC correlations (C-1^{III}/H-2^{II}, C-1^V/H-3^{II}, C-1^{IV}/H-3^{III}) confirmed the proposed structure. Once the structure of compound **4a** was secured we achieve full assignment of ^{1}H and ^{13}C NMR spectra of the parent compound **4** by analysis of COSY, HOHAHA, HMQC and ROESY experiments (Table 1). Compound **4** was identified from the above data as (25*R*)-5α-spirostan-3*β*,6*β*-diol 3-*O*-{*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside} (**4**).

Compound **3** showed a pseudomolecular ion peak at *m/z* 1049 $[M–H]^-$ in the negative ion FAB mass spectrum, 162 mass units less compared to **4**, thus suggesting the absence of one hexose sugar unit. Diagnostic fragmentation peaks at *m/z* 917 $[M–H\text{-}132]^-$ (loss of one pentose), *m/z* 887 $[M–H\text{-}162]^-$ (loss of one hexose) and at *m/z* 755 $[M–H\text{-}(132+162)]^-$ indicated a branched sugar moiety. The tetrasaccharide nature of compound **3** was clearly indicated by comparison of the ^{1}H and ^{13}C NMR spectra of **3** and **4**, which showed almost identical resonances (Table 1) for the aglycone moiety, differing in the absence in compound **3** of signals related to the fifth sugar residue. Acetylation of **3** and successive COSY and HOHAHA analysis of the acetylated derivative (**3a**) revealed a saccharide chain as that of **3** differing just for the absence of the terminal Glc IV. The structure of the sugar moiety was confirmed by chemical analysis. Thus **3** was submitted to acid methanolysis followed by silylation with trisil-Z. GC analysis of the released saccharides showed a sugar composition of glucose, galactose and xylose in a relative ratio 2:1:1. The above data defined the structure of compound **3** as (25*R*)-5α-spirostan-3*β*,6*β*-diol 3-*O*-{*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside}.

By assuming glucose, galactose and xylose to belong to the most commonly found D-form, the absolute stereochemistry of **3** and **4** could be defined.

The isolated compounds **1**–**4** when tested against the fungus *Fusarium culmorum* exhibited ED_{50}s of 30–35 μg/ml.

3. Experimental

3.1. General methods

FABMS (recorded in glycerol matrix) were measured on a VG Prospec (FISONS) mass spectrometer (Xe atoms of energy of 2–6 kV). ^{1}H and ^{13}C NMR measurements were performed on a Bruker AMX-500 spectrometer. Chemical shifts were referenced to the residual solvent signal (pyridine-d5: $\delta_H = 7.20$, $\delta_C = 77.0$; CD_3OD: $\delta_H = 3.34$, $\delta_C = 49.0$). Signals of methyl, methylene and methine carbon atoms were distinguished by DEPT experiments. One-bond heteronuclear ^1H–^{13}C connectivities were determined by means of HETCOR and HMQC (Bax & Subramanian, 1986) experiments ($^1J_{CH}$ of 125 Hz). During the HMQC acquisition time ^{13}C

Table 2
^{1}H NMR data[a] of compounds **3a** and **4a**

Position	**3a** δ_H (mult., *J*, Hz)	**4a** δ_H (mult., *J*, Hz)
3	3.51[b]	3.53 (m)
6	4.94 (bs)	4.96 (bs)
16	4.37 (m)	4.38[b]
18	0.79 (s)	0.80 (s)
19	1.00 (s)	1.02 (s)
21	0.96 (d, 7.1)	0.96 (d, 7.1)
26eq	3.46[b]	3.46 (dd, 11.0, 3.0)
ax	3.37 (dd, 10.8, 10.8)	3.34 (dd, 11.0, 11.0)
27	0.78 (d, 6.4)	0.78 (d, 6.4)
3-0-Gal^I		
1	4.41 (d, 7.8)	4.47 (d, 8.2)
2	5.35 (dd, 7.8, 9.2)	5.30 (dd, 8.2, 10.3)
3	4.89 (dd, 2.8, 10.2)	4.96 (bd, 10.3)
4	4.06 (bs)	4.15 (bs)
5	3.65 (m)	3.67 (m)
6a,b	4.21 (dd, 5.2, 11.4), 4.13[b]	4.13 (m)
Glc^{II}		
1	4.32 (d, 7.8)	4.44 (d, 7.8)
2	3.85 (dd, 9.2)	3.70 (dd,7.8, 9.2)
3	3.93 (dd, 9.2, 9.2)	3.93 (dd, 9.2, 9.2)
4	4.98 (dd, 9.2, 9.2)	4.89 (dd, 9.2, 9.2)
5	3.46[b]	3.45 (m)
6a,b	4.07 (m)	4.12 (bd, 12.3), 4.02 (dd, 12.3, 3.5)
Glc^{III}		
1	4.84 (d, 7.8)	4.79 (d, 8.2)
2	5.18 (dd, 7.8, 9.2)	5.14 (dd, 8.2, 9.2)
3	5.30 (m)	4.08 (dd, 8.8, 8.8)
4	5.29 (m)	5.26 (m)
5	3.74 (m)	3.75 (m)
6a,b	4.35[b], 4.25 (dd, 3.3, 12.3)	4.30 (dd, 7.5, 12.1), 4.20 (dd, 2.5, 12.1)
Glc^{IV}		
1		4.77 (d, 8.2)
2		4.94 (dd, 8.2, 9.2)
3		5.16 (dd. 9.2, 9.6)
4		5.08 (dd. 9.6, 9.9)
5		3.87 (ddd, 9.9, 3.5, 2.1)
6a,b		4.11[b], 4.02 (3.5, 12.4)
Xyl^V		
1	5.14 (d, 7.0)	4.90[b]
2	4.86 (dd, 2.8, 2.6)	4.91[b]
3	5.01 (dd, 2.8, 2.8)	5.13 (m)
4	4.76 (m)	4.86[b]
5eq	4.12[b]	4.07[b]
5ax	3.53 (dd, 4.1, 11.9)	3.36 (dd, 6.7, 12.4)

[a] The spectra are in $CDCl_3$ (Bruker AMX-500).
[b] Superimposed by other signals.

broad-band decoupling was performed by using the GARP sequence (Shaka, Barker, & Freeman, 1985). ^{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 (Bax & Davis, 1985). Nuclear Overhauser effect (nOe) measurements were performed by 2D ROESY experiments. MPLC was performed on a Buchi 861 apparatus using a $Si0_2$ (230–400 mesh) and RP-18 columns. HPLC was performed on a Varian apparatus equipped with UV and refractive index detectors. Hibar LiChrospher columns were used.

3.2. Plant material

Samples of *Allium porrum* L. were collected in May 1995 near Salerno (Campania, Italy). The plants were frozen immediately after collection and kept frozen until extraction. A reference specimen has been deposited at the Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II, Italy.

3.3. Isolation procedure

The collected plant samples (820 g, dry weight after extraction) were homogenized and extracted at room temperature with the following solvents: hexane, $CHCl_3$, $CHCl_3$/MeOH (9:1) and MeOH. The butanol soluble fraction of the MeOH extract was concentrated in vacuo to afford 5 g of a crude organic extract that was chromatographed by MPLC on a RP-18 column using a gradient solvent system from H_2O to MeOH. Fractions eluted with MeOH (290 mg), were purified by HPLC on a Hibar LiChrospher 100 RP-18 (10 μm) with a mobile phase MeOH–H_2O (85:15) to give fractions 6, 7 and 8 containing saponins.

Fraction 6 (50 mg) subjected to MPLC on a SiO_2 column (230–400 mesh) using a gradient solvent system from $CHCl_3$ to $CHCl_3$–MeOH–H_2O (65:45:10) afforded compound **4** (30 mg).

Fraction 7 (44 mg) subjected to the same treatment gave compounds **3** (10.3 mg) and **2** (13.4 mg). Both compounds were purified by HPLC on a Hibar LiChrospher 100 RP-18 (5 μm) with a mobile phase MeOH–H_2O (85:15) to give pure compounds **3** (5 mg) and **2** (6 mg).

Fraction 8 (22 mg) subjected to MPLC on a SiO_2 column (230–400 mesh) using a gradient solvent system from $CHCl_3$ to $CHCl_3$–MeOH–H_2O (65:45:10) afforded compound **1** (9 mg), which was purified by HPLC on a Hibar LiChrospher 100 RP-18 (5 μm) with a mobile phase MeOH–H_2O (85:15) to give pure compound **1** (4 mg).

3.4. Acetylation of saponins *3* and *4*

Acetylation was performed with Ac_2O and pyridine (1:1) overnight at room temp. The concd reaction mixture was subjected to HPLC (column: Hibar LiChrospher Si-60 5mm; eluent: hexane:EtOAc, 3:7). ^{1}H NMR data of the acetylated derivatives **3a** and **4a** are reported in Table 2.

3.5. Methanolysis of **3** and **4**: sugar analysis

A solution of **3** (1 mg) in anhydrous 2 M HCl in MeOH (0.5 ml) was heated at 80°C in a stoppered reaction vial for 8 h. After cooling, neutralization with Ag_2CO_3 and centrifugation, the supernatant was evaporated to dryness under N_2. The residue was trimethylsilylated with TRISIL Z (Pierce Chemical Co.) for 15 min at room temp. GLC analysis gave peaks which co-eluted with those of methylxyloside, methylgalactoside and methylglucoside. A solution of **4** (1 mg) subjected to the same treatment gave methylxyloside, methylgalactoside and methylglucoside.

(25*R*)-5α-Spirostan-3*β*,6*β*-diol 3-*O*-{*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside} (**3**): $[\alpha]_D^{25} = -57°$ (MeOH); FABMS (negative ion) *m*/*z* 1049 $[M-H]^-$; ^1H and ^{13}C NMR data are reported in Table 1.

(25*R*)-5α-Spirostan-3*β*,6*β*-diol 3-*O*-{*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside} (**4**): $[\alpha]_D^{25} = -56°$ (MeOH); FABMS (negative ion) *m*/*z* 1211 $[M-H]^-$; ^1H NMR and ^{13}C NMR data are reported in Table 1.

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