Two New Triterpene Saponins from *Acanthophyllum laxiusculum*

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Two new triterpene glycosides, **1** and **2**, together with three known ones, were isolated from roots of *Acanthophyllum laxiusculum* SCHIMAN-CZEIKA. The structures of the new compounds were established by extensive 1D- and 2D-NMR spectroscopic experiments and MS analyses as 23-*O*-*β*-D-galactopyranosylgypsogenic acid 28-*O*-{*β*-D-glucopyranosyl-(1→2)-6-*O*-[4-carboxy-3-hydroxy-3-methyl-1-oxobutyl]-*β*-D-glucopyranosyl-(1→6)}-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-galactopyranosyl ester (**1**) and gypsogenic acid 28-*O*-{*β*-D-glucopyranosyl-(1→2)-6-*O*-[4-carboxy-3-hydroxy-3-methyl-1-oxobutyl]-*β*-D-glucopyranosyl-(1→6)}-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-galactopyranosyl ester (**2**).

Introduction. – In a continuation of our studies on saponins from the plants of the Caryophyllaceae family [1–6], we have examined the saponins from the roots of *Acanthophyllum laxiusculum* SCHIMAN-CZEIKA (syn.: *Acanthophyllum heratense* SCHIMAN-CZEIKA). *Acanthophyllum* C.A.MEY is a genus with *ca.* 61 herbaceous species worldwide, of which 33 occur in Iran, with 23 being endemic [7]. Traditionally, all species of *Acanthophyllum* are used as soup in Khorasan Province, and the aqueous extract of their roots is used to make a special type of candy [5]. No previous phytochemical study has been reported on saponins of *A. laxiusculum.* Herein, we report the isolation and structure elucidation of two new triterpene saponins, **1** and **2** (*Fig.*), and the identification of three known ones, **3**–**5** (*Fig.*), from the H_2O extract of the roots of this plant.

Results and Discussion. – The H_2O extract of roots of *A. laxiusculum* was fractionated by vacuum liquid chromatography (VLC) and purified by repeated medium-pressure liquid chromatography (MPLC) on normal or reversed-phase (RP) silica gel to yield **1** and **2** (*Fig.*), and three known compounds. Their structures were elucidated by extensive NMR spectroscopy, including a series of 2D-NMR experiments (^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, and HMBC), and by mass spectrometry. The known saponins were identified by comparison of their spectral data with those reported in the literature as glanduloside C (**4**) from *Acanthophyllum glandulosum*, *Acanthophyllum sordidum*, *Acanthophyllum lilacinum*, and *Acanthophyllum elatius* [3][6], its prosapogenin, **3**, from *Gypsophila oldhamania* [8], and 3-*O*-

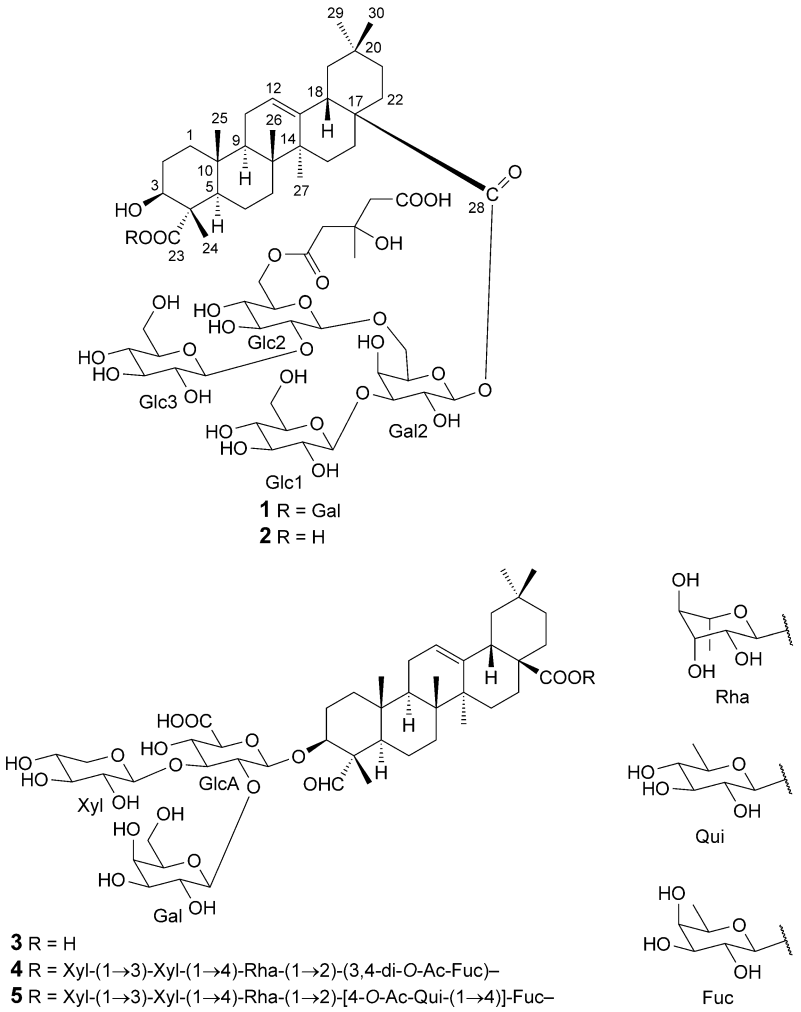

Figure. *Structures of* **1**–**5**

β-D-galactopyranosyl-(1 → 2)-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucuronopyranosyl-gypsogenin 28-*O*-*β*-D-xylopyranosyl-(1 → 3)-*β*-D-xylopyranosyl-(1 → 4)-*α*-L-rhamnopyranosyl-(1 → 2)-[(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1 → 4)]-*β*-D-fucopyranosyl ester (= *O*-*β*-D-xylopyranosyl-(1 → 3)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-6-deoxy-*α*-L-mannopyranosyl-(1 → 2)-*O*-4-*O*-acetyl-6-deoxy-*β*-D-glucopyranosyl-(1 → 4)-6-deoxy-1-*O*-[(3*β*)-3-[[*O*-*β*-D-galactopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucopyranuronosyl]oxy]-23,28-dioxoolean-12-en-28-yl]-*β*-D-galactopyranose; **5**) from *Gypsophila bicolor* [4].

Compounds **1** and **2** were isolated as white amorphous powder. The monosaccharides obtained by acid hydrolysis of each compound were identified as galactose and

glucose for **1** and **2** by TLC comparison with authentic samples. The absolute configurations were determined as D for all sugars (see the *Exper. Part*) by GC analysis [9].

The HR-ESI mass spectrum of **1** exhibited a *quasi*-molecular-ion peak at *m*/*z* 1463.6311 ($[M+Na]^+$) consistent with the molecular formula $C_{66}H_{104}O_{34}$. The positive-ion-mode ESI-MS of **1** exhibited two *quasi*-molecular-ion peaks at *m*/*z* 1479 ($[M+K]^+$) and 1463 ($[M+Na]^+$). The HSQC spectrum of the aglycone exhibited six Me signals at δ(H)/δ(C) 1.46 (*s*)/11.5 (C(24)), 0.82 (*s*)/15.7 (C(25)), 0.92 (*s*)/17.0 (C(26)), 1.03 (*s*)/25.6 (C(27)), 0.76 (*s*)/32.7 (C(29)), and 0.82 (*s*)/23.3 (C(30)), one olefinic CH signal at 5.32 (*s*)/123.1 (C(12)), and a C_q signal at 143.8 (C(13); *Table 1*). These data were indicative of an olean-12-ene-type aglycone [2][6]. The HMBC cross-peaks between δ(H) 1.46 (Me(24)), and δ(C) 74.8 (C(3)), 54.7 (C(4)), 51.6 (C(5)), and 177.5 (C(23)) indicated that one secondary OH and one ester group were located at C(3) and C(23), respectively. Another upfield-shifted carboxylate C-atom signal at δ(C) 176.3 (C(28)) evidenced that **1** was a bidesmosidic saponin with two glycosyl ester linkages at C(23) and C(28). An extensive analysis of 1D- and 2D-NMR spectroscopic data indicated that the aglycone of **1** was gypsogenic acid (=(3β)-3-hydroxyolean-12-ene-23,28-dioic acid), and they were in good agreement with those in [2] and [6]. The ^{1}H-NMR spectrum of **1** exhibited signals of five anomeric H-atoms at δ(H) 6.25 (*d*, *J* = 7.8), 6.06 (*d*, *J* = 7.3), 5.26 (*d*, *J* = 7.6), 4.88 (*d*, *J* = 7.6), and 5.23 (*d*, *J* = 7.8), which correlated in the HSQC spectrum with signals of five anomeric C-atoms at δ(C) 95.9, 94.9, 104.7, 102.4, and 104.5, respectively, indicating the presence of five sugar units. Complete assignments of each sugar were achieved by extensive 1D- and 2D-NMR analyses, allowing the identification of two β-galactopyranosyl (Gal1 and Gal2) and three β-glucopyranosyl (Glc1 – Glc3) units, respectively. The HMBC cross-peak 6.25 (H–C(1) of Gal1)/177.5 (C(23) of the aglycone unit (Agly)) established that the Gal1 unit was linked to C(23) of Agly. The HMBC cross-peaks at δ(H)/δ(C) 6.06 (H–C(1) of Gal2)/176.3 (C(28) of Agly); 5.26 (H–C(1) of Glc1)/86.9 (C(3) of Gal2); 4.88 (H–C(1) of Glc2)/69.1 (C(6) of Gal2); and 5.23 (H–C(1) of Glc3)/82.0 (C(2) of Glc2) indicated that the oligosaccharide sequence Glc3-(1 → 2)-Glc2-(1 → 6)-[Glc1-(1 → 3)]-Gal2 was linked to C(28) of Agly. These linkages were also confirmed by the following NOESY cross-peaks: δ(H) 5.26 (H–C(1) of Glc1)/δ(H) 4.25 (H–C(3) of Gal2); 4.88 (H–C(1) of Glc2)/4.25 (CH_2(6) of Gal2); and 5.23 (H–C(1) of Glc3)/4.05 (H–C(2) of Glc2). Furthermore, the deshielded signals of CH_2(6) of Glc2 at δ(H) 4.48, 4.82/δ(C) 63.9 indicated an acylation in this position. The presence of a dicrotalic acid moiety (= 3-hydroxy-3-methylpentanedioic acid) was ascertained by the observation of a set of additional signals in the 1D- and 2D-NMR spectra corresponding to a 4-carboxy-3-hydroxy-3-methyl-1-oxobutyl moiety (see *Table 2*), which were in good agreement with those reported in [10 – 12]. The linkage of this unit to CH_2(6) of Glc2 was confirmed by the HMBC δ(H) 4.48/δ(C) 170.8. Thus, **1** was elucidated as 23-*O*-β-D-galactopyranosylgypsogenic acid 28-*O*-{β-D-glucopyranosyl-(1 → 2)-6-*O*-[4-carboxy-3-hydroxy-3-methyl-1-oxobutyl]-β-D-glucopyranosyl-(1 → 6)}-[β-D-glucopyranosyl-(1 → 3)]-β-D-galactopyranosyl ester.

The positive-ion-mode HR-ESI-MS of **2** exhibited a *quasi*-molecular-ion peak at *m*/*z* 1301.5774 ($[M+Na]^+$) consistent with the molecular formula $C_{60}H_{94}O_{29}$. The positive-ion-mode ESI-MS of **2** exhibited a *quasi*-molecular-ion peak at *m*/*z* 1301

Table 1. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; in C_5D_5N) *of the Aglycone of* **1** *and* **2** *from 1D- and 2D-NMR Experiments*[a]). δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	0.96, 1.46	38.6	1.01, 1.47	38.7
2	1.81, –[b])	26.8	1.81, –[b])	26.9
3	4.53 (*dd*, *J* = 8.1, 6.9)	74.8	4.50	75.3
4		54.7		53.9
5	1.76	51.6	1.88	51.3
6	1.80, 1.86	22.9	1.83, 1.88	23.6
7	1.64, 1.76	32.1	1.66, 1.76	32.0
8		39.7		39.9
9	1.60	47.9	1.68–1.72 (*m*)	47.6
10		36.4		36.5
11	1.80, 1.88	23.5	1.81, 1.90	23.4
12	5.32 (br. *s*)	123.1	5.35 (br. *s*)	123.1
13		143.8		144.0
14		41.6		41.8
15	0.99, 2.04	27.8	1.06, 2.08–2.12 (*m*)	27.9
16	1.43, 1.55	21.2	1.50, 1.55	21.4
17		46.7		46.8
18	3.05 (br. *d*, *J* = 12.1)	41.3	3.07 (br. *d*, *J* = 12.0)	41.3
19	1.08–1.14 (*m*), 1.59	45.9	1.12, 1.62	46.0
20		30.6		30.4
21	1.04, 1.22	33.7	1.08, 1.22	33.6
22	1.20, 1.50	32.4	1.23, 1.52–1.58 (*m*)	32.2
23		177.5		183.0
24	1.46 (*s*)	11.5	1.47 (*s*)	12.5
25	0.82 (*s*)	15.7	0.86 (*s*)	15.8
26	0.92 (*s*)	17.0	0.98 (*s*)	17.2
27	1.03 (*s*)	25.6	1.12 (*s*)	25.8
28		176.3		176.4
29	0.76 (*s*)	32.7	0.77 (*s*)	32.8
30	0.82 (*s*)	23.3	0.82 (*s*)	23.4

[a]) Overlapped signals are reported without designated multiplicity. [b]) Not determined.

($[M + Na]^+$), 162 mass units lower than that for **1**. The ^{1}H- and ^{13}C-NMR assignments of **2** (*Tables 1* and *2*) accomplished by extensive 2D-NMR analyses were almost superimposable to those of **1** except for the disappearance of the signals of a terminal galactopyranosyl moiety. The characteristic upfield ^{13}C-NMR signal of an carboxylate C-atom in **1** at δ(C) 177.5 (C(23)) was replaced by a signal at 183.0 in **2**, indicative of a COOH group. This was confirmed by the HMBCs between δ(H) 1.47 (*s*, Me(24)), and δ(C) 183.0 (C(23)), 75.3 (C(3)), and 53.9 (C(4)). Thus, the structure of **2** was elucidated as gypsogenic acid 28-*O*-{β-D-glucopyranosyl-(1 → 2)-6-*O*-[4-carboxy-3-hydroxy-3-methyl-1-oxobutyl]-β-D-glucopyranosyl-(1 → 6)}-[β-D-glucopyranosyl-(1 → 3)]-β-D-galactopyranosyl ester.

A literature survey revealed that the sequence 23-*O*-Gal-gypsogenic acid 28-*O*-Gal with a terminal Gal moiety at C(23), and additional substitutions at C(3) and C(6) of

Table 2. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; in C_5D_5N) *of the Sugar Moieties of* **1** *and* **2** *from 1D- and 2D-NMR Experiments*[a]). δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
23-O-*Sugar*				
Gal1				
1	6.25 (*d*, *J* = 7.8)	95.9		
2	4.10	73.5		
3	4.20	77.9		
4	4.14	70.6		
5	3.93	78.7		
6	4.17, 4.32	61.6		
28-O-*Sugars*				
Gal2				
1	6.06 (*d*, *J* = 7.3)	94.9	6.06 (*d*, *J* = 7.3)	94.3
2	4.22	72.5	4.22	72.6
3	4.25	86.9	4.23	87.1
4	4.23	68.5	4.24	68.5
5	4.02	76.9	4.04	76.9
6	4.25, 4.43	69.1	4.25, 4.46	69.4
Glc1				
1	5.26 (*d*, *J* = 7.6)	104.7	5.23	104.8
2	4.02	75.4	4.03	75.3
3	4.06	77.5	4.09	77.3
4	3.96	71.3	3.95 (*dd*, *J* = 8.8, 8.0)	70.9
5	3.85–3.90 (*m*)	77.9	3.84–3.88 (*m*)	77.8
6	4.10, 4.42	62.0	4.24, 4.40	61.9
Glc2				
1	4.88 (*d*, *J* = 7.6)	102.4	4.89	102.5
2	4.05	82.0	4.08	82.0
3	4.16	77.4	4.14–4.18 (*m*)	77.3
4	3.93	70.8	3.96 (*dd*, *J* = 8.8, 8.0)	70.7
5	3.78–3.82 (*m*)	74.9	3.81–3.84 (*m*)	74.9
6	4.48 (*dd*, *J* = 10.7, 4.0), 4.82 (br. *d*, *J* = 10.4)	63.9	4.48, 4.89	64.0
Glc3				
1	5.23 (*d*, *J* = 7.8)	104.5	5.24	104.8
2	4.03	75.3	4.02	74.9
3	4.09	77.5	4.09	77.3
4	4.05	70.5	4.09	70.9
5	3.81–3.84 (*m*)	77.8	3.84–3.88 (*m*)	77.8
6	4.23, 4.42	62.0	4.10, 4.42	61.9
Acid at C(6) of Glc2				
1		170.8		171.1
2	2.80–2.86 (*m*, 2 H)	46.9	2.82–2.89 (*m*, 2 H)	47.0
3		70.0		69.9
4	2.70 (*d*, *J* = 15.2), 2.90 (*d*, *J* = 15.0)	47.7	2.72 (*d*, *J* = 15.4), 2.90	47.6
5		179.8		179.2
6	1.49 (*s*)	27.8	1.51 (*s*)	28.0

[a]) Overlapped signals are reported without multiplicity.

Gal at C(28) in **1** occurs in several saponins isolated only from *Acanthophyllum* species of Caryophyllaceae, such as *A. glandulosum*, *A. sordidum*, and *A. lilacinum* [3][6]. These conclusions suggested that this sequence might represent a chemotaxonomic marker for the genus *Acanthophyllum*.

Experimental Part

General. TLC and HP-TLC: silica gel *60* F_{254} (SiO_2; *Merck*); identification of saponins with 1% vanillin in EtOH/H_2SO_4 50:1. VLC/MPLC: SiO_2 *60* (15–40 μm; *Merck*), *RP-18* (75–200 μm; *SiliCycle*). MPLC: *Alltech* pump, *Büchi* column (460 × 15 mm and 230 × 15 mm), *Büchi* precolumn (110 × 15 mm). GC: *ThermoQuest* gas chromatograph, *DB-1701* cap. column (30 m × 0.25 mm i.d; *J&W Scientific*); detector, FID; detector temp., 250°; injection temp., 230°; initial temp., 80° for 5 min and then increased to 270° at a rate of 15°/min; carrier gas, He [12]. Optical rotations: *AA-OR* automatic polarimeter. ^{1}H- and ^{13}C-NMR spectra: *Varian Unity-600* and *Inova-600* instruments equipped with a *Sun-4-L-X* computer system (at 600 and 150 MHz, resp.); for details see [1]; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS (pos.): *MicrOTOF* spectrometer; in *m*/*z*. HR-ESI-MS (pos.): *Q-TOF-1 Micromass* spectrometer; in *m*/*z*.

Plant Material. The roots of *A. laxiusculum* SCHIMAN-CZEIKA were collected from Torbat-e Heydarieh, Khorasan Province, Iran, in July 2012, and identified by Dr. *Atefeh Pirani*, plant taxonomist at the Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, where a voucher specimen (No. 09092013) was deposited.

Extraction and Isolation. Air-dried powdered roots of *A. laxiusculum* (30 g) were extracted with H_2O (3 × 500 ml) for 6 h under reflux to yield 8.5 g of a crude H_2O extract after evaporation. An aliquot of this extract (1.96 g) was submitted to VLC (SiO_2 *60*; $CHCl_3$/MeOH/H_2O 60:32:7 and 0:100:0 (300 ml each)) to give six fractions, *Frs. 1–6*. *Fr. 2* (186 mg; eluted with $CHCl_3$/MeOH/H_2O 60:32:7) was separated by MPLC (SiO_2 *60*; $CHCl_3$/MeOH/H_2O 60:32:7) to give 15 subfractions, *Frs 2.1–2.15* (*Fr. 2.14*: **2** (7 mg) and *Fr. 2.9*: **5** (12 mg)). Furthermore, *Fr. 4* (350 mg) was subjected to VLC (*RP-18*; MeOH/H_2O 0:100 → 100:0) to give six subfractions, *Frs. 4.1–4.6*. *Frs. 4.4* and *4.5* were combined (210 mg) and separated by MPLC (SiO_2 *60*; $CHCl_3$/MeOH/H_2O 60:32:7) to give ten subfractions, *Frs. 4.4.1–4.4.10*. *Fr. 4.4.6* was purified by MPLC (*RP-18*; MeOH/H_2O 20:80 → 60:40) to give **1** (11 mg). *Fr. 2.5* (59 mg) was separated by MPLC (*RP-18*; MeOH/H_2O 30:70 → 80:20) to give **3** (7 mg) and **4** (9 mg).

*23-*O*-β-*D*-Galactopyranosylgypsogenic Acid 28-*O*-[β-*D*-Glucopyranosyl-(1 → 2)-6-*O*-[4-carboxy-3-hydroxy-3-methyl-1-oxobutyl]-β-*D*-glucopyranosyl-(1 → 6)]-[β-*D*-glucopyranosyl-(1 → 3)]-β-*D*-galactopyranosyl Ester* (= O-β-D-*Glucopyranosyl-(1 → 3)*-O-[O-β-D-*glucopyranosyl-(1 → 2)-6*-O-*(4-carboxy-3-hydroxy-3-methyl-1-oxobutyl)-β-D-glucopyranosyl-(1 → 6)]-1*-O-*[(3β)-23-(β-D-galactopyranosyloxy)-3-hydroxy-23,28-dioxoolean-12-en-28-yl]-β-D-galactopyranose*; **1**). White amorphous powder. $[\alpha]_D^{25} = -10.2$ ($c = 0.09$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 1479 ($[M+K]^+$), 1463 ($[M+Na]^+$). HR-ESI-MS (pos.): 1463.6311 ($[M+Na]^+$, $C_{66}H_{104}NaO_{34}^+$; calc. 1463.6301).

*Gypsogenic Acid 28-*O*-[β-*D*-Glucopyranosyl-(1 → 2)-6-*O*-[4-carboxy-3-hydroxy-3-methyl-1-oxobutyl]-β-*D*-glucopyranosyl-(1 → 6)]-[β-*D*-glucopyranosyl-(1 → 3)]-β-*D*-galactopyranosyl Ester* (= O-β-D-*Glucopyranosyl-(1 → 3)*-O-[O-β-D-*glucopyranosyl-(1 → 2)-6*-O-*(4-carboxy-3-hydroxy-3-methyl-1-oxobutyl)-β-D-glucopyranosyl-(1 → 6)]-1*-O-*[(3β)-3,23-dihydroxy-23,28-dioxoolean-12-en-28-yl]-β-D-galactopyranose*; **2**). White amorphous powder. $[\alpha]_D^{25} = -16.8$ ($c = 0.07$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 1301 ($[M+Na]^+$). HR-ESI-MS (pos.): 1301.5774 ($[M+Na]^+$, $C_{60}H_{94}NaO_{29}^+$; calc. 1301.5773).

Acid Hydrolysis and GC Analysis. Compounds **1** and **2** (3 mg) were hydrolyzed with 2N aq. CF_3COOH (5 ml) for 3 h at 95°. After extraction with CH_2Cl_2 (3 × 5 ml), the aq. layer was repeatedly evaporated to dryness until neutral by addition of MeOH, and then analyzed by TLC (SiO_2; $CHCl_3$/MeOH/H_2O 8:5:1), followed by comparison with authentic samples. Further, the residue of sugars was dissolved in anh. pyridine (100 μl), and L-cysteine methyl ester hydrochloride (0.06 mol l^{-1}) was added. The mixture was stirred at 60° for 1 h, then 150 μl of hexamethyldisilazane (HMDS)/Me_3SiCl; 3:1) were

added, and the mixture was stirred at 60° for another 30 min. The precipitate was centrifuged, and the supernatant was concentrated under N_2. The residue was partitioned between hexane and H_2O (0.1 ml each), and the hexane layer (1 µl) was analyzed by GC [9]. The absolute configurations were determined by comparing the t_R values with those of the thiazoline derivatives prepared in a similar way from standard sugars (*Sigma–Aldrich*): t_R (D-galactose) 19.6 and t_R (D-glucose) 18.6 min for **1** and **2**.

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