Antimicrobial Triterpenoid Glycosides from *Cephalaria scoparia*

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Five new oleanane-type triterpenoid glycosides (**1**−**5**), named scoposides A−E, along with one known bisdesmosidic triterpene glycoside, were isolated from the aerial parts of *Cephalaria scoparia*. Three prosapogenins (**2a**−**4a**) were also obtained after alkaline hydrolysis of the bisdesmosidic compounds **2**−**4**. The structures of compounds **1**−**5** were determined by spectroscopic (1D and 2D NMR, HRESIMS) and chemical methods. The antimicrobial effects of compounds **1**−**5** were evaluated against a small panel of Gram-positive and Gram-negative bacteria.

The genus *Cephalaria* (Dipsacaceae) consists of 93 species that are found mainly in the Mediterranean region and the Middle East. In Turkey, there are 39 *Cephalaria* species and 23 of them are endemic. *Cephalaria scoparia* "Contrandr. & Quézel", one of these endemic species, occurs in southwestern Anatolia.[1] Many *Cephalaria* species have been used in traditional medicine due to their antimicrobial,[2] antifungal,[2] antioxidant,[3] and cytotoxic[4] effects. Previous phytochemical investigations on this genus have reported a number of triterpene saponins,[2,4−9] iridoids,[10] flavonoids,[11] and lignan glycosides.[12] In the present work, the isolation and structure elucidation of five new oleanane-type triterpenoid glycosides (**1**−**5**), named scoposides A−E, along with three prosapogenins (**2a**−**4a**), are reported from the aerial parts of *C. scoparia*. Additionally, one known bisdesmosidic triterpene glycoside (lycicoside-II)[13] and one known prosapogenin (3-*O*-α-L-arabinopyranosyloleanolic acid)[14] were obtained.

The dried aerial parts of *C. scoparia* were extracted with MeOH and then with *n*-BuOH−H_2O (1:1). The *n*-BuOH phase was found to be much more active in an antimicrobial assay than other fractions and was fractionated by RP-MPLC and repeated silica gel column chromatography, which allowed the purification of five triterpenoid saponins. The structures of all new compounds (**1**−**5**) were elucidated on the basis of their chemical and spectroscopic data. Antimicrobial effects of **1**−**5** were examined against different bacteria strains using a MIC method.

Results and Discussion

Compound **1** was obtained as an amorphous powder. The ESIMS of **1** exhibited a protonated molecular ion $[M - H]^-$ at *m*/*z* 1057.6, compatible with the molecular formula, $C_{53}H_{101}O_{20}$. This was confirmed by HRESIMS (negative-ion mode) at *m*/*z* 1057.6870 $[M - H]^-$ and supported by the NMR spectroscopic data for this compound (Tables 1 and 2). The IR spectrum of **1** showed absorptions for olefin (1634 cm^{-1}), hydroxy (3434 cm^{-1}), and ester (1728, 1280 cm^{-1}) functionalities. The 1D NMR data of **1** (Tables 1 and 2) revealed the presence of seven methyl groups at δ 1.04 (H_3-27), 0.91 (H_3-23), 0.84 (H_3-25), 0.84 (H_3-29), 0.84 (H_3-30), 0.72 (H_3-24), and 0.66 (H_3-26), one olefinic proton at δ 5.14 (1H, brs) with two typical olefinic carbon signals at δ_C 122.3 and 144.2, and one carbonyl signal at δ 176.0, indicating olean-12-enoic acid as an aglycon. The sugar part of **1** was found to consist of four anomeric proton signals for four sugar moieties that resonated at δ_H 5.21 (d, $J = 8.0$ Hz), 5.01 (brs), 4.28 (d, $J = 5.6$ Hz), and 4.19 (d, $J = 8.0$ Hz) and one secondary methyl carbon of rhamnose at δ_H 1.05 (d, $J = 6.4$ Hz) in the ^{1}H NMR spectrum. The coupling constants confirmed the β-glycosidic linkages for two glucose units and α-glycosidic linkages for one rhamnose unit and one arabinose unit. A microacid hydrolysis experiment on TLC and GC-MS analysis of **1** also confirmed the types of sugar units. The HMQC spectrum of **1** helped clarify the proton−carbon correlations of the compound. After alkaline hydrolysis of **1**, two glucose and one rhamnose unit were determined in the aqueous phase of the hydrolysate by a microhydrolysis method and GC-MS analysis. According to the ^{1}H NMR data of the *n*-BuOH phase of the alkaline hydrolysis, one arabinose unit remained in the structure of prosapogenin, **1a**.[14] All these findings indicated that two glucose moieties and one rhamnose unit are affixed at the C-28 position of the aglycon through an ester linkage. In the HMBC spectrum of **1**, specific correlations were observed between signals at δ_H 4.28 (H-1 of Ara) and δ_C 88.5 (C-3 of the aglycon), δ_H 5.21 (H-1 of Glc) and δ_C 176.0 (C-28 of the aglycon), δ_H 5.01 (H-1 of Rha) and δ_C 75.3 (C-2 of Glc), and δ_H 4.19 (H-1 of Glc′) and δ_C 68.5 (C-6 of Glc), showing the linkage points of the sugar moieties to the aglycon and to each other. Hence, the structure of this glycoside was established as 3-*O*-α-L-arabinopyranosylolean-12-ene 28-*O*-β-D-glucopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl ester (scoposide A).

Compound **2** was obtained as an amorphous powder. Its sodiated molecular ion, $C_{64}H_{104}O_{30}Na$, could be proposed by the positive-ion HRESIMS at *m*/*z* 1375.6566 $[M + Na]^+$. The IR and NMR spectroscopic features of **2** were similar to those of **1** except for the sugar units (see Experimental Section and Tables 1 and 2). Analysis of the NMR data indicated that compound **2** is an oleanane-type triterpene saponin with six sugars that were identified as glucose, xylose, and rhamnose through cochromatographic and GC-MS analysis. Alkaline hydrolysis also indicated that compound **2** is a bisdesmosidic triterpenoid glycoside with two glucose moieties linked to the C-28 position of the aglycon, with glucose, rhamnose, and two xylose moieties linked to the C-3 position of the aglycon. The linkage points of the sugar units to each other and to C-3 of the aglycon were established from the HMBC correlations of signals at δ_H 4.24 (H-1 of Glc) with δ_C 88.5 (C-3 of the aglycon), δ_H 5.01 (H-1 of Rha) with δ_C 74.5 (C-4 Glc), δ_H 4.31 (H-1 of Xyl) with δ_C 81.8 (C-3 of Rha), and δ_H 4.26 (H-1 of Xyl′) with δ_C 76.9 (C-4 of Xyl). The sugar sequence at C-28 was determined by the following HMBC correlations: δ_H 5.20 (H-1 of Glc′) with δ_C 176.0 (C-28 of the aglycon) and δ_H 4.45 (H-1 of Glc″) with δ_C 68.8 (C-6 of Glc′). Accordingly, the structure of **2** was assigned as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosylolean-12-ene 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (scoposide B).

Compound **3** was an amorphous powder and displayed a $[M + Na]^+$ ion at *m*/*z* 1507.7 in the ESIMS. The HRESIMS exhibited a sodiated molecular ion peak at *m*/*z* 1507.7011, to indicate a molecular formula of $C_{69}H_{112}O_{34}$. The IR and NMR data of the aglycon moiety of **3** were identical to the other new compounds

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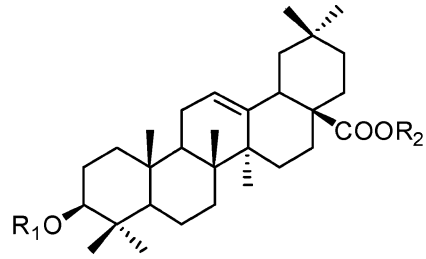
Chart 1



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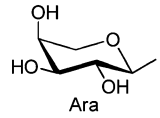
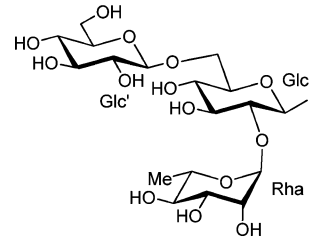
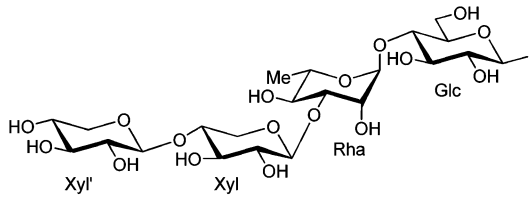
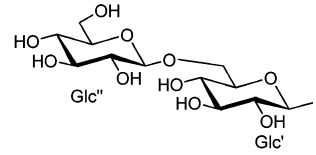
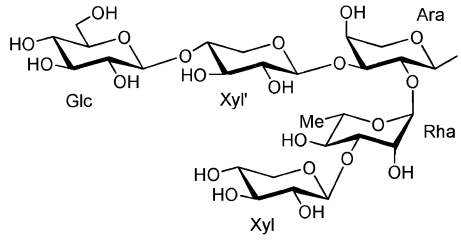
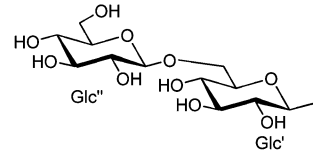
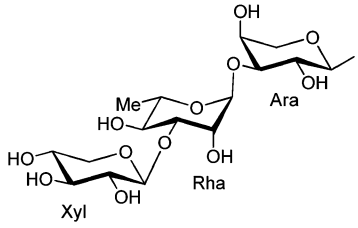
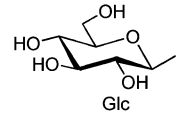
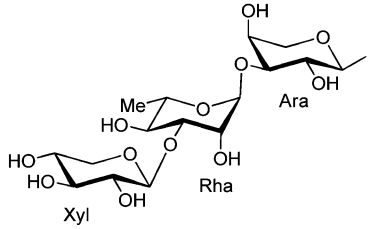
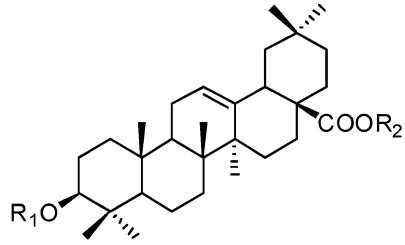
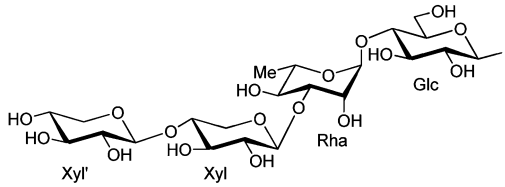
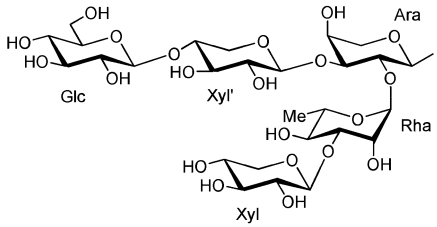
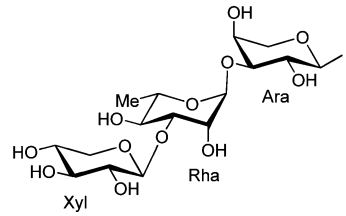
1		
2		
3		
4		
5		H-

(**1**−**5**). Seven anomeric protons [δ_H 5.21 (d, J = 7.8 Hz), 5.11 (brs), 4.31 (d, J = 7.6 Hz), 4.31 (d, J = 7.6 Hz), 4.26 (d, J = 4.2 Hz), 4.22 (d, J = 7.6 Hz), and 4.18 (d, J = 8.0 Hz)] and seven anomeric carbons [δ_C 106.0, 104.5, 104.0, 103.5, 100.8, 100.2, and 94.5] were observed in the NMR spectra of compound **3**. GC-MS and TOCSY analysis of **3** confirmed the NMR data of the sugar moieties, indicating the units as β-glucose, α-arabinose, α-rhamnose, and β-xylose (Table 1). In the HMBC spectrum, the specific correlations of the C-28 and C-3 carbon atoms with different sugar moieties showed that compound **3** has two sugar chains linked to the aglycon at the C-28 and C-3 carbons. In this spectrum, the linkage of the sugar units to aglycon were observed between δ_H 5.21 (H-1 of Glc′) and δ_C 175.7 (C-28 of the aglycon) and between δ_H 4.26 (H-1 of Ara) and δ_C 88.3 (C-3 of the aglycon). The linkage positions of the sugar moieties to each other were established from the HMBC correlations between signals at δ_H 5.11 (H-1 of Rha)

Chart 2



	R_1	R_2
2a		H-
3a		H-
4a		H-

and δ_C 74.3 (C-2 of Ara), δ_H 4.31 (H-1 of Xyl) and δ_C 81.6 (C-3 of Rha), δ_H 4.31 (H-1 of Xyl′) and δ_C 76.7 (C-3 of Ara), δ_H 4.22 (H-1 of Glc) and δ_C 79.6 (C-4 of Xyl′), and δ_H 4.18 (H-1 of Glc″) and δ_C 68.3 (C-6 of Glc′). Therefore, compound **3** (scoposide C) was determined as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)[-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosylolean-12-ene 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Compound **4** was isolated as an amorphous powder. The ESIMS of **4** exhibited a pseudomolecular ion $[M + Na]^+$ at *m*/*z* 1051.6. Its molecular formula, $C_{52}H_{84}O_{20}$, was assigned by HRESIMS (positive-ion mode) at *m*/*z* 1051.5402 $[M + Na]^+$. In the NMR spectra of compound **4**, four anomeric proton [δ_H 5.21 (d, $J = 8.4$ Hz), 5.14 (brs), 4.27 (d, $J = 7.2$ Hz), 4.26 (d, $J = 4.2$ Hz)] and four anomeric carbon [δ_C 106.3, 104.5, 100.1, and 94.6] signals were observed, corresponding to α-arabinose, α-rhamnose, β-glucose, and β-xylose. In the HMBC spectrum, the main correlations from H-1 of glucose to C-28 and from H-1 of arabinose to C-3 confirmed that the sugar units were located at C-28 and C-3 of the aglycon, respectively. Other correlations were observed between signals at δ_H 5.14 (H-1 of Rha) and δ_C 74.3 (C-3 of Ara) and between δ_H 4.27 (H-1 of Xyl) and δ_C 81.6 (C-3 of Rha). Alkaline hydrolysis and GC-MS experiments also confirmed that compound **4** is a bisdesmosidic triterpenoid glycoside. Thus, scoposide D (**4**) was elucidated as 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-arabinopyranosylolean-12-ene 28-*O*-β-D-glucopyranosyl ester.

Compound **5** displayed a $[M - H]^-$ ion at *m*/*z* 865.6 in the ESIMS and was assigned a molecular formula of $C_{46}H_{73}O_{15}$ as determined as *m*/*z* 865.4938 in the HRESIMS (negative-ion mode). The NMR data (Tables 1 and 2) indicated the presence of three sugars that were identified as α-arabinose (4.24, d, $J = 6.6$ Hz), β-xylose (4.26, d, $J = 7.8$ Hz), and α-rhamnose (5.21, brs). Compound **5** was not affected by alkaline hydrolysis, so this compound occurred as a C-3 monodesmosidic triterpene glycoside. The GC-MS analysis of the hydrolysate of **5** confirmed the type of sugar moieties. The sugar chain was assigned to C-3 of the aglycon since a free carboxyl group at C-28 was indicated by the chemical shift at δ_C 181.4. The HMBC correlations between signals at δ_H 4.24 (H-1 of Ara) and δ_C 88.6 (C-3 of the aglycon), δ_H 5.21 (H-1 of Rha) and δ_C 73.6 (C-3 of Ara), and δ_H 4.26 (H-1 of Xyl) and δ_C 81.8 (C-3 of Rha) defined the locations of the sugars in the molecule. Therefore, compound **5** (scoposide E) was assigned as 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-arabinopyranosylolean-12-en-28-oic acid.

The organic layers of the alkaline hydrolysis of the pure compounds **1**−**4** afforded three new prosapogenins, namely, 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosylolean-12-en-28-oic acid (**2a**), 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)[-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosylolean-12-en-28-oic acid (**3a**), and 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-arabinopyranosylolean-12-en-28-oic acid (**4a**) along with one known prosapogenin, **1a**[14] (3-*O*-α-L-arabinopyranosyloleanolic acid).

The antimicrobial effects of the scoposides A−E (**1**−**5**) were examined using the MIC method (Table 3).[17] According to these results, compound **5** was found to be most active for both Gram-positive and Gram-negative bacteria, whereas compounds **1**−**4** were moderately active. The most active substance, scoposide E (**5**), showed equal activity with gentamicin against *E. faecalis*.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Rudolph Research Analytical Autopol I automatic polarimeter fitted with a sodium lamp. IR spectra were recorded using on an ATI Mattson 1000 Genesis series FT-IR instrument. NMR measurements were performed on Varian AS 400 MHz and Varian ASP 600 MHz NMR spectrometers in DMSO-d_6. TMS was used as an internal standard, and *J* values are reported in Hz. Mass spectrometric analysis was carried out using a Bruker HCT Ultra ESIMS ion trap instrument using the positive mode. HRESIMS was measured on a Bruker LC micro-Q-TOF mass spectrometer. MPLC was carried out on a Buchi C-605 MPLC instrument. GC-MS analysis was performed by a HP 6890-5973 instrument with a HP-5MS column. For thin-layer chromatography, silica gel F_{254} (Merck 5554) and RP-18 F_{254s} (Merck 5560) precoated plates were used. Silica gel 60 (0.063−0.200 mm, Merck), Sephadex-LH 20 (25−100 μm, Sigma-Aldrich), and LiChroprep RP-18 (25−40 μm, Merck) were used for both column chromatography and MPLC. TLC plates were developed by spraying with 20% H_2SO_4, followed by heating at 120 °C.

Plant Material. *Cephalaria scoparia* was collected from Antalya-Elmalı, about 1200 m in altitude, in August 2007, and identified by H. Sümbül and R. S. Göktürk (Department of Biology, Faculty of Art and Science, Akdeniz University). A voucher specimen (No. R. S. Göktürk 6094) has been deposited at the Herbarium Research and Application Centre of Akdeniz University.

Extraction and Isolation. Air-dried and powdered aerial parts of *C. scoparia* (1.63 kg) were extracted with MeOH (3 × 2 L) at room temperature. The extract was concentrated to dryness at ca. 40 °C (305 g). Then, a portion (100 g) of the obtained brown syrup was extracted (3 × 100 mL) with *n*-BuOH−H_2O (1:1). Each extraction step was carried out overnight. The dried *n*-BuOH phase (37 g) was extracted with hexane (10 × 50 mL) to remove chlorophyll and oily substances and was fractionated by RP-MPLC (LiChroprep RP-18; 49 mm × 230 mm, MeOH−H_2O, 0:100 → 100:0% MeOH, flow rate 20 mL/min). At the end of the column elution, 11 fractions were obtained, and fractions 8 and 9 (20% H_2O and 10% H_2O) were combined and subjected to column chromatography over silica gel eluted with $CHCl_3$−MeOH−H_2O (90:10:1 to 61:32:7 + 20% MeOH), to yield 31

Table 1. ^{1}H NMR Spectroscopic Data for Compounds **1**−**5**[a]

position	**1**	**2**	**3**	**4**	**5**
3	2.95, m	3.01, m	3.00, m	2.99, m	3.02, m
5	0.76, s	0.66, s	0.74, s	0.74, s	0.76, s
9	1.48, m	1.46, m	1.47, m	1.48, m	1.44, m
12	5.14, brs	5.14, brs	5.14, brs	5.14, brs	5.01, brs
23	0.91, s	0.93, s	0.93, s	0.93, s	0.93, s
24	0.72, s	0.74, s	0.74, s	0.74, s	0.75, s
25	0.84, s	0.84, s	0.84, s	0.84, s	0.84, s
26	0.66, s	0.67, s	0.67, s	0.67, s	0.71, s
27	1.04, s	1.05, s	1.06, s	1.06, s	1.03, s
29	0.84, s	0.84, s	0.85, s	0.86, s	0.81, s
30	0.84, s	0.84, s	0.84, s	0.85, s	0.84, s
	Ara at C-3	Glc at C-3	Ara at C-3	Ara at C-3	Ara at C-3
1	4.28, d (5.6)	4.24, d (7.6)	4.26, d (4.2)	4.26, d (4.2)	4.24, d (6.6)
2	3.52, m	3.53, m	3.54, m	3.40, m	3.48, m
3	3.66, m	3.50, m	3.50, m	3.54, m	3.58, m
4	3.57, m	3.54, m	3.26, m	3.10, m	3.66, m
5	3.28, 3.30, m	3.14, m	3.10, 3.87, m	3.40, 3.58, m	3.26, 3.36, m
6		3.38, 3.63, m			
	Glc at C-28	Rha	Rha	Rha	Rha
1	5.21, d (8.0)	5.01, brs	5.11, brs	5.14, brs	5.21, brs
2	3.54, m	3.78, m	3.02, m	3.78, m	3.75, m
3	3.02, m	3.62, m	3.60, m	3.59, m	3.60, m
4	3.07, m	3.40, m	3.40, m	3.41, m	3.40, m
5	3.11, m	3.55, m	3.57, m	3.56, m	3.54, m
6	3.58, m 3.88, d (10.8)	1.07, d (6.4)	1.08, d (6.6)	1.08, d (6.0)	1.08, d (6.0)
	Rha	Xyl	Xyl	Xyl	Xyl
1	5.01, brs	4.31, d (7.6)	4.31, d (7.6)	4.27, d (7.2)	4.26, d (7.8)
2	3.18, m	3.09, m	3.12, m	3.54, m	3.02, m
3	3.43, m	3.10, m	3.20, m	3.09, m	3.08, m
4	3.11, m	3.05, m	3.11, m	3.12, m	3.64, m
5	3.62, m	3.08, 3.70, m	3.06, 3.70, m	2.96, 3.66, m	2.92, 3.65, m
6	1.05, d (6.4)				
	Glc′	Xyl′	Xyl′	Glc at C-28	
1	4.19, d (8.0)	4.26, d (7.6)	4.31, d (7.6)	5.21, d (8.4)	
2	2.93, m	3.80, m	2.96, m	3.52, m	
3	3.31, m	3.42, m	3.28, m	3.18, m	
4	3.35, m	3.27, m	3.30, m	3.08, m	
5	3.25, m	3.36, 3.66, m	3.35, 3.64, m	3.11, m	
6	3.40, 3.55, m			3.46, m, nd	
		Glc′ at C-28	Glc		
1		5.20, d (8.0)	4.22, d (7.6)		
2		3.10, m	3.04, m		
3		3.28, m	3.12, m		
4		3.22, m	3.02, m		
5		3.32, m	3.15, m		
6		3.77, 3.92, m	3.57, 3.72, m		
		Glc″	Glc′ at C-28		
1		4.45, d (7.6)	5.21, d (7.8)		
2		3.00, m	3.26, m		
3		3.23, m	3.32, m		
4		3.04, m	3.09, m		
5		3.16, m	3.10, m		
6		3.44, 3.68, m	nd, 3.9, m		
			Glc″		
1			4.18, d (8.0)		
2			2.93, m		
3			3.02, m		
4			3.20, m		
5			3.09, m		
6			3.42, 3.62, m		

[a] ^{1}H NMR data (δ) were measured in DMSO-d_6 at 400 MHz for **1** and **2** and at 600 MHz for **3**−**5**. Coupling constants (J) in Hz are given in parentheses. The assignments are based on DEPT, COSY, TOCSY, NOESY, HMQC, and HMBC experiments. nd: not detected.

further fractions (Frs. 1−31). Frs. 17, 21, 27, and 30 afforded compounds **1** (89 mg), **2** (203 mg), **3** (80 mg), and a known bisdesmosidic triterpene glycoside, lycicoside-II (186 mg). Fr. 25 was chromatographed by column chromatography using as solvent system $CHCl_3$−MeOH−H_2O (61:32:7), to yield compound **4** (13.7 mg). Fr. 10 and Fr. 11 were combined and purified by silica gel column chromatography using $CHCl_3$−MeOH−H_2O (90:10:1) as solvent system, yielding compound **5** (15.3 mg).

Scoposide A (1): white, amorphous powder; $[\alpha]^{26}_D$ −3.3 (*c* 1.8, MeOH); IR (KBr) ν_{max} 3434, 2950, 1728, 1634, 1460, 1390, 1280, 1076 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see Tables 1 and 2, respectively; negative-ion ESIMS *m*/*z* 1057.6 $[M - H]^-$; negative-ion HRESIMS *m*/*z* 1057.6870 $[M - H]^-$ (calcd for $C_{53}H_{101}O_{20}$, 1057.6892).

Scoposide B (2): white, amorphous powder; $[\alpha]^{26}_D$ −4.5 (*c* 2.0, MeOH); IR (KBr) ν_{max} 3434, 2945, 1725, 1634, 1457, 1389, 1314, 1256, 1074 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m*/*z* 1375.9 $[M + Na]^+$; positive-ion HRESIMS *m*/*z* 1375.6566 $[M + Na]^+$ (calcd for $C_{64}H_{104}O_{30}Na$, 1375.6505).

Scoposide C (3): white, amorphous powder; $[\alpha]^{26}_D$ −2.8 (*c* 1.6, MeOH); IR (KBr) ν_{max} 3400, 2946, 2881, 1726, 1638, 1387, 1257,

Table 2. ^{13}C NMR Spectroscopic Data for Compounds **1**−**5**[a]

position	1	2	3	4	5
1	39.0	39.1	38.9	38.8	39.1
2	26.2	26.5	26.2	27.6	26.4
3	88.5	88.5	88.3	88.3	88.6
4	39.4	39.4	39.2	39.1	39.3
5	55.8	55.9	55.7	55.7	56.0
6	18.4	18.4	23.0	22.0	18.4
7	33.0	33.0	33.0	32.1	33.2
8	39.7	39.7	39.5	39.4	40.5
9	47.8	47.8	47.6	47.6	47.8
10	37.0	37.0	36.8	36.8	36.8
11	23.6	23.6	23.8	26.2	23.9
12	122.3	122.3	122.1	122.1	120.4
13	144.2	144.2	144.0	143.9	146.2
14	42.0	42.0	41.8	41.7	42.1
15	27.9	27.9	27.7	29.4	28.1
16	23.4	23.2	23.4	23.4	23.4
17	46.2	46.3	46.0	46.0	46.1
18	41.4	41.4	41.2	41.2	41.9
19	46.7	46.6	46.4	46.4	47.4
20	31.0	31.0	30.7	30.8	31.1
21	33.9	33.9	33.7	33.7	34.7
22	32.4	32.3	32.7	32.7	33.3
23	28.1	28.1	27.8	27.8	27.9
24	16.9	17.0	16.8	16.8	16.8
25	15.9	15.9	15.7	15.7	15.7
26	17.4	17.4	17.1	17.1	17.6
27	26.2	26.2	25.9	25.9	26.1
28	176.0	176.0	175.7	175.7	181.4
29	33.4	33.5	33.2	33.2	33.8
30	24.1	24.1	23.9	23.8	24.1
	Ara at C-3	Glc at C-3	Ara at C-3	Ara at C-3	Ara at C-3
1	104.4	102.3	104.5	104.5	104.8
2	72.7	73.7	74.3	72.7	70.5
3	71.1	77.1	76.7	74.3	73.6
4	68.0	74.5	67.9	69.9	65.4
5	64.3	77.7	63.7	61.1	63.5
6		62.0			
	Glc at C-28	Rha	Rha	Rha	Rha
1	94.8	100.4	100.2	100.1	99.9
2	75.3	70.6	70.6	70.4	70.6
3	77.4	81.8	81.6	81.6	81.8
4	70.6	71.6	71.4	71.4	71.6
5	77.5	68.7	68.4	68.4	68.7
6	68.5	18.4	18.2	18.2	18.2
	Rha	Xyl	Xyl	Xyl	Xyl
1	100.7	106.1	106.0	106.3	106.6
2	69.9	73.0	74.2	74.1	74.5
3	71.1	74.3	77.0	76.8	77.1
4	73.0	76.9	71.1	70.0	70.9
5	69.0	64.1	66.3	66.2	66.4
6	18.5				
	Glc′	Xyl′	Xyl′	Glc at C-28	
1	103.7	104.8	100.8	94.6	
2	74.2	71.9	73.7	73.6	
3	77.4	75.0	75.6	77.1	
4	71.2	68.1	79.6	72.9	
5	77.3	65.2	65.0	78.2	
6	61.6			60.7	
		Glc′ at C-28	Glc		
1		94.8	104.0		
2		71.3	73.1		
3		75.1	77.2		
4		69.8	70.4		
5		77.0	77.3		
6		68.8	60.6		
		Glc″	Glc′ at C-28		
1		101.8	94.5		
2		73.4	74.7		
3		77.1	76.8		
4		70.8	72.7		
5		77.1	77.1		
6		61.8	68.3		
			Glc″		
1			103.5		
2			73.9		
3			77.3		
4			69.8		
5			78.2		
6			61.4		

[a] ^{13}C NMR data (δ) were measured in DMSO-d_6 at 100 MHz for **1** and **2** and at 150 MHz for **3**−**5**. The assignments are based on DEPT, COSY, TOCSY, NOESY, HMQC, and HMBC experiments.

1158, 1060 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m/z* 1507.7 $[M + Na]^+$; positive-ion HRESIMS *m/z* 1507.7011 $[M + Na]^+$ (calcd for $C_{69}H_{112}O_{34}Na$, 1507.6933).

Table 3. Antimicrobial Activity Results for Compounds **1**−**5** by the MIC Method (μg/mL)

microorganism	1	2	3	4	5	gentamycin
S. aureus	128	256	64	64	32	1
S. epidermidis	64	128	64	32	16	1
S. typhimurium	128	64	64	128	32	1
E. coli	8	128	128	32	16	1
B. cereus	16	16	32	32	16	4
K. pneumonie	64	64	64	32	64	4
E. faecalis	32	64	32	32	16	16
P. aeruginosa	64	64	128	64	16	2

Scoposide D (4): white, amorphous powder; $[\alpha]^{26}_D$ −2.4 (*c* 1.7, MeOH); IR (KBr) ν_{max} 3423, 2918, 2852, 1723, 1643, 1468, 1388, 1353, 1270, 1048, 1026 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m/z* 1051.6 $[M + Na]^+$; positive-ion HRESIMS *m/z* 1051.5402 $[M + Na]^+$ (calcd for $C_{52}H_{84}O_{20}Na$, 1051.5448).

Scoposide E (5): white, amorphous powder; $[\alpha]^{26}_D$ −4.0 (*c* 1.5, MeOH); IR (KBr) ν_{max} 3305, 2925, 2852, 1732, 1671, 1460, 1389, 1287, 1125, 1073, 1054 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Tables 1 and 2, respectively; negative-ion ESIMS *m/z* 865.6 $[M - H]^-$; negative-ion HRESIMS *m/z* 865.4938 $[M - H]^-$ (calcd for $C_{46}H_{73}O_{15}$, 865.4949).

Alkaline Hydrolysis. Pure compounds **1**−**5** (10−20 mg each) were refluxed in 5% KOH solution (pH 12−13) at 80 °C for 1 h. The reaction mixtures were neutralized with 5% HCl solution and then concentrated to dryness.[15] The residues were extracted with *n*-BuOH, and the organic layers of pure compounds were analyzed by NMR spectroscopy. Hydrolysis of **1** afforded a known triterpene glycoside,[14] while **2**−**4** produced three new prosapogenins, compounds **2a**−**4a**, while compound **5** was not affected by alkaline hydrolysis.

Sugar Analysis. The sugar analysis of the glycosides was performed using a microhydrolysis technique on a TLC plate and GC-MS analysis with authentic samples.[8,16] Each compound (5 mg) was hydrolyzed with 1 N HCl (2 mL) for 6 h at 90 °C. After extraction with $CHCl_3$ (3 × 5 mL), the aqueous layer was evaporated to dryness and then analyzed by TLC over silica gel ($CHCl_3$−MeOH−H_2O−gAcOH, 16:9:2:2) by comparison with authentic samples. Furthermore, the residue of the sugars was dissolved in anhydrous pyridine (1 mL), and then 1 mL of HMDS−TMCS (hexamethyldisilazane−trimethylchlorosilane, 1:1) was added, and the mixture was stirred at 70 °C for 1 h. The mixture was concentrated under N_2 stream, solved with *n*-hexane (1 μL), and analyzed by GC-MS.[8] L-Arabinose, L-rhamnose, D-xylose, and D-glucose were detected by co-injection of the hydrolysate with standard silylated sugars. Identification of L-arabinose, L-rhamnose, and D-glucose was detected for **1**, giving peaks at 14.75, 15.16, and 30.64 min, respectively. In turn, peaks at 15.18, 18.28, and 30.67 min from **2**, for L-rhamnose, D-xylose, and D-glucose, peaks at 14.75, 15.15, 18.26, and 30.64 min from **3** and at 14.74, 15.15, 18.25, and 30.64 min from **4**, for L-arabinose, L-rhamnose, D-xylose, and D-glucose, and at 14.75, 15.15, and 18.26 min from **5**, for L-arabinose, L-rhamnose, and D-xylose were obtained.

3-*O*-*β*-D-Xylopyranosyl-(1→4)-*β*-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosylolean-12-en-28-oic acid (2a): white, amorphous powder; 17.5 mg; $[\alpha]^{26}_D$ − 1.1 (*c* 1.8, MeOH); ^1H NMR (DMSO-d_6, 400 MHz) aglycon, δ_H 3.03 (1H, m, H-3), 0.66 (1H, s, H-5), 1.47 (1H, m, H-9), 5.15 (1H, s, H-12), 0.94 (3H, s, H-23), 0.75 (3H, s, H-24), 0.85 (3H, s, H-25), 0.70 (3H, s, H-26), 1.06 (3H, s, H-27), 0.85 (3H, s, H-29), 0.85 (3H, s, H-30); sugars, δ_H 4.25 (1H, d, *J* = 7.6 Hz, H-1 of Glc), 5.09 (1H, brs, H-1 of Rha), 4.30 (1H, d, *J* = 7.6 Hz, H-1 of Xyl), 4.26 (1H, d, *J* = 7.6 Hz, H-1 of Xyl′); negative-ion HRESIMS *m/z* 1027.5471 $[M - H]^-$ (calcd for $C_{52}H_{83}O_{20}$, 1027.5483).

3-*O*-*β*-D-Glucopyranosyl-(1→4)-*β*-D-xylopyranosyl-(1→3)[-*β*-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosylolean-12-en-28-oic acid (3a): white, amorphous powder; 6.9 mg; $[\alpha]^{26}_D$ −2.9 (*c* 0.7, MeOH); ^1H NMR (DMSO-d_6, 400 MHz) aglycon, δ_H 3.01 (1H, m, H-3), 0.74 (1H, s, H-5), 1.47 (1H, m, H-9), 5.23 (1H, brs, H-12), 0.95 (3H, s, H-23), 0.77 (3H, s, H-24), 0.85 (3H, s, H-25), 0.71 (3H, s, H-26), 1.08 (3H, s, H-27), 0.86 (3H, s, H-29), 0.85 (3H, s, H-30); sugars, δ_H 4.28 (1H, d, *J* = 4.2 Hz, H-1 of Ara), 5.06 (1H,

brs, H-1 of Rha), 4.33 (1H, d, $J = 7.6$ Hz, H-1 of Xyl), 4.33 (1H, d, $J = 7.6$ Hz, H-1 of Xyl′), 4.24 (1H, d, $J = 7.6$ Hz, H-1 of Glc); negative-ion HRESIMS *m/z* 1159.5937 $[M - H]^-$ (calcd for $C_{57}H_{91}O_{24}$, 1159.5906).

3-*O*-*β*-D-Xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-arabinopyranosylolean-12-en-28-oic acid (4a): white, amorphous powder; 1.8 mg; $[\alpha]^{26}_D$ −4.0 (*c* 0.2, MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) aglycon, δ_H 3.01 (1H, m, H-3), 0.76 (1H, s, H-5), 1.46 (1H, m, H-9), 5.17 (1H, brs, H-12), 0.96 (3H, s, H-23), 0.78 (3H, s, H-24), 0.86 (3H, s, H-25), 0.72 (3H, s, H-26), 1.09 (3H, s, H-27), 0.86 (3H, s, H-29), 0.86 (3H, s, H-30); sugars, δ_H 4.28 (1H, d, $J = 4.2$ Hz, H-1 of Ara), 5.13 (1H, brs, H-1 of Rha), 4.29 (1H, d, $J = 7.2$ Hz, H-1 of Xyl); negative-ion HRESIMS *m/z* 865.4932 $[M - H]^-$ (calcd for $C_{46}H_{73}O_{15}$, 865.4955).

Antimicrobial Assay. In vitro antibacterial activities were investigated using a microdilution assay.[17] Four Gram-negative [*Escherichia coli* (ATCC 23999), *Pseudomonas aeroginosa* (ATCC 27853), *Salmonella thyphimirium* (CCM 5445), *Klebsiella pneumonie* (CCM 2318)] and four Gram-positive [*Staphylococcus aureus* (ATCC 6538-P), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (ATCC 7064), *Enterococcus faecalis* (ATCC 29212)] bacterial strains were used in the assays. The bacterial strains were inoculated on Mueller−Hinton broth (Difco) and incubated for 24 h at 37 ± 0.1 °C. The inocula were from 24 h broth cultures, and suspensions were adjusted to 0.5 McFarland standards and diluted 1:100 (v/v) in Mueller−Hinton broth. Dilution series of the compounds were prepared in test tubes, then transferred to the broth in 96-well microtiter plates. Final concentrations were 256 to 0.5 μg/mL in the medium. The last well, containing 100 μL of nutrient broth without compounds and 10 μL of the inocula on each strip, was used as a negative control. All plates were covered with a sterile plate sealer and incubated at 37 °C for 24 h. The MIC is defined as the lowest concentration that appeared clear against a black background (no visible growth). Samples from clear wells were subcultured by plotting onto Mueller−Hinton agar. Gentamycin (Sigma) was used as the positive control. Dilutions were prepared from 128 to 0.25 μg/mL concentrations in microtiter plates. All of the assays were performed in triplicate.

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Supporting Information Available: 1D and 2D NMR of compounds **1**−**5** and HRESIMS of compounds **1**−**5** and prosaponins **2a**−**4a** are available free of charge via the Internet at http://pubs.acs.org.

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