Thalicosides A1−A3, Minor Cycloartane Bisdesmosides from *Thalictrum minus*

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Three new cycloartane bisdesmosides, two of which are based on a new genin, were isolated from the above-ground parts of *Thalictrum minus*. Thalicosides A1−A3 (**1**−**3**) were characterized as 3-*O*-*β*-D-galactopyranosyl-29-*O*-*β*-D-glucopyranosyl-3*β*,16*β*,29-trihydroxy-22(*S*),25-epoxycycloartane (**1**); 3-*O*-α-L-arabinopyranosyl-29-*O*-*β*-D-glucopyranosyl-3*β*,16*β*,29,22(*S*)-tetrahydroxycycloart-24-ene (**2**); and 3-*O*-α-L-arabinopyranosyl-29-*O*-*β*-D-glucopyranosyl-3*β*,16*β*,29-trihydroxy-22(*S*),25-epoxycycloartane (**3**), respectively. The structural assignments of these new compounds were based on interpretation of spectroscopic data. Thalicoside A2 showed in vitro inhibition of the fungus *Candida albicans* and also activity against *Staphylococcus aureus*.

Previous studies on the chemical constituents of *Thalictrum minus* have resulted in the isolation of eight cycloartane and oleanane glycosides, some of which possess potential antitumor and contraceptive activities.[1] In this paper, we report the isolation and structure determination of three minor cycloartane bisdesmosides, two of which are based on a new genin, from the above-ground parts of *T. minus* L. (Ranunculaceae), collected in eastern Siberia (Russian Federation). Saponins A1−A3 are bisdesmosides and contain identical polycyclic fragments (rings A, B, C, and D). Thalicosides A1 (**1**) and A3 (**3**) differ only in the nature of the sugar units linked to C-3 of the genin. Compounds **2** and **3** have the same sugar moieties, but their genins differ in the fact that **2** has a secondary hydroxyl group at C-22 and a double bond at C-24.

Results and Discussion

Glycosides A1−A3 (**1**−**3**) were isolated and purified from the crude saponin mixture obtained from *T. minus*, as described in the Experimental Section. The ^{1}H and ^{13}C NMR signals of compounds **1**−**3** were assigned using COSY, ROESY, DEPT, and HMBC spectra. The carbon resonances were associated with the corresponding proton signals using ^{1}H−^{13}C HETCOR experiments, and the results are shown in Table 1.

The IR spectra of compounds **1**−**3** contained the following prominent absorption bands: 3400−3411 cm^{-1} (hydroxyl group); 3043 cm^{-1} (CH_2 of cyclopropane group); 2940, 2888 cm^{-1} (aliphatic CH); 1122 cm^{-1} (C−O−C group), and 1054 cm^{-1} (C−O of secondary, alicyclic alcohol).

The molecular formula of thalicoside A1 (**1**) was determined to be $C_{42}H_{70}O_{14}$ by HRFABMS and ^{13}C NMR. In addition to the quasimolecular ion at *m/z* 797 [M − H]$^-$, the negative FABMS of **1** showed prominent peaks at *m/z* 635 [M − 163]$^-$, corresponding to the loss of a hexose from the parent molecular ion, and at *m/z* 473 [M − 325]$^-$, representing the loss of two hexose units.

There were two anomeric protons observed in the ^{1}H NMR spectrum of **1**, with shift values of *δ* 5.34 (d, *J* = 7.8 Hz) and 5.50 (d, *J* = 7.8 Hz), with corresponding ^{13}C NMR shifts of *δ* 106.77 and 105.74. These data suggested that thalicoside A1 (**1**) consists of a triterpenoid skeleton with a molecular weight of 474 Da and two residues of hexoses. Acid hydrolysis of **1** produced a previously unreported genin, thalicogenin A1 (**1a**), and two sugars, galactose and glucose, as determined by TLC analysis. A molecular weight of 474.3709 Da for **1a** was determined by HREIMS, corresponding to the molecular formula $C_{30}H_{50}O_4$. This formula suggests six degrees of unsaturation, implying six saturated rings in **1a**. The ^{1}H NMR spectrum of **1a** showed three geminal methine proton signals and a signal from one methylene group on an oxygen-bearing carbon atom (H-3, H-16, H-22 and 2H-29, respectively). The resonances for the oxygenated carbons are consistent with the presence of three oxymethine and one oxymethylene carbons [*δ* 73.37 (C-3), *δ* 71.30 (C-16), *δ* 82.19 (C-22), and *δ* 66.90 (C-29)], and one oxygenated quaternary carbon [*δ* 80.12 (C-25)]. These data are consistent with a genin having one primary and two secondary hydroxyl groups and a 22,25 epoxy-ring side chain.

A careful comparison of the ^{13}C NMR spectrum of **1a** [C-30 (*δ* 11.09), C-29 (*δ* 69.90), and C-3 (*δ* 73.38)] with those of thalicogenin A [C-30 (*δ* 11.4), C-29 (*δ* 68.5), C-3 (*δ* 74.0)][2] and squarrogenin I [C-30 (*δ* 64.5), C-29 (*δ* 18.4), C-3 (*δ* 80.1)][3] indicated the presence of a secondary hydroxyl group at the 3*β*-position and a primary hydroxyl group at the 4α-position. The ^{1}H and ^{13}C NMR signals of the five-membered ring D of **1a** [C-15 (*δ* 2.04, 1.68, and 47.86), C-16 (*δ* 4.61 and 71.30)] indicated the presence of an additional secondary hydroxyl group on the C-16*β* position.[4] The presence of a cross-peak between Hα-16 and Hα-17 and between Hα-16 and CH_3-28 in the ROESY spectrum of **1a** confirmed the assigned configuration of the hydroxyl group at C-16*β*.

The ^{1}H NMR spectrum of **1a** showed signals at *δ* 0.39 and 0.65 (d, *J* = 4.0 Hz, H-19a and H-19b), characteristic of a methylene proton AX system on a cyclopropane ring. The epoxy ring exhibited two tertiary methyl groups at C-25 [(δ_H 1.19 and 1.28) and (δ_C 27.35 and 28.31)]. The configuration at the C-22 chiral center of the genin was

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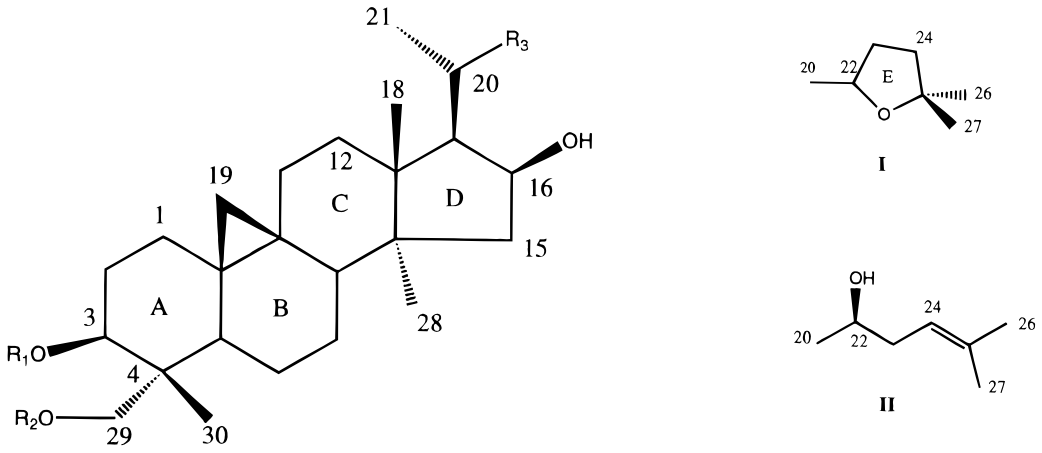

1	R_1 = β-D-galactopyranoside	R_2 = β-D-glucopyranoside	R_3 = I	(Thalicoside A1)
1a	R_1 = H	R_2 = H	R_3 = I	(Thalicogenin A1)
2	R_1 = α-L-arabinopyranoside	R_2 = β-D-glucopyranoside	R_3 = II	(Thalicoside A2)
3	R_1 = α-L-arabinopyranoside	R_2 = β-D-glucopyranoside	R_3 = I	(Thalicoside A3)

Figure 1.

determined by comparing the ^{13}C NMR chemical shift of **1a** with that of an artifact of thalicogenin,[5] for which a 22-(*S*)-configuration was determined by X-ray analysis.[6] The ^{13}C NMR chemical shifts for both compounds were similar for rings D and E, and, consequently, **1a** has been assigned a 22(*S*) configuration. Hence, the structure of the new genin, called thalicogenin A1 (**1a**), is 3*β*,16*β*,29-trihydroxy-22(*S*),25-epoxycycloartane.

The observed ^{1}H and ^{13}C NMR spectra supported the assignment of the terminal sugar moieties in **1** (Table 1) as *β*-D-galactopyranoside and *β*-D-glucopyranoside. The *β*-configuration at the anomeric centers was confirmed by the large *J* coupling (7.8 Hz for H-1 of both sugar units). The low-field ^{13}C NMR chemical shifts of the C-3 and C-29 signals (*δ* 81.75 and *δ* 71.57) supported the conclusion that **1** is a bisdesmosidic glycoside. The attachment sites of the sugar residues were determined by HMBC experiments; cross-peaks between H-1 of the galactopyranoside (*δ* 5.50) and C-3 of the genin (*δ* 81.75) and between H-1 of the glucopyranoside (*δ* 5.34) and C-29 of the genin (*δ* 71.57) indicated that the galactose and glucose units are linked to C-3 and C-29, respectively, of the aglycon. ROESY cross-peaks found between H-1 of the galactopyranoside (*δ* 5.50) and H-3 of the genin (*δ* 4.46) and between H-1 of the glucopyranoside (*δ* 5.34) and 2H-29 of the genin (*δ* 4.09, 4.42) confirmed the linkage point for the two carbohydrates to the genin. Hence, **1** was established as 3-*O*-*β*-D-galactopyranosyl-29-*O*-*β*-D-glucopyranosyl-3*β*,16*β*,29-trihydroxy-22(*S*),25-epoxycycloartane (**1**).

The molecular weight of thalicoside A2 (**2**) was determined by HRFABMS and corresponds to a molecular formula $C_{41}H_{68}O_{13}$. This molecular formula indicated that compound **2** contains seven saturated ring systems, five of which are associated with the genin. The negative ion FABMS of thalicoside A2 gave a quasimolecular ion peak at *m/z* 767 $[M - H]^-$, as well as prominent peaks at *m/z* 635 $[M - 133]^-$ and *m/z* 605 $[M - 163]^-$ due to the loss of a pentose or hexose, respectively, from the parent molecular ion, and at *m/z* 473 $[M - (133 + 163) + H]^-$, representing an additional loss of a pentose or a hexose. These data suggested that **2** is a bisdesmoside containing a pentose and a hexose, not connected in sequence, but attached to the genin at different carbons.

A comparison of the ^{1}H and ^{13}C NMR spectra of compounds **2** and **1** showed that the chemical shifts of both the hydrogen and carbon atoms in the polycyclic portion (rings A−D) of the genin are very similar. This strongly suggested that the structure of this fragment is the same for the two compounds, and that the structures of **1** and **2** only differ in the nature of the side chains attached to the C-17 of the genin. A detailed comparison of the NMR spectra of glycoside **2** with that of thalicogenin[2] revealed them to be identical.

The absolute configuration of the C-22 chiral center was determined by comparing the ^{13}C NMR spectral data of compound **2** with that of the known compounds, senexdiolic acid, diol-10,[7] and thalicogenin.[2] The C-22 atom of senexdiolic acid and thalicogenin has an *S*-configuration and resonates in the range *δ* 73.3−75.8. In contrast, the C-22 atom of diol-10 has the *R*-configuration and resonates at *δ* 70.8. The C-22 atom of **2** has a chemical shift of 75.23, and thus it was assigned the *S* configuration.

The ^{1}H and ^{13}C NMR spectral data of the carbohydrate part of **2** (Table 1) suggested the terminal sugar units to be α-L-arabinopyranoside and *β*-D-gucopyranoside. The coupling constants (J = 7.4 Hz and J = 8.0 Hz) of the anomeric centers of α-L-arabinopyranoside and *β*-D-glucopyranoside supported this assignment. The cross-peaks in the HMBC spectrum between H-1 of the arabinopyranoside (*δ* 5.38) and C-3 of the genin (*δ* 81.11) and between H-1 of the glucopyranoside (*δ* 5.28) and C-29 of the genin (*δ* 71.14) and the corresponding cross-peaks in the ROESY spectrum confirmed that the arabinopyranoside residue is attached to C-3 of the genin and the glucopyranoside residue to C-29 of the genin. Hence, **2** was assigned as 3-*O*-α-L-arabinopyranosyl-29-*O*-*β*-D-glucopyranosyl-3*β*,-16*β*,29,22(*S*)-tetrahydroxycycloart-24-ene.

A molecular weight of 791.457 Da for thalicoside A3 (**3**) was determined by HRFABMS, corresponding to the quasi-molecular ion $C_{41}H_{68}O_{13}Na$. The fragmentation in the negative ion FABMS of **3** was similar to that of **2**, suggesting that compound **3** is a bisdesmoside containing a pentose and a hexose. The molecular weight of the genin of **3** is 474 Da. The ^{1}H and ^{13}C NMR spectra of this genin were identical with those of the corresponding genin for **1** (Table 1). On the basis of the foregoing data, the genin of glycoside **3** was established as thalicogenin A1 (**1a**).

The ^{1}H and ^{13}C NMR spectra of the carbohydrate portion of **3** are the same as that of **2** (Table 1). COSY spectral data confirmed this conclusion by showing that

Table 1. NMR Data for Thalicosides A1−A3 (**1**−**3**) (125 and 500 MHz, C_5D_5N)

	thalicoside A1 (**1**)		thalicoside A2 (**2**)		thalicoside A3 (**3**)	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	32.29	0.98,[a] 1.12[a]	32.06	1.12,[a] 1.26[a]	32.42	[b]
2	29.80	2.02,[a] 2.44	29.51	2.02,[a] 2.39[a]	29.85	2.02,[a] 2.38[a]
3	81.75	4.46 dd (4.9, 1.1)	81.11	4.48 dd (4.3, 11.5)	81.43	4.47 dd (4.5, 11.6)
4	45.25		45.86		45.30	
5	40.89	2.00 dd (4.1, 11.1)	40.67	2.00[a]	41.03	2.08[a]
6	20.95	0.61 dddd (2.3, 11.1, 12.2, 12.5), 1.78[a]	20.65	0.64 dddd (2.1, 12.2, 12.7, 12.9), 1.82[a]	20.98	0.71 dddd (1.7, 11.5, 11.6, 12.3), 1.87[a]
7	26.63	0.96,[a] 1.11[a]	26.35	0.98,[a] 1.11[a]	26.69	[b]
8	48.52	1.54 dd (4.4, 12.5)	46.57	1.57 dd (4.3, 12.5)	48.83	1.61 dd (4.5, 12.5)
9	19.78		19.63		19.88	
10	25.93		25.70		26.01	
11	26.69	1.07 m, 1.80[a]	26.41	1.04,[a] 1.90[a]	26.69	1.05,[a] 1.87[a]
12	33.71	1.34,[a], 1.62[a]	33.55	1.51,[a] 1.59[a]	33.76	[b]
13	46.27		44.96		46.31	
14	47.28		47.23		47.30	
15	48.52	1.63,[a] 2.01	48.83	1.7, 2.04	48.56	1.67, 2.08
16	71.94	4.64 st (4.6, 7.8)	71.66	4.83 st (4.9, 7.3)	71.97	4.65 st (5.1, 7.4)
17	53.09	2.02[a]	52.97	2.36 dd (4.9, 11.0)	53.14	2.06
18	19.72	1.34 s	19.69	1.46 s	19.80	1.39 s
19	30.84	0.23 d (3.8), 0.43 d (3.8)	30.56	0.33 d (3.5), 0.54 d (3.5)	30.87	0.33 d (4.0), 0.53 d (4.0)
20	33.36	2.50 m	36.02	2.60 m	33.37	2.52 m
21	15.16	0.96 d (6.5)	14.55	1.21 d (7.0)	15.22	1.01 d (6.5)
22	82.80	4.30 dd (2.2, 7.8)	75.23	4.32 dd (5.0, 11.0)	82.86	4.31 m
23	27.66	1.76,[a] 1.79[a]	33.49	2.46 m, 2.58 m	27.66	1.88a, 1.88b
24	38.87	1.61a, 1.61b	123.86	5.60 br t (6.7)	38.89	1.63a, 1.63b
25	80.79		132.03		80.81	
26	28.99	1.26 s	18.07	1.66 br s	29.02	1.30 s
27	28.03	1.17 s	25.95	1.68 br s	28.02	1.21 s
28	20.84	0.83 s	20.56	0.89 s	20.87	0.88 s
29	71.57	4.09 d (9.8), 4.42 d (9.8)	71.14	4.11 d (10.2), 4.36 d (10.2)	71.44	4.11 d (9.7), 4.34 d (9.7)
30	11.59	0.92 s	11.67	0.94 s	12.00	0.96 s
Glc 1′	105.74	5.34 d (7.8)	105.13	5.28 d (8.0)	105.65	5.21 d (7.7)
2′	75.82	4.18 d (7.8)	75.36	4.14 br t (8.0)	75.74	4.12[a]
3′	78.96	4.27[a]	78.67	4.27[a]	79.07	4.24[a]
4′	72.09	4.24[a]	71.76	4.26[a]	72.15	4.23[a]
5′	78.73	4.03 m	78.39	4.0 ddd (2.5, 5.0, 9.0)	78.79	4.08 m
6′	62.72	4.41,[a] 4.52[a]	62.82	4.41 dd (2.5, 11.6), 4.52 dd (5.0, 11.6)	63.20	4.39 dd (5.2, 11.8), 4.52 dd (2.4, 11.8)
Gal 1″	106.77	5.50 d (7.8)				
2″	73.61	4.43[a]				
3″	75.87	4.27[a]				
4″	70.81	4.55[a]				
5″	76.67	4.33 m				
6″	63.15	4.43,[a] 4.53[a]				
Ara 1‴			106.43	5.38 d (7.4)	106.79	5.35 d (7.4)
2‴			73.19	4.45 d (7.4)	73.56	4.42 d (7.4)
3‴			74.90	4.25[a]	75.27	4.22[a]
4‴			70.01	4.26[a]	70.44	4.23[a]
5‴			66.99	4.05 dd (2.5, 11.9), 4.28 dd (4.0, 11.9)	67.35	4.02 br d (12.0), 4.29 dd (4.0, 12.0)

[a] Overlapping signals, determined by COSY, HETCOR, HMBC, and ROESY spectral data. [b] Obscured.

the spin systems of the carbohydrate residues in **3** and **2** are identical. Thus, the structure of thalicoside **3** is proposed as 3-*O*-α-L-arabinopyranosyl-29-*O*-*β*-D-glucopyranosyl-3*β*,16*β*,29-trihydroxy-22(*S*),25-epoxycycloartane.

Thalicosides A1−A3 were tested in assays to detect growth inhibition of a fungus (*Candida albicans*) and two bacteria strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). They were also tested for cytotoxicity against a human cervical cancer cell line (HeLa). Thalicoside A2 showed inhibitory activity against *C. albicans* (78.7%) and against *S. aureus* (45.7%), both at a concentration of 1 mg mL^{-1}.

Experimental Section

General Experimental Procedures. Melting points were measured on a Bristoline apparatus and are uncorrected. Optical rotation data were obtained using a Perkin-Elmer 241 polarimeter. IR spectra were measured on a Mattson Polaris spectrometer. ^1H and ^{13}C NMR, COSY, DEPT, HETCOR, HMBC, and ROESY spectra were recorded on a Varian VXR-500S spectrometer (^1H, 500 MHz; ^{13}C, 125 MHz) equipped with a SUN SPARC 20 workstation. Chemical shifts are given in *δ* (ppm); TMS was used as a reference. HRFABMS were obtained using a JEOL SX 102A mass spectrometer in the negative or positive ion mode using a glycerol or thioglycerol matrix. The target was bombarded with 6 kV Xe atoms.

Column chromatography was carried out using chromatographic Si gel 200−425 mesh (Fisher), and TLC experiments utilized Whatman Al SilG/UV. The following TLC and Si gel column chromatography solvent systems were used: $CHCl_3$−MeOH−H_2O [100:12:1 (1), 70:15:1 (2), 70:23:1 (3), 70:23:4 (4), and 80:36:7 (5)]. The spray reagent used for saponins was 0.5% vanilin in 50% H_3PO_4.

Plant Material. The whole plant of *T. minus* L. was collected at the time of its flowering (July) near the upper Kitoi river (a tributary of the Angara river, eastern Siberia, Russian Federation). A herbarium sample is deposited in the M. Popov Herbarium of the Siberian Institute of Plant Physiology and Biochemistry of the RAS, Irkutsk, Russian Federation.

Extraction and Isolation. The air-dried, above-ground parts of *T. minus* (4.5 kg) were exhaustively extracted with 80% aqueous MeOH. The MeOH was removed under reduced pressure. Fats and oils were removed by extracting the aqueous residue with $CHCl_3$. The defatted aqueous solution was exhaustively extracted using *n*-BuOH. The organic phase was removed and taken to dryness at a temperature not exceeding 50 °C, under reduced pressure. The residue (321 g) was fractionated over a Si gel column and eluted by solvent systems 1−5, in sequence. Fractions were monitored by TLC using solvent systems 3−5. The fraction eluted by solvent systems 3 and 4 gave 300 mg of nonpolar crude saponins. This fraction was further separated by column chromatography over Si gel to yield a mixture of **2** and **3** (fractions 8−15) and **1** and **2** (fractions 16−25). Fractions 8−15 were further separated by repeatedly passing over a Si gel column with systems 1 and 2 to yield pure compounds **2** (25 mg) and **3** (8 mg). Fractions 16−25 were separated with systems 2−4 to give pure compounds **1** (41 mg) and **2** (30 mg).

Thalicoside A1 (1): white needles (from system 3); mp 300−301 °C; $[\alpha]_D^{25}$ +3.6° (*c* 0.66, C_5H_5N); UV, no absorption above 210 nm; IR (KBr, disk) ν_{max} 3411, 3043, 2944, 2888, 1122, 1054 cm^{-1}; ^1H and ^{13}C NMR spectra, see Table 1; FABMS (negative ion mode) *m*/*z* 797 $[M - H]^-$, 635$[M - 163]^-$, 474 $[M - 325]^-$; HRFABMS *m*/*z* 797.4686 (calcd for $C_{42}H_{69}O_{14}$, 797.4687).

Thalicoside A2 (2): white platelets (system 2); mp 272−274 °C, $[\alpha]_D^{25}$ +10.6°(*c* 0.9, MeOH−$CHCl_3$,1:1); UV, no absorption above 210 nm; IR (KBr, disk) ν_{max} 3400, 3045, 2940, 2890, 1125, 1055 cm^{-1}; ^1H and ^{13}C NMR spectra, see Table 1; FABMS (negative ion mode) *m*/*z* 767 $[M - H]^-$, 635$[M - 133]^-$, 605 $[M - 163]^-$, 473 $[M - 295]^-$; HRFABMS *m*/*z* 767.4586 (calcd for $C_{41}H_{67}O_{13}$, 767.4582).

Thalicoside A3 (**3**) was obtained as white needles (system 1); mp 253−255 °C; $[\alpha]_D^{25}$ +1.1° (*c* 0.57, MeOH−$CHCl_3$,1:1); UV, no absorption above 210 nm; IR (KBr, disk) ν_{max} 3406, 3050, 2945, 2890, 1456, 1382, 1120, 1050 cm^{-1}; ^1H and ^{13}C NMR spectra, see Table 1; FABMS (negative ion mode) *m*/*z* 767 $[M - H]^-$, 635[$(M - 133]^-$, 605 $[M - 163]^-$, 473 $[M - 295]^-$; HRFABMS (positive ion mode) *m*/*z* 791.4565 (calcd for $C_{41}H_{68}O_{13}Na$, 791.4557).

Acid Hydrolysis 1−3. Solutions of compounds **1**−**3** (5 mg each) in 10% HCl (dioxane−H_2O, 1:1, 3 mL) were heated in a boiling water bath for 4 h. Each reaction mixture was extracted by $CHCl_3$. The aqueous layer was then passed through an Amberlite IRA-68 column and concentrated to 2 mL under reduced pressure. The residue was analyzed by Si gel TLC with $CHCl_3$−MeOH−H_2O (7:4:0.8) and compared with standard sugars. Glucose (R_f 0.23) and galactose (R_f 0.29) were identified from **1**, and arabinose (R_f 0.67) and glucose (R_f 0.23) were identified from **2** and **3**. In addition, 50 mg of saponin **1** in 10 mL 5% HCl in dioxane−H_2O (1:1) were heated at 80 °C for 6 h. After the dioxane was removed, the residue was extracted with $CHCl_3$ (1 mL × 3). The $CHCl_3$ portion was washed with H_2O, concentrated, and chromatographed on Si gel using $CHCl_3$ containing 1−5% MeOH as eluent to yield the aglycon **1a** (9 mg).

Thalicogenin A1 (1a): white prisms (from C_6H_6−Me_2CO, 3:1); mp 332−333 °C, $[\alpha]_D^{25}$ +7.1° (*c* 2.0, MeOH−$CHCl_3$, 1:1); UV, no absorption above 210 nm; IR (KBr, disk) ν_{max} 3410, 3043, 2940, 2893 cm^{-1}; ^1H NMR (C_5D_5N, 50 °C, 500 MHz) δ 4.61 (1H, m, J = 4.8, 7.5 Hz, H-16), 4.28 (1H, m, H-3 overlapped H-22), 4.26 (1H, m, H-22), 4.18 (1H, d, J = 10.5 Hz, Ha-29), 3.76 (1H, d, J = 10.5 Hz, Hb-29), 2.52 (1H, m, H-20), 2.08 (1H, H-17, overlapped H-2), 2.04 (1H, Ha-15, overlapped H-5), 1.68 (1H, Hb-15, overlapped 2H-12), 1.42 (3H, s, CH_3-18), 1.28 (3H, s, CH_3-26), 1.19 (3H, s, CH_3-27), 1.15 (3H, s, CH_3-30), 1.00 (3H, d, J = 7.0 Hz, CH_3-21), 0.94 (3H, s, CH_3-28), 0.65 (1H, d, J = 4.0 Hz, Ha-19), 0.39 (1H, d, J = 4.0, Hb-19); ^{13}C NMR (C_5D_5N, 125 MHz) δ 82.19 (C-22), 80.12 (C-25), 73.37 (C-3), 71.30 (C-16), 66.90 (C-29), 52.43 (C-17), 48.15 (C-8), 47.86 (C-15), 46.63 (C-14), 45.65 (C-13), 44.43 (C-4), 41.20 (C-5), 38.19 (C-24), 33.18 (C-12), 32.69 (C-20), 31.98 (C-1), 30.56 (C-2), 30.01 (C-19), 28.31 (C-26), 27.35 (C-27), 26.96 (C-23), 26.11 (C-7), 26.00 (C-11), 25.77 (C-10), 20.83 (C-6), 20.11 (C-28), 19.46 (C-9), 19.08 (C-18), 14.57 (C-21), 11.09 (C-30); EIMS (70 eV) *m*/*z* 474 (4), 441 (8), 329 (7), 173 (10), 159 (11), 126 (123), 99 (100), 81 (53); HREIMS *m*/*z* 474.3709 $[M]^+$ (calcd for $C_{30}H_{50}O_4$, 474.3709).

Biological Assays. Antimicrobial assays were carried out in triplicate utilizing 96-well microtiter plates. The plates were seeded with *C. albicans* (ATCC 90028) or *S. aureus* (ATCC 6538P) and the optical density (OD) measured at 600 nm using an automated spectrophotometric plate reader (CERES UV900 HDi). The drug solutions were added, and the plates were then incubated at 35 °C for 24 h and the OD again read. The difference between the initial and final OD readings as compared with the controls represented growth inhibition.[8] Cytotoxicity assays were conducted on HeLa cells (ATCC XC12) by the method of Skehan et al. using sulphorhodamine B to dye viable cells.[9]

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