Isolation and Structures of Avicins D and G: In Vitro Tumor-Inhibitory Saponins Derived from *Acacia victoriae*

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Two new saponins named avicins D (**1**) and G (**2**) were isolated from the seed pods of the desert legume plant *Acacia victoriae*. The structures, elucidated by 1D and 2D NMR studies and by chemical means, were characterized as acacic acid-bearing oligosaccharides at C-3 and C-28 with a side chain linked to C-21 comprised of two monoterpene carboxylic acids and a quinovose moiety. Both compounds exhibited potent cytotoxicity (apoptosis) against human T-cell leukemia (Jurkat cells) in vitro.

Efforts to identify plant compounds that may selectively induce apoptosis of cancer cells revealed that an aqueous MeOH extract of the seed pods of *A. victoriae* induced significant (IC_{50} 0.38 μg/mL) cell cycle arrest in Jurkat cells in vitro.[1] *Acacia victoriae* Benth. (Leguminosae) thrives in semiarid and arid lands, typically along dry waterways (rivers, streams, and arroyos) that are subject to intermittent rains and flooding, and tolerates relatively poor clay-like and sandy soils having potentially high salinity.[2] The traditional use of *Acacia victoriae* as a medicinal plant is not documented, whereas several related *Acacia* species have had a long history of medicinal use. *Acacia concinna* Wall. (Leguminosae) fruits, for example, have been used as a purgative, emetic, and expectorant.[3] Several acacic acid-based saponins with cytotoxic properties have been isolated from other *Acacia* species.[4−6] The present paper describes the isolation, structure elucidation, and evaluation of the biological activities of the most active components, designated avicin D (**1**) and avicin G (**2**), from the aqueous MeOH extract. Several prosaponins prepared from avicin D (**1**) by chemical hydrolysis were also tested for cytotoxicity.

Results and Discussion

The ground pods of *Acacia victoriae* were extracted with MeOH−H_2O (1:4). The extract was fractionated by C_{18} flash chromatography. The 75% MeOH−H_2O eluate (named F094), which had the most potent cytotoxicity against Jurkat cells in vitro, was shown by HPLC analysis to consist of a complex mixture of compounds. After further fractionation by C_{18} flash chromatography and preparative HPLC on a perfluorophenyl (PFP) column, the fraction F094 yielded avicins D and G as the most active components (Figure 1).

Avicin D (**1**) was obtained as a white amorphous solid and analyzed for $C_{98}H_{155}NO_{46}$ using HRFABMS and MALDI mass spectrometry data at *m/z* 2104 $[M + Na]^+$. The presence of 13 methyl groups, eight anomeric protons, five olefinic bonds, and four carbonyl groups in the ^1H and ^{13}C NMR spectra (Tables 1−3) of **1** suggested a triterpene saponin with two monoterpenoid units. Hydrolysis of **1** with MeOH−HCl (for composition analysis) and with butanol−HCl (for configurational analysis),[7] followed by GC/MS analysis of the trimethylsilyl ethers of the monosaccharides, confirmed the presence of D-xylose, L-rhamnose, L-arabinose, D-fucose, D-quinovose, D-glucose (two units), and D-*N*-acetylglucosamine. After mild alkaline hydrolysis (0.5 N NH_4OH for 1 h) of **1**, the hydrolysate was separated by C_{18} preparative HPLC to give the outer monoterpene **3** and prosaponin **4** (Figure 2). By NMR spectral analysis,

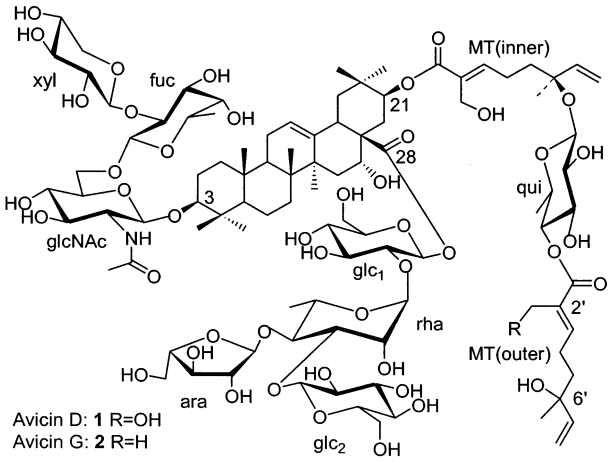

Figure 1. Structures of avicins D (**1**) and G (**2**).

Table 1. ^{13}C NMR Data in CD_3OD for the Aglycon Moieties of Avicin A (**1**) and Avicin G (**2**)

carbon	1	2	carbon	1	2
1	39.9	39.7	16	74.3	74.3
2	27.2	27.1	17	52.3	52.3
3	89.9	89.8	18	41.6	41.6
4	40.1	40.1	19	48.7	48.3
5	57.1	57.1	20	35.9	35.9
6	19.5	19.5	21	78.6	78.6
7	34.6	34.6	22	36.1	36.1
8	40.8	40.8	23	28.6	28.6
9	48.1	48.1	24	17.1	17.1
10	37.9	37.9	25	16.2	16.2
11	24.5	24.5	26	17.7	17.7
12	124.0	124.0	27	27.4	27.3
13	143.7	143.7	28	173.6	175.3
14	42.6	42.6	29	29.4	29.4
15	36.2	36.4	30	19.4	19.4

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Table 2. ^{13}C NMR Data in CD_3OD for the Monoterpene Units of Avicin A (**1**) and Avicin G (**2**)

	inner monoterpene		outer monoterpene	
carbon	**1**	**2**	**1**	**2**
1	168.2	169.0	168.7	168.7
2	132.5	128.5	132.9	132.9
3	148.0	145.9	148.5	148.0
4	24.3	24.3	24.5	24.6
5	41.3	41.3	41.9	41.3
6	81.0	81.0	73.6	73.6
7	144.0	144.4	145.9	144.0
8	116.0	116.0	112.5	112.4
9	56.6	56.6	56.5	12.5
10	23.8	23.7	27.9	27.8

Table 3. ^{13}C NMR Data in CD_3OD for Sugar Residues of Avicins **1** and **2**

carbon	**1**	**2**	carbon	**1**	**2**
qui-1	99.4	99.3	glc_1-1	95.3	95.3
qui-2	76.0	76.0	glc_1-2	81.0	81.3
qui-3	75.3	75.3	glc_1-3	78.6	78.7
qui-4	76.4	77.3	glc_1-4	71.5	71.7
qui-5	70.9	70.9	glc_1-5	75.6	75.7
qui-6	18.3	18.2	glc_1-6	62.3	62.2
glcNAc-1	104.8	104.8	rha-1	101.3	101.3
glcNAc-2	57.9	57.9	rha-2	71.2	71.2
glcNAc-3	76.4	76.4	rha-3	82.6	82.6
glcNAc-4	71.1	71.2	rha-4	78.2	78.6
glcNAc-5	77.7	77.1	rha-5	69.1	69.1
glcNAc-6	69.9	69.9	rha-6	18.6	18.6
glcNAc-Ac	173.3, 23.2	173.4, 23.3			
fuc-1	103.8	103.8	glc_2-1	105.8	105.8
fuc-2	82.3	82.3	glc_2-2	75.5	75.6
fuc-3	74.9	74.9	glc_2-3	77.6	78.2
fuc-4	72.7	72.7	glc_2-4	72.2	71.7
fuc-5	71.7	72.2	glc_2-5	77.5	77.5
fuc-6	16.7	16.7	glc_2-6	62.2	62.3
xyl-1	106.9	106.9	ara-1	111.0	111.0
xyl-2	75.5	75.7	ara-2	85.6	85.6
xyl-3	77.1	77.7	ara-3	79.1	79.1
xyl-4	71.1	71.1	ara-4	83.9	83.9
xyl-5	67.3	67.2	ara-5	63.1	63.1

the monoterpene **3** was identified as (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoic acid.[8] The *E* configuration of the double bond was confirmed by the proton chemical shift of H-3 (δ_H 6.92).[9,10] Due to degradation, the amount of **3** obtained was insufficient to determine the stereochemistry at the asymmetric center. The sugar analysis of prosaponin **4** indicated that the same eight sugars in **1** were still present and lacked one monoterpene, as evidenced by the loss of proton NMR signals for one unit of monoterpene and by FABMS. An HBMC correlation in **1** between the quinovose H-4 (δ_H, 4.63) and the carboxyl carbon (δ_C, 168.7) of the outer monoterpene suggested an ester linkage.

More aggressive basic hydrolysis (1 N NH_4OH for 22 h) produced tetrasaccharide **5**, the inner monoterpene glycoside **6**, and prosaponin **7**, which were isolated by C_{18} semipreparative HPLC (Figure 2). After acid hydrolysis, prosaponin **7** yielded xylose, fucose, and *N*-acetylglucosamine, as component sugars, and acacic acid lactone (**8**). The identity of the latter was confirmed by comparison of the NMR data of its open lactone with reported values for acacic acid.[11] Extensive NMR (COSY, TOCSY, NOESY) and MS studies on prosaponin **7** revealed the connectivity among the three sugars and to the triterpene skeleton as described for prosaponin julibroside A_3 obtained from *Albizia julibrissin*.[11] By analysis of the NMR spectral data, and literature rotation data for its methyl ester, the monoterpenoid **6** was determined as the previously known (6*S*)-(2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoic acid 6-*O*-β-D-quinovopyranoside.[8] β-Glucosidase hydrolysis of **6** liberated the inner monoterpene, which was identical to the outer monoterpene. The rotation data of the inner monoterpene further confirmed the *S*-configuration at the asymmetric carbon.[9] The linkage of the inner monoterpene to acacic acid was defined by the HMBC correlations in **1** between the H-21 proton (δ_H, 5.48) and the monoterpene ester carbonyl carbon (δ_C, 168.2). Tandem mass spectrometry of the sodium adduct $[M + Na]^+$ of the tetrasaccharide **5**, observed at *m/z* 643, produced daughter ions at *m/z* 511 and 463 corresponding to the loss of ara and glc, respectively. Fragmentation of the daughter ion observed at *m/z* 511 (MS^3) produced an ion signal corresponding to the sodium adduct of a disaccharide, glc-rha, at *m/z* 313. Fragmentation of the daughter ion observed at *m/z* 463 (MS^3) produced ion signals corresponding to the sodium adducts of two disaccharides, ara-rha and glc-rha, at *m/z* 283 and 313, respectively. These data are consistent with a central rhamnose unit with two glucoses and one arabinose attached to it. The ^{13}C NMR signals for the component sugars in **1** matched well with the tetrasaccharide unit in kinmoonosides A−C[6] and elliptosides A, E, and F.[12] The chemical shift of the anomeric cabon of arabinose (δ_C, 111.0) indicated its α-furanose form.[13] The stereochemistry at the glycosidic position of other sugars was defined by the coupling constants observed for the respective anomeric proton. Therefore, the tetrasaccharide unit was characterized as α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl. In **1**, the anomeric proton of a glucose residue (δ_H, 5.32) had a HMBC correlation to the C-28 carbonyl (δ_C, 173.6), thus establishing the linkage between the acacic acid moiety and the tetrasaccharide unit. The complete structure of avicin D (**1**) was deduced as 21-*O*-[(6*S*)-(2*E*)-2-hydroxymethyl-6-methyl-6-*O*-{(2′*E*)-2′-hydroxymethyl-6′-hydroxy-6′-methyl-2′,7′-octadienoyl-(1′→4)-β-D-quinovopyrano-syl}-2,7-octadienoyl]-3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-β-D-2-acetamido-2-deoxyglucopyranosylacacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester.

Avicin G (**2**) displayed an intense $[M + Na]^+$ peak at *m/z* 2088 by MALDI MS, which is 16 mass units less than avicin D (**1**). The HRESIMS analysis allowed the determination of the molecular formula of **2** as $C_{98}H_{155}NO_{45}$. The NMR data were similar to those of avicin D except for an additional methyl group (δ_H 1.82, δ_C 12.5) and a missing hydroxymethyl signal (δ_H 4.31, δ_C 56.5), indicating hydroxylation in one of the methyl groups. After subjection to the degradative procedures described earlier, it was found that the outer monoterpene in **2** was (2*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid.[9] As in the case of avicin D, the rotation of the outer monoterpene could not be determined due to its conversion to other cyclic derivatives during the hydrolysis, resulting in insufficient material. The NMR data of isolated inner monoterpene from avicin G (**2**) matched well with the monoterpene **3**, and its rotation data indicated the *S*-configuration at the asymmetric center. The sugar composition of **2** was identical to that of avicin D (**1**). On the basis of the above results, the structure of **2** was elucidated as 21-*O*-[(6*S*)-(2*E*)-2-hydroxymethyl-6-methyl-6-*O*-{(2′*E*)-2′,6′-dimethyl-6′-hydroxy-2′,7′-octadienoyl-(1′→4)-β-D-quinovopyranosyl}-2,7-octadienoyl]-3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-β-D-2-acetamido-2-deoxyglucopyranosylacacic acid 28-*O*-α-

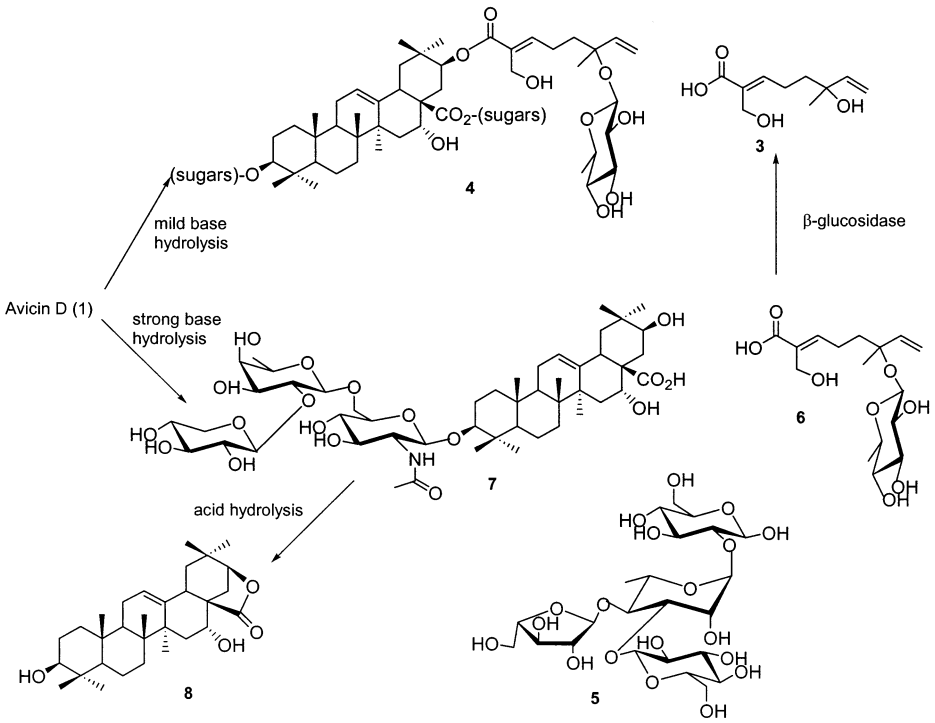

Figure 2. Hydrolysis of avicin D (**1**).

L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester.

Saponins with a variety of monoterpene esters located at C-28 or C-21 on acacic acid have been reported from various plant genera, including *Acacia*.[4−6,11,12,14−19] Elliptosides E and A have the same sugars and monoterpenes with attachments as in avicins D (**1**) and G (**2**), respectively.[12] However, authentic samples of elliptosides were found to have different HPLC retention times when spiked with the avicins. The configuration of the inner monoterpene in elliptosides is not reported.[12] However, the NMR data, especially for H-7, H-10, and C-5 of the inner monoterpene of elliptoside A, closely resembled those of the *R*-isomer in kinmoonoside A rather than the *S*-isomer.[6] The chiral center of the outer monoterpenes in elliptosides is reported to be in the *R*-configuration, and their C-2 olefinic bonds have a *Z*-configuration as depicted in a previous publication.[12] Therefore, the main differences between the avicins and the elliptosides lie in the stereochemistry of the monoterpenes. The cytotoxic kinmoonosides,[6] julibrosides,[15,17] and pithedulosides[18] differ from the avicins in having changes in the trisaccharide portion, the monoterpene structures, or both and in having additional sugars on the C-21 side chain.

The cytotoxicity of compounds **1**−**2**, **4**, and **7**−**8** was examined against Jurkat cells. Although prosaponin **7** and acacic acid lactone **8** were inactive (IC_{50} >100 μg/mL), the novel avicins **1** and **2** and prosaponin **4** displayed significant cytotoxicity, with IC_{50} values of 0.58, 0.22, and 5.8 μg/mL, respectively. The crude extract (F094) had a value of 0.38 μg/mL. Paclitaxel was employed as a positive control.

We have reported that the avicins induce tumor cell apoptosis by direct perturbation of the mitochondria.[1] Recent unpublished data suggest that the avicins interact directly with the outer mitochondrial membrane. In addition to the pro-apoptotic effects, the avicins suppress the development of DMBA-induced skin carcinogenesis and exhibit an antimutagenic and antioxidant effect.[20] Furthermore, the avicins inhibit the activation of NF-κB, probably due to the interaction of one or more Michael reaction acceptor sites on the monoterpene side chain with a critical sulfhydral group on NF-κB.[21] Finally, the avicins also may suppress tumor growth by inhibition of PI-3K signaling.[22] Thus, these new triterpene saponins represent an exciting new class of cytotoxic compounds with pro-apoptotic, antiinflammatory biological properties.

Experimental Section

General Experimental Procedures. Optical rotations were measured on an Autopol II Rudolph polarimeter. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD or pyridine-d_5 either on a JEOL Eclipse 400 or on a 500 MHz Varian-Inova 500. Mass spectra were recorded on Micromass Platform LC-MS (ESIMS), VG Autospec (HRFAB), Micromass Q-TOF Ultima (HRESI), and Perspective DE-STR (MALDI) instruments. MS/MS studies were performed on a Hitachi M 800 ion trap. GC−MS data were obtained on a HP5985 system fitted with a DB-1 column (0.25 mm i.d. × 30 m) (J&W Scientific); column temp, 160−200 °C (2 °C/min) and then 200−260 °C (10 °C/min); carrier gas, He at a flow rate of 50 mL/min. Preparative HPLC separations were performed on a NovaPrep 800 system. Analytical HPLC separations (column: Intersil RP_{18}, 4.6 × 150 mm) with a CH_3CN-H_2O solvent system (30−42% in 42 min, flow rate 1.0 mL/min) were carried out on a Hitachi D-6000 instrument equipped with a diode array detector. The detector in the HPLC systems was set at 220 nm. TFA (0.1%) was added to H_2O used in the purification and analytical steps.

Plant Material. *A. victoriae* flowers, stems, leaves, and seed pods were collected in July 1996 in Tucson, AZ. A voucher specimen (No. 258232) has been deposited in the herbarium of the University of Arizona in Tucson. The plant was identified by F. C. Vasek in September 1968 in Northern Territory, Australia (personal collection number 680915-37).

Extraction and Isolation. The ground seed pods of *A. victoriae* (2.5 kg) were extracted in 20% aqueous MeOH (40 L) at 50 °C for 20 h. The dried extract (614 g) was fractionated on a Biotage-75 radial compression module with a C_{18} cartridge (7.5 × 30 cm) system in increments of 150−200 g of extract per run. The column was eluted with increasing amounts of MeOH−H_2O (50%; 11 L, 60%, 65%, 70%, 75%, 80%; each 5 L).

The fractions were analyzed by HPLC, and the 75% aqueous MeOH eluate, with F094 (90 g) being selected for further fractionation based on the observed bioactivity. The F094 extract (70 g) was refractionated (500 mL fractions) over the same Biotage cartridge with the MeOH−H_2O solvent system (60%; 5 L, 70%; 13 L, 75%; 15 L, 80%; 2 L) to give two combined fractions (D-pool, 27.3 g; G-pool, 11.4 g). The D-pool in 1.0 g aliquots was separated by preparative HPLC (Flurosep-RP phenyl column, 50 × 250 mm, 10 μm, flow rate 80 mL/min) with 28% aqueous CH_3CN. The avicin D-rich fractions in 100 mg aliquots were further purified on the same preparative HPLC column with 56% aqueous MeOH to give avicin D (**1**, 1.1 g). The G-pool was similarly subjected to repeated preparative HPLC purification employing as solvent systems 61% aqueous MeOH followed by 30% aqueous CH_3CN to furnish avicin G (**2**, 0.35 g).

Avicin D (1): white solid; $[\alpha]^{25}_D$ −30° (*c* 0.4, MeOH); ^{1}H NMR (partial, CD_3OD, 500 MHz) δ 0.76 (6H, s, H_3-24, H_3-26), 0.86 (3H, s, H_3-29), 0.95 (3H, s, H_3-25), 0.98 (3H, s, H_3-23), 1.04 (3H, s, H_3-30), 1.11 (3H, d, J = 6.4 Hz, H_3-qui-6), 1.26 (3H, d, J = 6.8 Hz, H_3-fuc-6), 1.27 (3H, s, H_3-outerMT-10), 1.32 (3H, d, J = 6.4 Hz, H_3-rha-6), 1.38 (3H, s, H_3-innerMT-10), 1.43 (3H, s, H_3-27), 1.94 (3H, s, H_3-NHAc), 3.25(1H, m, H-3), 4.31 (4H, s, H_2-each MT-9), 4.42 (1H, d, J = 7.8 Hz, H-qui-1), 4.43 (1H, d, J = 8.7 Hz, H-glcNAc-1), 4.46 (1H, d, J = 7.3 Hz, H-glc$_2$-1), 4.48 (2H, m, H-fuc-1, H-16), 4.50 (1H, d, J = 8.7 Hz, H-xyl-1), 4.63 (1H, d, J = 9.3 Hz, H-qui-4), 5.05, 5.20 (2H, m, H_2-outerMT-8), 5.23 5.27 (2H, m, H_2-innerMT-8), 5.31 (1H, d, J = 7.6 Hz, H-rha-1), 5.32 (1H, d, J = 7.6 Hz, H-glc$_1$-1), 5.33 (1H, d, J = 2.1 Hz, H-ara-1), 5.34 (1H, m, H-12), 5.48 (1H, dd, J = 5.4, 11.0 Hz, H-21), 5.90 (1H, dd, J = 11.0, 18.0 Hz, H-outerMT-7), 5.95 (1H, dd, J = 11.0, 18.0 Hz, H-innerMT-7), 6.90 (1H, t, J = 7.8 Hz, H-outerMT-3), 6.94 (1H, t, J = 7.8 Hz, H-innerMT-3); ^{13}C NMR data, see Tables 1−3; MALDI MS (positive ion mode) $[M + Na]^+$ 2104; HRFABMS *m*/*z* 2104.9651 (calcd for $C_{98}H_{155}NO_{46}Na$ $[M + Na]^+$, 2104.9717); t_R (min) 15.2 (avicin D), 12.5 (elliptoside E).

Avicin G (2): white solid; $[\alpha]^{25}_D$ −26.9° (*c* 0.4, MeOH); ^{1}H NMR (partial, CD_3OD, 500 MHz) δ 0.76 (6H, s, H_3-24, H_3-26), 0.86 (3H, s, H_3-29), 0.95 (3H, s, H_3-25), 0.97 (3H, s, H_3-23), 1.04 (3H, s, H_3-30), 1.08 (3H, d, J = 6.0 Hz, H_3-qui-6), 1.26 (3H, d, J = 6.5 Hz, H_3-fuc-6), 1.27 (3H, s, H_3-outerMT-10), 1.32 (3H, d, J = 6.0 Hz, H_3-rha-6), 1.38 (3H, s, H_3-innerMT-10), 1.43 (3H, s, H_3-27), 1.82 (3H, s, H_3-outerMT-9), 1.94 (3H, s, H_3-NHAc), 3.24 (1H, m, H-3), 4.32 (2H, s, H_2-innerMT-9); 4.41 (1H, d, J = 7.8 Hz, H-qui-1), 4.43 (1H, d, J = 7.8 Hz, H-glcNAc-1), 4.46 (1H, d, J = 7.3 Hz, H-glc$_2$-1), 4.48 (2H, m, H-fuc-1, H-16), 4.49 (1H, d, J = 7.7 Hz, H-xyl-1), 4.61 (1H, t, J = 9.5 Hz, H-qui-4), 5.04, 5.19 (2H, m, H_2-outerMT-8), 5.22, 5.27 (2H, m, H_2-innerMT-8), 5.33 (2H, d, J = 7.7 Hz, H-rha-1, H-glc$_1$-1), 5.34 (2H, d, J = 2.1 Hz, H-ara-1, H-12), 5.48 (1H, dd, J = 5.5, 11.0 Hz, H-21), 5.91 (1H, dd, J = 10.7, 17.8 Hz, H-outerMT-7), 5.94 (1H, dd, J = 10.7, 17.8 Hz, H-innerMT-7), 6.79 (1H, dt, J = 7.8, 1.5 Hz, H-outerMT-3), 6.89 (1H, t, J = 7.5 Hz, H-innerMT-3); ^{13}C NMR data, see Tables 1−3; MALDI MS (positive ion mode) $[M + Na]^+$ 2088; HRESIMS *m*/*z* 2088.9668 (calcd for $C_{98}H_{155}NO_{45}Na$ $[M + Na]^+$, 2088.9770); t_R (min) 29.1 (avicin G), 26.0 (elliptoside A).

Sugar Determinations. Each sample (20 mg) was hydrolyzed by 2 N HCl (10 mL) at 100 °C for 2 h. The hydrolysate was diluted with H_2O and basified to pH 5−6 with dilute NaOH. The mixture was loaded onto a 3 mL BakerBond PolarPlus C_{18} SPE column and washed with H_2O. The washings were lyophilized to give a residue that was converted to Me_3Si ethers with Tri-Sil and analyzed by GC−MS for the sugar composition. For configuration analysis, the sample was first hydrolyzed by butanolysis using *R*-(−)-2-butanol HCl (80 °C for 16 h) and then *N*-acetylated with Ac_2O−pyridine (80 °C for 30 min) prior to Tri-Sil treatment.[23] The derivatives of D-xylose, L-arabinose, L-rhamnose, D-fucose, D-quinovose, D-glucose, and D-*N*-acetylglucosamine in both **1** and **2** were detected as follows: t_R (min) 11.16 (derivative of D-xylose); 14.82 (derivative of L-arabinose); 15.01 (derivative of L-rhamnose); 16.49 (derivative of D-fucose); 18.95 (derivative of D-quinovose); 24.92 (derivative of D-glucose); 31.77 (derivative of D-*N*-acetylglucosamine).

Mild Alkaline Hydrolysis. Compound **1** (72 mg) was treated with 0.5 N NH_4OH for 1 h at room temperature. After adjusting to pH 3.0 with formic acid, the mixture was passed through a 3 mL BakerBond PolarPlus C_{18} SPE column. The column was first washed with H_2O to remove salts and finally eluted with MeOH. The MeOH eluent was evaporated and chromatographed by C_{18} semipreparative HPLC (Waters, 25 × 100 mm, 6.0 μm; MeOH−H_2O gradient, 54−60%, 30 min, 30 mL/min) to yield **3** (4 mg) and **4** (12 mg). The outer monoterpene **3** was identified as (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoic acid by comparison with reported NMR data.[9]

Prosaponin 4: white solid; ^{1}H NMR (partial, CD_3OD, 400 MHz) δ 0.76 (6H, s, H_3-24, H_3-26), 0.86 (3H, s, H_3-29), 0.95 (3H, s, H_3-25), 0.98 (3H, s, H_3-23), 1.04 (3H, s, H_3-30), 1.22 (3H, d, J = 5.9 Hz, H_3-qui), 1.26 (3H, d, J = 6.5 Hz, H_3-fuc), 1.32 (3H, d, J = 6.24 Hz, H_3-rha), 1.37 (3H, s, H_3-MT-10), 1.43 (3H, s, H_3-27), 1.94 (3H, s, H_3-NHAc), 4.31 (2H, s, H_2-MT-9); 4.34 (1H, d, J = 8.0 Hz, qui-1), 4.63 (1H, d, J = 9.3 Hz, qui-4), 5.20, 5.27 (2H, m, H_2-MT-8), 5.34 (1H, m, H-12), 5.48 (1H, m, H-21), 5.93 (1H, dd, J = 11.0, 18.0 Hz, H-MT-7), 6.89 (1H, t, J = 7.6 Hz, H-MT-3); MALDI MS (positive-ion mode) *m*/*z* 1922 $[M + Na]^+$. Carbohydrate analysis resulted in the same compositional sugars as in **1**.

Strong Alkaline Hydrolysis. A solution of **1** (100 mg) in 1.0 N NH_4OH in a sealed bottle was kept for 22 h at 60 °C. After acidifying to pH 3.0 with formic acid, the mixture was passed through a 6 mL BakerBond PolarPlus C_{18} SPE column. The column was first washed with H_2O and finally eluted with MeOH. The H_2O eluent was lyophilized to give tetrasaccharide **5** (10.2 mg) as a white solid. The MeOH eluent was concentrated to 2.0 mL and chromatographed in two aliquots by C_{18} semipreparative HPLC (Waters, 25 × 100 mm, 6.0 μm; MeOH−H_2O gradient, 15−60%, 50 min, 30 mL/min) to yield a small amount of **3** (1 mg), the monoterpene glycoside **6** (10 mg), and prosaponin **7** (20 mg).

Monoterpene glycoside 6: colorless oil; $[\alpha]^{25}_D$ −24 ° (*c* 0.4, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 1.22 (3H, d, J = 11.3 Hz, H_3-qui-6), 1.36 (3H, s, H_3-10), 1.72 (2H, m, H_2-5), 2.42 (2H, m, H_2-4), 2.97 (H, t, J = 9.2 Hz, H-qui-4), 3.16 (H, dd, J = 9.2, 7.8 Hz, H-qui-2), 3.27 (H, t, J = 9.2 Hz, H-qui-3), 4.29 (2H, s, H_2-9), 4.34 (H, d, J = 7.0 Hz, H-qui-1), 5.20, 5.27 (2H, dd, J = 11.0, 1.1 Hz and dd, J = 17.6, 1.1 Hz, H_2-8), 5.93 (H, dd, J = 17.8, 11.0 Hz, H-7), 6.92 (H, t, J = 7.7 Hz, H_3-3); ESIMS (negative-ion mode) *m*/*z* 345 $[M - H]^-$.

Enzymatic Hydrolysis of 6. Compound **6** (15 mg) and β-glucosidase (100 units from Sigma) were dissolved in acetate buffer (100 mM, pH 5.0, 1.0 mL) and incubated at 37 °C for 6 h. The reaction mixture was separated by C_{18} semipreparative HPLC (Waters, 25 × 100 mm, 6.0 μm; MeOH−H_2O gradient, 54−60%, 30 min, 30 mL/min) to give inner monoterpene **3** (8 mg): oil; $[\alpha]^{25}_D$ +16° (*c* 0.4, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 1.27 (3H, s, H_3-10), 1.63 (2H, m, H_2-5), 2.35 (2H, m, H_2-4), 4.29 (2H, s, H_2-9), 5.05, 5.22 (2H, dd, J = 17.6, 10.6 Hz, H_2-8), 5.90 (H, dd, J = 17.6, 10.6 Hz, H-7), 6.92 (H, t, J = 7.7 Hz, H-3); ESIMS (negative-ion mode) *m*/*z* 199 $[M - H]^-$.

Outer Monoterpene in Avicin G (2) [(2*E*)-2,6-Dimethyl-6-hydroxy-2,7-octadienoic Acid]. Avicin G (**2**) with mild base treatment as described above yielded a monoterpene: oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.25 (3H, s, H_3-10), 1.59 (2H, m, H_2-5), 1.78 (3H, d, J = 1.0 Hz, H_3-9), 2.13 (2H, m, H_2-4), 5.02, 5.20 (2H, dd, J = 17.2, 10.4 Hz, H_2-8), 5.90 (H, dd, J = 17.2, 10.4 Hz, H-7), 6.40 (H, t, J = 6.4 Hz, H-3); ESIMS (negative-ion mode) *m*/*z* 183 $[M - H]^-$.

Prosaponin 7: white amorphous powder; ^{1}H NMR (partial, CD_3OD, 400 MHz) δ 0.76, 0.91, 0.94, 0.97, 0.98, 0.99, 1.25 (21H, s, 7 × Me of acacic acid), 1.26 (3H, d, J = 4.8 Hz, H_3-fuc), 1.94 (3H, s, H_3-NHAc), 3.27 (1H, m, H-3), 3.89 (1H, dd, J = 4.7, 12.0 Hz, H-21), 4.25 (1H, bd, J = 5.5 Hz, H-16), 4.42 (1H, d, J = 8.4 Hz, H-glcNAc-1), 4.45 (1H, d, J = 7.3 Hz, H-fuc-1), 4.50 (1H, d, J = 7.0 Hz, H-xyl-1), 5.40 (1H, m, H-12); MALDI MS (positive-ion mode) *m*/*z* 992 $[M + Na]^+$.

Cytotoxicity Assays. Assays were performed as previously described.[22]

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