Bioactive Saponins from *Acacia tenuifolia* from the Suriname Rainforest[1]

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Bioassay-guided fractionation of the MeOH extract of *Acacia tenuifolia* using the engineered yeast strains 1138, 1140, 1353, and Sc7 as the bioassay tool resulted in the isolation of the three new saponins **3**, **5**, and **6** and the three known saponins **1**, **2**, and **4**. The structures of the new compounds were established on the basis of HRMS, 1D and 2D NMR spectral data on the intact saponins, and GC−MS analyses of the sugars. Compounds **1**,**2** and **5**,**6** showed cytotoxicity against mammalian cell lines.

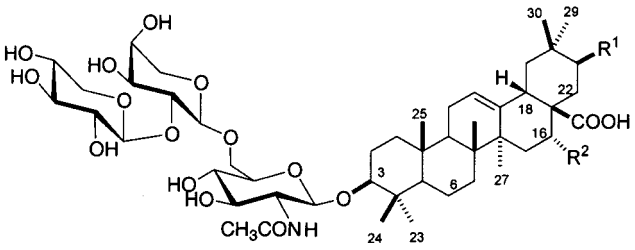
In recent years we have been engaged in a program to discover bioactive compounds from the Suriname rain forest using a mechanism-based bioassay involving genetically engineered mutants of the yeast *Saccharomyces* cerevisiae;[2] this work has been reviewed.[3] As a part of this study the plant *Acacia tenuifolia* (L.) Willd. (Mimosaceae) was collected in central Suriname. There are three species of *A. tenuifolia* reported in Suriname; two of them are climbing shrubs, and the third is a small tree. The name of *A. tenuifolia* in the local Saramaca language is bhe akamakka.

Acacia species have been extensively investigated and have yielded diterpenoids,[4] triterpenoids,[5] triterpenoid saponins,[6] and flavonols,[7] among other compounds. Some *Acacia* constituents have interesting biological activities, such as the Tie2 kinase inhibitory activity of a triterpenoid from *Acacia aulacocarpa*[5] and the triterpenoid saponins from *Acacia victoriae* that inhibit tumor cell growth, induce apoptosis,[6] and prevent chemically induced carcinogenesis in mice.[8]

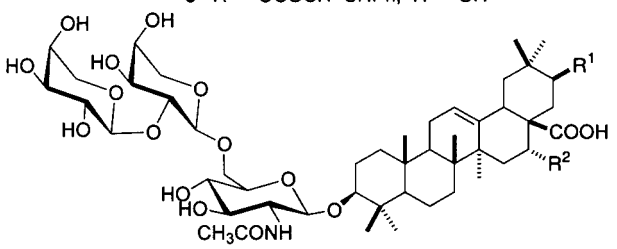
A methanol extract of *A. tenuifolia* was found to show reproducible activity in our yeast assay. Herein we report the isolation, structure determination, and bioactivity of the new saponins **3**, **5**, and **6** and the known saponins **1**, **2**, and **4** from this plant.

Results and Discussion

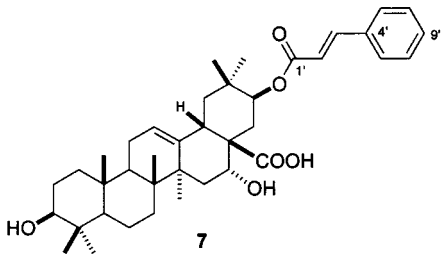
The MeOH extracts of *A. tenuifolia* had IC_{12} values of 6000, 5500, 5200, and 4000 μg/mL against the 1138, 1140, 1353, and Sc-7 yeast strains, respectively. Fractionation of these extracts by liquid−liquid partition caused the bioactivity to partition successively into the aqueous MeOH phase of a hexane−80% aqueous MeOH partition, then into the $CHCl_3$ phase of a $CHCl_3$−50% aqueous MeOH partition, and then finally into the *n*-BuOH phase of an *n*-BuOH−water partition. Purification of the *n*-BuOH-soluble fraction using a C_{18} reversed-phase column, followed by preparative TLC, resulted in the isolation of triterpene saponins **1**−**6**.



1 $R^1 = R^2 = H$
3 $R^1 = H$, $R^2 = OH$
5 $R^1 = OCOCH{=}CHPh$, $R^2 = OH$



2 $R^1 = R^2 = H$
4 $R^1 = H$, $R^2 = OH$
6 $R^1 = OCOCH{=}CHPh$, $R^2 = OH$



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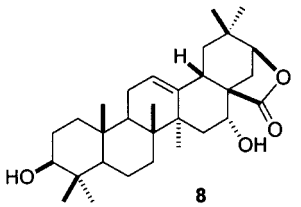

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Compound **1** was isolated as a white solid that analyzed for $C_{48}H_{77}NO_{16}$ by a combination of HRFABMS and ^{13}C NMR spectrometry. It showed IR absorption bands at ν_{max}

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1670 and 1550 cm^{-1} and had three characteristic signals in its ^{13}C NMR spectrum at δ 23.2, 57.7, and 173.5, diagnostic for $CHNHCOCH_3$. Comparison of its ^1H and ^{13}C NMR spectroscopic data with those of albiziatrioside A (3-*O*-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl]oleanolic acid) recently isolated from *Albizia subdimidiata*[9] indicated that it was the same compound, and this was confirmed by a direct TLC comparison of the samples.

Compound **2** had spectroscopic data that were almost identical with those of **1**. The ^{13}C NMR data of the triterpenoid portion identified it as oleanolic acid, and the presence of three sugar moieties was also indicated by spectroscopic data. The ^1H and ^{13}C NMR spectra were identical to those of 3-*O*-[α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl]oleanolic acid, available from *Albizia subdimidiata*[9] and also found in *Calliandra anomala*[10] and *Pithecellobium racemosum*.[11]

Two closely related metabolites, compounds **3** and **4**, were isolated as white solids. The molecular formulas of **3** and **4** were both $C_{48}H_{77}NO_{17}$, as determined by combined HRFAB mass and ^{13}C NMR spectrometry. The NMR spectral data of the former were very similar to those obtained for **1**, while those of the latter were very close to those derived from **2**. The only differences in the ^{13}C NMR spectra were replacement of upfield signals at δ 24.5 in **1** and **2** with oxymethine carbon signals at δ 75.7 in **3** and **4**. Corresponding differences were also found in the ^1H NMR spectra, in which new oxymethine proton signals appeared at δ 4.36 (1H, t, J = 3.6 Hz) and 4.37 (1H, t, J = 3.5 Hz) in **3** and **4**, respectively.

The position of this oxymethine group was assigned to C-16 by a combination of $^1H-^1H$ COSY and HMQC experiments. Acid hydrolysis of **3** and **4** with 3 N HCl gave echinocystic acid as the aglycon, and this was identified by mass spectroscopic analysis and by comparison of its ^1H NMR spectral data with the literature data.[12] The sugars present were positively identified by hydrolysis with methanolic HCl, followed by reduction and acetylation to form the corresponding alditol acetates. GC analysis of the alditol acetates and direct comparison with alditol acetates of standard sugars[13] indicated the presence of L-arabinose, D-xylose, and *N*-acetyl-D-glucosamine; the absolute stereochemistries of the sugars were assigned by analogy with related compounds of this class.[14] Further evidence for the nature of the sugar linkages was derived from analysis of the MS fragmentation of the sugars after methylation, hydrolysis, reduction, acetylation, and GC separation (Figure 1, Supporting Information).[15]

These experiments identified compounds **3** and **4** as 3-*O*-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl]echinocystic acid (**3**) and 3-*O*-[α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl]echinocystic acid (**4**). Compound **4** has been previously reported,[9a,16] and its ^1H and ^{13}C NMR spectral data measured in the same NMR solvent were in good agreement with the reported data. Compound **3** is reported for the first time from nature and was given the name acacioside A.

Closely related compounds **5** and **6** were obtained as white solids. HRFAB mass and ^{13}C NMR spectral data of both compounds showed the same molecular formula $C_{57}H_{83}NO_{19}$. Spectral data of **5** and **6** were very similar to those for **3** and **4**, and careful examination of their NMR data revealed that these compounds possessed the same sugar units and A−D rings as **3** and **4**. However, there were significant differences in the ^{13}C NMR spectrum of ring E of the aglycon. Signals of the methylene carbons at δ 36.5 and 36.6 in **3** and **4**, respectively, were replaced by signals for oxygen-bearing methines at δ 79.8 in **5** and **6**. Corresponding changes were found in the ^1H NMR spectra in which new downfield signals appeared at δ 5.57 (1H, dd, J = 11.4, 5.6 Hz) and 5.56 (1H, dd, J = 11.5, 5.4 Hz) in **5** and **6**, respectively, suggesting that these oxygen-bearing methines were esterified. In addition, seven downfield carbon signals at δ 120−170 and corresponding proton signals indicated the existence of a *trans*-cinnamoyl group, and $^1H-^1H$ COSY, HMQC, and HMBC experiments confirmed that it was connected to the C-21 position of the aglycon of each compound.

The structure of the aglycon part of **5** and **6** was identified as the sapogenin, **7**, designated acaciagenin, which was obtained on acid hydrolysis of each compound; its molecular formula was established as $C_{39}H_{54}O_4$ by HRFABMS. The identification of acaciagenin was confirmed by a chemical transformation; treatment of **7** with 0.5 N KOH in MeOH followed by acidification with 3 N HCl afforded acacic acid lactone (**8**), which was identified by comparison of ^1H and ^{13}C NMR spectral data with the literature data[14c] and by mass spectral analysis. The structures of **5** and **6** were thus established as 3-*O*-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-21-*O*-*trans*-cinnamoylacacic acid (**5**) and 3-*O*-[α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-21-*O*-*trans*-cinnamoylacacic acid (**6**).

Compounds **1**,**2** and **5**,**6** exhibited weak activity in our yeast bioassay (Table 4). In cytotoxicity tests, **1** and **2** showed significant activity against the M 109 lung cancer cell line, with IC_{50} values of 1 μM, while **5** and **6** showed weak activity against the A 2780 ovarian cancer cell line.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra were recorded on a Nicolet Impact 400 FT-IR spectrophotometer. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded in CD_3OD on a JEOL Eclipse 500 instrument at 500.1624 for ^1H and 125.7778 MHz for ^{13}C, and a Varian Unity 400 NMR instrument at 399.951 MHz for ^1H and 100.578 MHz for ^{13}C NMR, using standard pulse sequence programs. Mass spectral data were obtained at the Nebraska Center for Mass Spectrometry. UV spectra were measured on a Shimadzu UV1201 spectrophotometer.

Yeast Bioassay. The bioassay was carried out as previously described.[2c]

Cytotoxicity Bioassay. The in vitro antitumor cytotoxicity assays were performed at Bristol-Myers Squibb Pharmaceutical Research Institute using the Madison Lung Carcinoma (M109)[17] murine cell line as previously described,[18] or at Virginia Polytechnic Institute and State University using the A2780 ovarian cancer cell line as previously described.[19]

Plant Collection and Extraction. *Acacia tenuifolia* (L.) Willd. (Mimosaceae) was collected at Botopasi, Sipaliwini District, Suriname, by I. Derveld on November 17, 1997. It is a climbing shrub, armed with recurved prickles. Voucher specimens have been deposited in the National Herbarium of Suriname (voucher number CI 0846). The dried stems were ground to a powder and extracted with MeOH for 24 h at

Table 1. Selected ^{1}H NMR Spectral Data for Compounds **1**−**7**[a]

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
3	3.20 (1H, m)	3.20 (1H, m)	3.20 (1H, m)	3.20 (1H. m)	3.21 (1H, m)	3.20 (1H, m)	3.15 (1H, m)
12	5.21 (1H, s)	5.21 (1H, s)	5.31 (1H, t, 3.1)	5.31 (1H, t, 3.1)	5.31 (1H, t, 3.4)	5.31 (1H, t, 3.5)	5.32 (1H, t, 3.4)
16			4.36 (1H, t, 3.6)	4.37 (1H, t, 3.5)	4.55 (1H, m)	4.55 (1H, m)	4.57 (1H, m)
21					5.57 (1H, dd, 11.4, 5.6)	5.56 (1H, dd, 11.5, 5.4)	5.58 (1H, dd, 11.1, 5.6)
23	0.96 (3H, s)	0.96 (3H, s)	0.96 (3H, s)	0.96 (3H, s)	0.96 (3H, s)	0.96 (3H, s)	0.96 (3H, s)
24	0.75 (3H, s)	0.75 (3H, s)	0.75 (3H, s)	0.75 (3H, s)	0.75 (3H, s)	0.75 (3H, s)	0.75 (3H, s)
25	0.94 (3H, s)	0.93 (3H, s)	0.93 (3H, s)	0.93 (3H, s)	0.94 (3H, s)	0.94 (3H, s)	0.93 (3H, s)
26	0.86 (3H, s)	0.85 (3H, s)	0.84 (3H, s)	0.83 (3H, s)	0.84 (3H, s)	0.84 (3H, s)	0.83 (3H, s)
27	1.15 (3H, s)	1.15 (3H, s)	1.33 (3H, s)	1.33 (3H, s)	1.40 (3H, s)	1.39 (3H, s)	1.33 (3H, s)
29	0.88 (3H, s)	0.88 (3H, s)	0.86 (3H, s)	0.86 (3H, s)	0.87 (3H, s)	0.87 (3H, s)	0.86 (3H, s)
30	0.94 (3H, s)	0.94 (3H, s)	0.97 (3H, s)	0.97 (3H, s)	1.12 (3H, s)	1.12 (3H, s)	0.97 (3H, s)
2′					7.64 (1H, d, 16.1)	7.63 (1H, d, 16.1)	7.64 (1H, d, 16.1)
3′					6.49 (1H, d, 16.1)	6.49 (1H, d, 16.1)	6.50 (1H, d, 16.1)
GlcNAc H-1	4.44 (1H, d, 8.5)	4.43 (1H, d, 8.4)	4.43 (3H, d, 8.6)	4.43 (1H, d, 8.5)	4.44 (1H, d, 8.7)	4.43 (1H, d, 8.3)	
Ara H-1	4.52 (1H, d, 5.5)	4.55 (1H, d, 5.8)	4.54 (1H, d, 5.4)	4.52 (1H, d, 6.0)	4.55 (1H, d, 5.1)	4.53 (1H, d, 5.6)	
terminal xyl H-1	4.44 (1H, d, 7.2)		4.43 (1H, d, 7.2)		4.44 (1H, d, 7.2)		
terminal ara H-1		4.49 (1H, d, 6.7)		4.49 (1H, d, 6.8)		4.49 (1H, d, 6.8)	
CH_3CONH	1.95 (3H, s)	1.95 (3H, s)	1.95 (3H, s)	1.95 (3H, s)	1.95 (3H, s)	1.95 (3H, s)	

[a] Obtained in CD_3OD at 400 MHz; coupling constants in Hz.

Table 2. ^{13}C NMR Spectral Data for Aglycon Moieties of Saponins **1**−**6** and Sapogenin (**7**)[a]

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	40.0	40.0	39.8	39.8	40.0	40.0	39.9
2	27.1	27.0	27.1	27.0	27.1	27.1	27.4
3	90.3	90.6	90.3	90.7	90.5	90.8	79.8
4	39.7	39.7	40.0	40.0	39.8	39.8	39.8
5	56.9	56.9	57.1	57.0	57.1	57.1	56.9
6	19.4	19.4	19.4	19.4	19.4	19.4	19.5
7	34.1	34.1	34.3	34.3	34.5	34.5	34.5
8	40.5	40.5	40.9	40.8	40.6	40.6	40.6
9	49.1	49.1	48.4	48.4	48.3	48.3	48.3
10	37.9	37.9	37.9	37.9	38.0	38.0	38.2
11	24.6	24.6	24.5	24.5	24.6	24.6	24.5
12	123.0	123.0	122.8	122.9	123.0	123.0	123.0
13	146.0	146.0	145.5	145.5	145.4	145.4	145.5
14	42.9	42.9	42.9	42.8	42.6	42.6	42.6
15	35.2	35.3	36.1	36.1	36.3	36.3	36.3
16	24.5	24.5	75.7	75.7	76.2	76.2	76.1
17	47.7	47.7	49.3	49.4	53.6	53.6	53.6
18	43.1	43.1	42.7	42.6	42.0	42.0	42.0
19	47.8	47.8	48.0	48.0	49.0	49.0	49.0
20	31.7	31.7	31.3	31.3	36.1	36.1	36.1
21	34.1	34.1	36.5	36.6	79.8	79.8	79.7
22	29.1	29.1	31.3	31.3	38.5	38.5	38.5
23	28.6	28.6	28.6	28.5	28.6	28.6	28.8
24	17.1	17.1	17.1	17.1	17.1	17.1	16.3
25	16.0	16.0	16.2	16.2	16.0	16.0	16.0
26	18.0	18.0	18.2	18.2	18.2	18.2	18.2
27	26.6	26.6	27.7	27.6	27.5	27.4	27.5
28	184.7	184.7	185.4	185.0	183.4	183.4	180.5
29	33.8	33.8	33.6	33.6	29.7	29.7	29.7
30	24.2	24.2	26.1	26.0	19.6	19.6	19.5
1′					168.9	168.9	168.9
2′					119.8	119.8	119.7
3′					145.7	145.7	145.7
4					135.9	135.9	135.9
5′, 6′					129.2	129.2	129.2
7′, 8′					130.0	130.0	130.0
9′					131.3	131.3	131.4

[a] Measured in CD_3OD at 125 MHz. Assignment made by combination of DEPT, ^{1}H COSY, HMQC, and HMBC, and comparison with the related compounds.

Table 3. ^{13}C NMR Data for Sugar Moieties of Saponins **1**−**6** in CD_3OD[a]

no.	**1**	**2**	**3**	**4**	**5**	**6**
GluNAc 1	104.9	104.9	104.9	104.9	104.8	104.9
2	57.7	57.6	57.8	57.7	57.8	57.7
3	76.6	76.3	76.6	76.4	76.7	76.5
4	72.2	72.0	72.3	72.0	72.4	72.1
5	75.7	75.7	75.7	75.7	75.7	75.8
6	69.6	69.5	69.6	69.5	69.6	69.4
Ara 1	103.3	103.4	103.3	103.4	103.3	103.4
2	81.3	80.4	81.3	80.4	81.2	80.3
3	73.1	73.4	73.1	73.4	73.1	73.4
4	68.5	68.8	68.5	68.7	68.5	68.8
5	65.4	65.7	65.4	65.7	65.4	65.7
Terminal xyl 1	106.5		106.5		106.4	
2	75.7		75.7		75.8	
3	77.5		77.5		77.6	
4	71.1		71.1		71.1	
5	67.2		67.2		67.2	
terminal ara 1		105.8		105.8		105.8
2		73.0		72.9		72.9
3		74.2		74.2		74.2
4		70.0		69.8		69.7
5		67.1		67.1		67.1
$NHCOCH_3$	23.2	23.2	23.2	23.1	23.1	23.1
$NHCOCH_3$	173.5	173.5	173.4	173.5	173.4	173.4

[a] Recorded at 125 MHz.

Table 4. Bioactivities of Saponins **1**,**2** and **5**,**6**

	IC_{12} values (μg/mL) in *S. cerevisiae* strains				IC_{50} values (μg/mL) in mammalian cell lines	
saponin	1138	1140	1353	Sc7	M 109	A 2780
1	30	35	35	30	1	NT
2	25	40	30	45	1	NT
5	150	220	220	280	NT	13.5
6	160	215	220	275	NT	13.0

Bedrijf Geneesmiddelen Voorziening Suriname (BGVS), and then solvent was removed in vacuo. Extraction of 1.5 kg of dried plant material with MeOH yielded 55 g of extract as BGVS M980145.

Isolation of the Bioactive Saponins. The MeOH extract of *A. tenuifolia* (1.280 g) was weakly active against the mutant 1138, 1140, 1353, and Sc7 yeast strains. The four strains were used to determine the IC_{12} values of the total extract and pure isolates, while the activity was monitored during the partition using the 1138 mutant strain. The MeOH extracts were partitioned between 80% aqueous MeOH (250 mL) and *n*-hexane, giving an inactive hexane fraction (0.076 g). The aqueous MeOH fraction was diluted to 50% aqueous MeOH with water and partitioned with $CHCl_3$. The aqueous MeOH layer, after evaporation to dryness, was further partitioned between *n*-BuOH and water to afford a bioactive *n*-BuOH fraction (0.570 g, IC_{12} 800 μg/mL), which was subjected to C_{18} reversed-phase vacuum flash chromatography eluting with stepwise gradient mixtures of MeOH and H_2O (50%, 60%, 70%, 80%, 90% aqueous MeOH, and 100% MeOH).

Of these only fractions 3−5 (0.420 g) were found to be bioactive (IC_{12} 1000, 150, and 20 μg/mL, respectively).

Further purification of fraction 3 by RP-PTLC on a C-18 column with aqueous 70% MeOH as eluent followed by PTLC on a Si gel plate with the solvent system EtOAc/EtOH/H_2O (7:2:1) afforded compounds **3** (16.6 mg) and **4** (24.0 mg). Fraction 4 was subjected to RP-PTLC on a C_{18} plate with elution by aqueous 70% MeOH. Compounds **5** (3.6 mg) and **6** (4.2 mg) were isolated from a more polar fraction of RP-PTLC by continuous PTLC on a Si gel plate with the solvent systems $CHCl_3$/MeOH/H_2O (13:7:1) and $CHCl_3$/EtOH/H_2O (7:10:1), respectively. Fraction 5 from the above C_{18} reversed-phase column and a less polar fraction from RP-PTLC on C_{18} were combined on the basis of their TLC behavior and subjected to PTLC on Si gel with the solvent $CHCl_3$/EtOH/H_2O (7:10:1) to give compounds **1** (6.5 mg) and **2** (8.5 mg).

Albiziatrioside A 3-*O*-[*β*-D-xylonopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl]oleanolic acid (1): white amorphous solid, mp 275 (dec) °C; $[\alpha]^{25}_D$ +39.0° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3480 (br), 2940, 1670 (COOH), 1640, 1550 (NHCO) cm^{-1}; NMR, see Tables 1−3; HRFABMS *m/z* 946.5150 $(M + Na)^+$ (calcd for $C_{48}H_{77}NO_{16}Na$, 946.5140).

3-*O*-[α-L-Arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl]oleanolic acid (2): NMR, see Tables 1−3; FABMS *m/z* 660 (M^+ + H, 10), 659 (M^+, 4), 660 (M^+ − H, 6), 455 ($C_{30}H_{47}O_3$, 16), 454 (36), 453 (100), 439 ($C_{30}H_{47}O_3$, 32), 437 (42).

Acacioside A {**3-*O*-[*β*-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl]echinocystic acid**} **(3)**: white amorphous solid, mp 280 (dec) °C; $[\alpha]^{25}_D$ −0.4° (*c* 0.26, MeOH); IR (KBr) ν_{max} 3420 (br), 2940, 2920, 1700, 1675, 1650, 1630, 1560 cm^{-1}; NMR, see Tables 1−3; HRFABMS *m/z* 962.5090 $(M + Na)^+$ (calcd for $C_{48}H_{77}NO_{17}Na$, 962.5090).

3-*O*-[α-L-Arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl]echinocystic acid (4): white amorphous solid, mp 220 (dec) °C (lit.[12b] mp 211−212 (dec) °C); $[\alpha]^{25}_D$ +5.6° (*c* 0.26, MeOH)) (lit.[14a] $[\alpha]^{26}_D$ +7.03°); IR (KBr) ν_{max} 3410 (br), 1700, 1670, 1650, 1630, 1560 cm^{-1}; NMR, see Tables 1−3; HRFABMS *m/z* 962.5090 $(M + Na)^+$ (calcd for $C_{48}H_{77}NO_{17}Na$, 962.5090).

Acacioside B {**3-*O*-[*β*-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl]-21-*O*-*trans*-cinnamoylacacic acid**} **(5)**: white amorphous solid, mp 282 (dec) °C; $[\alpha]^{25}_D$ +19.0° (*c* 3.40, MeOH); UV (MeOH) λ_{max} (log ϵ) 276 (3.51); IR (KBr) ν_{max} 3430 (br), 2940, 2920, 1700, 1660, 1640, 1560 cm^{-1}; NMR, see Tables 1−3; HRFABMS *m/z* 1108.5443 $(M + Na)^+$ (calcd for $C_{57}H_{83}NO_{19}Na$, 1108.5456).

Acacioside C {**3-*O*-[α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl]-21-*O*-*trans*-cinnamoylacacic acid**} **(6)**: white amorphous solid, mp 278(dec) °C; $[\alpha]^{25}_D$ +14.8° (*c* 3.40, MeOH); UV (MeOH) λ_{max} (log ϵ) 276 (3.48); IR (KBr) ν_{max} 3430 (br), 2945 2920, 1700, 1650, 1565 cm^{-1}; NMR, see Tables 1−3; HRFABMS *m/z* 1108.5443 $(M + Na)^+$ (calcd for $C_{57}H_{83}NO_{19}Na$, 1108.5456).

Echinocystic Acid. A solution of **3** (6.6 mg) in 2 mL of 1 N CF_3COOH was refluxed for 3 h. The solution was extracted with $CHCl_3$, and the organic layer was evaporated to dryness and then subjected to PTLC on Si gel with the solvent $CHCl_3$/MeOH (49:1) to give echinocystic acid (2.5 mg): white amorphous solid, mp 275 (dec) °C (lit.[12b] mp 279−281 (dec); $[\alpha]^{25}_D$ +24.8° (*c* 0.26, MeOH) (lit.[12b] $[\alpha]^{26}_D$ + 20.4°); ^1H NMR (C_5D_5N, 400 MHz) δ 5.65 (1H, brs, H-12), 5.23 (1H, brs, H-16), 3.75 (1H, dd, J = 14.0, 2.8 Hz, H-18), 3.45 (1H, dd, J = 10.3, 5.4 Hz, H-3), 1.76 (3H, s), 1.22 (3H, s), 1.19 (3H, s), 1.03 (6H, s), 1.00 (3H, s), 0.92 (3H, s); HRFABMS *m/z* 495.3468 $(M + Na)^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3498).

21-*O*-*trans*-Cinnamoylacacic Acid (7). To a solution of **6** (6.0 mg) in MeOH (1 mL) was added an equal volume of 3 N HCl, and the mixture was stirred at 80 °C for 4 h. After cooling, the solution was evaporated to dryness and the residue was partitioned between EtOAc and H_2O. The organic layer was separated on Si gel PTLC with the solvent $CHCl_3$/MeOH (49:1) as developing system to yield 2.5 mg of 21-*O*-*trans*-cinnamoylacacic acid (**7**): white amorphous solid, mp 292 (dec) °C; $[\alpha]^{25}_D$ +8.3° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 276 (3.74); IR (KBr) ν_{max} 3400 (br), 2940, 2920, 1690, 1660, 1640, 1580, 1560, 1460, 1420, 1360, 1210, 1190, 1030 cm^{-1}; HR-FABMS *m/z* 677.8323 $(M + Na)^+$ (calcd for $C_{39}H_{54}O_6Na$, 677.8323).

Formation of Acacic Acid Lactone (8) from Compound 7. Compound **7** (1.8 mg) was dissolved into 0.5 N KOH in MeOH (1.5 mL), and the solution was stirred for 1 h at room temperature. After removal of MeOH, the residue in MeOH (1 mL) was treated with 3 N HCl (1.5 mL) and stirred at 85 °C for 1.5 h. After drying the solvent, the reaction mixture was subjected to PTLC on a Si gel with the solvent $CHCl_3$/MeOH (49:1) to give acacic acid lactone (**8**, 0.2 mg): white amorphous solid; mp 250−254 °C (lit.[14c] 249−253 °C); FABMS *m/z* 471 (M^+ + H, 100), 461 (41), 453 (44), 435 (33), 369 (91), 332 (32); ^1H NMR (C_5D_5, 400 MHz) δ 5.37 (1H, t, J = 3.4 Hz, H-12), 4.54 (1H, dd, J = 11.2, 5.2 Hz, H-16), 4.24 (1H, d, J = 5.6 Hz, H-21), 3.42 (1H, dd, J = 10.1, 5.8 Hz, H-3), 1.33 (3H, s), 1.22 (3H, s), 1.05 (3H, s), 1.03 (3H, s), 0.93 (3H, s), 0.86 (3H, s), 0.82 (3H, s).

Sugar Identification by GC−MS. The procedure described previously was used to prepare and analyze the alditol acetates of the sugars.[9] Retention times of 10.6 and 15.6 min were observed for the alditol acetates of arabinose and *N*-acetylglucosamine, respectively.

Linkage Analysis. A solution of 1 mg of saponins **1**−**6** in anhydrous DMSO (1 mL) was treated with 100 mg of finely powdered dry NaOH, and the mixture was stirred for 1 h. Methyl iodide (400 μL) was added, and the solution was allowed to react for 40 min. The reactions were then quenched by the addition of 10 mL of $Na_2S_2O_3$ solution in water (100 mg/mL) and extracted with 2 mL of CH_2Cl_2. The organic layers were washed two times with water and evaporated under a stream of N_2 at 40 °C. To each sample 1 mL of 0.25 M sulfuric acid in 93% aqueous HOAc was then added and the solution incubated at 80 °C for 2.5 h. The solution was neutralized with 350 μL of 2 M NH_4OH and dried. Reduction was then achieved using 500 μL of 2.5 M NH_4OH and 500 μL of 1 M $NaBD_4$ in NH_4OH, with incubation at 60 °C for 1 h. The reaction mixture was quenched by the addition of 150 μL of HOAc and the sample was dried. The sample was then treated with 200 μL of 1-methylimidazole and 2 mL of Ac_2O for 10 min, quenched with water, and extracted with CH_2Cl_2 (2 × 1 mL). The combined organic layer was then washed with water, dried under a stream of N_2, and dissolved separately in MeOH. The MeOH solution was subjected to GC−MS analysis using a 30 × 0.32 mm i.d. HP5 column (5% phenyl methyl silicone) connected to the VG7070 E-HF mass spectrometer with an initial temperature of 95 °C and then temperature programming to 285 °C at a rate of 10 °C/min.

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Supporting Information Available: Figure showing sugar analysis by GC−MS. This material is available free of charge via the Internet at http://pubs.acs.org.

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