Antifungal and Molluscicidal Saponins from *Serjania salzmanniana*

Opinya A. Ekabo and Norman R. Farnsworth*

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

Thomas O. Henderson

Department of Biochemistry, College of Medicine, University of Illinois at Chicago, Chicago, Illinois 60612

Guohua Mao

Shaman Pharmaceuticals, Inc., 213 East Grand Avenue, South San Francisco, California 94080

Rabindranath Mukherjee

Laboratorio de Technologia Farmaceutica, Universidade Federal da Paraiba, Joao Pessoa, Brazil

Received November 23, 1994[⊗]

An investigation of *Serjania salzmanniana* for biologically active substances has led to the isolation of two novel saponins, salzmannianoside A (3-*O*-[[*β*-D-glucopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]gypsogenin) [**3**] and salzmannianoside B (3-*O*-[[*β*-D-glucopyranosyl-(1→4)]-[α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]hederagenin) (**4**). Two known saponins, pulsatilla saponin D (3-*O*-[[*β*-D-glucopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]hederagenin) (**1**) and 3-*O*-[[*β*-D-glucopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]oleanolic acid (**2**) were also isolated from this plant. The structures of **3** and **4** were elucidated by FABMS and 2D NMR techniques. All these four saponins were mollusicidal, causing 70−100% mortality at 10 ppm against *Biomphalaria alexandrina*, a vector of *Schistosoma mansoni* in the Nile Valley. The saponins also showed antifungal activity against *Cryptococcus neoformans* and *Candida albicans* at minimal inhibitory concentrations of 8 and 16 *μ*g/mL, respectively.

Schistosomiasis infects some 250 million people in Asia, Africa, and South America.[1] A number of synthetic drugs have been developed and used successfully in chemotherapy and snail control.[2] However, their development on a large scale is hampered by their environmental effect, especially on nontarget organisms, by the costs of the drugs to developing countries hard hit by the disease, and by snail resistance to the synthetic molluscicides.[3] These concerns have prompted a drive to develop alternative methods for controlling the disease.

Plant molluscicides have emerged as a viable alternative for this purpose. *Phytolacca dodecandra* L'Herit (Endod) was the first plant used successfully to control schistosomiasis in an endemic area of Ethiopia. Since the discovery of saponins as the potent molluscicidal principles in the fruits of *P. dodecandra*, several saponins and plant extracts, many of them in the Sapindaceae, have been evaluated for molluscicidal activity.[4−7] In light of the previous use of sapindaceous plants as molluscicides,[8,9] we were encouraged to evaluate the saponins from *Serjania salzmanniana* Schlecht. (Sapindaceae) for their molluscicidal action against *Biomphalaria alexandrina*.

Acid hydrolysis of **1** afforded the aglycon hederagenin, which was identified by comparing its ^{1}H-NMR and ^{13}C-NMR data with published values.[9,10] L-arabinose, D-glucose, and L-rhamnose were detected in the saponin hydrolysate by GC−MS analysis. Three anomeric peaks at 101.50, 104.35, and 106.60 ppm in the ^{13}C-NMR of **1** confirmed the presence of the three sugar residues. A molecular ion $[M + H]^+$, recorded in the HRFABMS, at *m/z* 913.5158 was in accordance with the elemental formula $C_{47}H_{77}O_{17}$ (calcd 913.5161).

The successive elimination of rhamnose, glucose, and arabinose from **1** (M^-, *m/z* 912, 100%) in the FABMS was indicated by the fragment ions at *m/z* 766 $[M - 146]^-$ (19%), 750 $[M - 162]^-$ (31%), 603 $[M - 309]^-$ (18%), and 470 $[M - 442]^-$. It appeared from this fragmentation scheme that the trisaccharide was branched with glucose and rhamnose tentatively placed at the termini of the chain.[11] The exact sequence of the sugar residues was elucidated by the CIMS of **1** acetate,[12] which exhibited intense peaks at *m/z* 331 (75%) and 273 (100%), each corresponding to peracetylated glucose and rhamnose, thus confirming their location at the termini. Results from both the CIMS and FABMS experiments enabled the gross structure rha-(glc)-ara-hederagenin to be proposed for **1**.

Interglycosidic linkages were established by NMR experiments. The assignment of the sugar proton resonances was achieved with COSY and NOESY NMR spectra. The reporter signals for rhamnose (H-1, H-6), glucose (H-1, H-5), arabinose (H-1), and the aglycon (H-3, H-12, H-18) served as the entry points in the analysis of the cross peaks of the 2D NMR spectra. In selective INEPT NMR experiments, presaturation of H-1′, H-1″, and H-1‴ caused polarization transfer to C-3, C-2′, and C-4′, in that order. These results pointed to the attachment of glucose to C-4′ and rhamnose to C-2′ of arabi-

⊗ Abstract published in *Advance ACS Abstracts,* March 15, 1996.

0163-3864/96/3259-0431$12.00/0

nose. All these data taken together permitted the identification of **1** as 3-*O*-([β-D-glucopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)hederagenin.

An antifungal prosapogenin of **1**, hederagenin 3-*O*-α-L-arabinopyranoside, was recently reported from *Collinsonia canadensis* (Labiatae).[10] Kang[13] and Mineo *et al.*[14] have isolated from *Pulsatilla koreana* and *Pulsatilla cerma* pulsatilla saponin D, which is identical to **1**. The previous structural elucidation of **1** was based largely on chemical degradation and limited NMR spectrosopy.

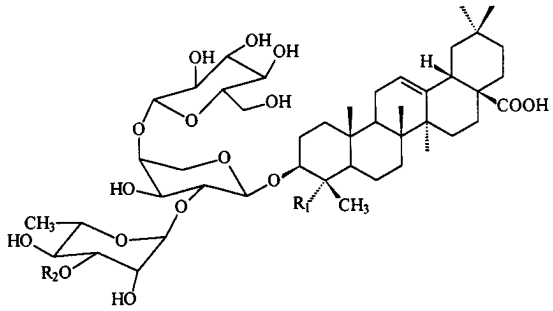
The second saponin (**2**) had a molecular ion, $[M + Na]^+$, at *m/z* 919 in the LRFABMS. The $[M - H]^-$ ion fragmented, further liberating identical sugar moieties as in **1** and the aglycon ions at *m/z* 455 [oleanolic acid $- H]^-$ and 439 [oleanolic acid $- H_2O]^-$. Comparison of the ^{1}H-NMR and ^{13}C-NMR spectra of **2** with those of **1** revealed that the trisaccharide was made up of β-D-glucose, α-L-rhamnose, and α-L-arabinose with the same interglycosidic linkages. The two compounds, **1** and **2**, were essentially identical except that **2** had oleanolic acid for a genin. The complete ^{1}H-NMR and ^{13}C-NMR assignments of **2** have been verified by ^{1}H−^{1}H-COSY and ^{1}H−^{13}C-HETCOR NMR methods. The structure of **2** was identified as 3-*O*-[[β-D-glucopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]oleanolic acid. A search of the literature showed that **2** was reported earlier by Schenkel *et al.* from a Brazilian ichthyotoxic plant, *Thinuoia coriacea* Britton (Sapindaceae).[15]

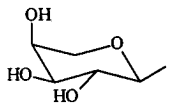
Saponin **3** had a NMR profile similar to that of **1**. The pattern of the ^{13}C-NMR signals of **3** aglycon was clearly indicative of a triterpene of the oleanene type but lacked in the resonance at 65.32 ppm for C-23 (hydroxymethyl) in hederagenin. However, peaks for a gypsogenin aldehydic group at C-23 and a tertiary methyl at C-24 were observed at 207.68 and 10.70 ppm, respectively. The molecular formula $C_{47}H_{74}O_7$ was estimated from the ^{13}C-NMR data and confirmed by a molecular ion, $[M + Na]^+$, in the HRFABMS at *m/z* 933.4813 (calcd 933.4824). The overall gross structure of **3** was similar to that of **1**, except that the aglycon was gypsogenin, which was confirmed by an aldehyde proton signal at 9.72 ppm in the ^{1}H-NMR spectrum. Further simplification of the ^{1}H-NMR spectrum was best achieved by partial relaxation of the sugar protons using the standard T_1 pulse sequence. This method resolved rhamnose H-3′, H-4″, H-5″, arabinose H-1′, H-3′, H-4′, and the aglycon H-3.

The HETCOR NMR spectrum of **3** showed glycosidation shifts for the aglycon C-3, arabinose C-2′, and C-4′. It was inferred that the interglycosidic linkages were similar to those of **1**. This was confirmed by selective INEPT experiments carried out in pyridine and MeOH. The structure, 3-*O*-[[β-D-glucopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]gypsogenin has been assigned to **3**, which we have named salzmannianoside A.

Compound **4** was given the trivial name salzmannianoside B and was assigned the molecular formula $C_{52}H_{85}O_{21}$ on the basis of its NMR data and a pseudomolecular ion, $[M + Na]^+$ in the HRFABMS at *m/z* 1067.5409 (calcd 1067.5403). The negative ion FABMS had a quasi-parent ion, $[M - H]^-$, at *m/z* 1043, which fragmented with elimination of 2 mol of arabinose and 1 mol each of glucose and rhamnose. The sugars were identified by comparing the NMR spectra of **4** with those of **1** and **2** and published data.[16] Intense fragment peaks in the CIMS of **4** acetate at *m/z* 331 (40%) and 259 (62%) suggested a branched tetrasaccharide with glucose and arabinose at the termini.

The ^{13}C-NMR spectrum of **4** in pyridine-d_5 exhibited four anomeric peaks at 106.83, 106.67, 104.40, and 101.06 ppm for four sugar units consistent with the FABMS data. A casual examination of the ^{13}C-NMR spectrum of **4** indicated that the linkages of the sugars and their anomeric configuration were the same as in **1**. An unambiguous assignment of the interglycosidic linkages was made possible by application of the Hartman−Hart method (HOHAHA), HETCOR, and selective INEPT NMR experiments.



	R_1	R_2
1	CH_2OH	H
2	CH_3	H
3	CHO	H
4	CH_2OH	

The HETCOR spectrum of **4** indicated correlations between H-3″, H-3, H-2′, H-4′, and their respective carbons downfield at 82.57, 81.05, 75.37, and 80.63 ppm. The other interglycosidic linkages were established by selective INEPT NMR studies. Irradiation of the anomeric protons H-1‴ and H-1⁗ caused polarization transfer from these protons to rhamnose C-4′ and C-3′, respectively. Polarization transfer from H-1″ to C-3″, C-5″, and C-2′ were consistent with the glycosidation shifts observed in **1** and **2**. It appears from these results that the terminal arabinose was bound to C-3″ of rhamnose, which in turn was attached to the inner arabinose on one side at C-2′, while glucose was attached on the other side at C-4′. Saponin **4** was accordingly identified as 3-*O*-[[β-D-glucopyranosyl-(1→4)]-[α-L-arabinopyranosyl-(1→3)-α-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]hederagenin.

The monodesmosidic saponins **1**−**4** were evaluated for molluscicidal activities against *B. alexandrina*, a vector of *Schistosoma mansoni*, and for antifungal activity against *C. albicans* (CA, ATCC 10259), *C. neoformans* (CN, ATCC 36556), and *Aspergillus fumigatus* (AF, ATTC 13073). The results of the molluscicidal and antifungal assays are summarized in Tables 3 and 4.

Table 1. ^{1}H-NMR Data of **1**−**4** in Pyridine-d_5[a]

sugar unit	proton	**1**	**2**	**3**	**3**[b]	**4**
arabinose	1′	4.99, d, J = 6.8	4.81, m	4.64, d, J = 6.2	4.23, d, J = 6.1	4.92, m
	2′	4.47−4.55, m	4.65, m	4.42−4.47, m	3.64, m	4.51, m
	3′	4.06, dd, J = 4.0	4.29, m	4.18, dd, J = 2.6, 5.3	3.73, m	3.87 = 3.89, m
	4′	4.16−4.26, m	4.22−4.30	4.27, m	3.86, m	4.17, m
	5′	3.66, d, J = 11.6, 4.47−4.55, m	3.85, d, J = 11.7, 4.43, m	3.73, d, J = 11.6, 4.42−4.47, m	3.49, d, J = 11.8, 4.14, dd, J = 3.2, 11.8	4.17−4.25, m, 3.75, d, J = 11.5
rhamnose	1″	6.28, s	6.21, s	6.16, s	5.17, s	6.35, s
	2″	4.72−4.76, m	4.78−4.81, m	4.69, s	3.86, m	4.94, m
	3″	4.64, dd, J = 3.2, 6.3	4.65, m	4.59−4.63, m	3.68, m	4.74−4.77, m
	4″	4.31, dd, J = 9.2, 9.2	4.35, m	4.32, dd, J = 9.3, 9.3	3.38, m	4.47,m
	5″	4.72−4.76, m	4.65, m	4.59−4.63, m	3.86, m	4.74−4.77, m
	6″	1.65, d, J = 6.1	1.67, d, J = 5.9	1.72, d, J = 6.3	1.26, d, J = 6.1	1.58, d, J = 6.0
glucose	1‴	5.13,d, J = 7.7	5.17, d, J = 7.6	5.12, d, J = 7.7	4.43, d, J = 7.6	5.10, d, J = 7.7
	2‴	4.02, dd, J = 8.3, 8.3	4.07, dd, J = 7.8, 7.8	4.04, dd, J = 7.7, 7.7	3.31, m	4.01, dd, J = 8.2, 8.2
	3‴	4.13−4.19, m	4.22−4.30, m	4.21−4.24, m	3.38, m	4.16, m
	4‴	4.16−4.25, m	4.22−4.30, m	4.21−4.24, m	3.31, m	4.17, m
	5‴	3.88−3.92, m	3.93, m	3.91−3.94, m	3.38, m	3.87−3.89, m
	6‴	4.36−4.40, m 4.47−4.55, m		4.53, d, J = 11.1, 4.36−4.47, m	3.53−3.62, m, 3.8, m	4.51, m, 4.36, m
terminal arabinose	1⁗					5.31, d, J = 7.2
	2⁗					4.54, m
	3⁗					4.11, m
	4″					4.17−4.25, m
	5⁗					3.58, d, J = 12.9, 4.34−4.38, m

[a] Spectra were recorded at room temperature at 300 or 360 MHz except for **3**. The internal standard was TMS. J values are in Hz. [b] Spectra recorded in MeOH-d_4.

Table 2. ^{13}C-NMR Data of **1**−**4** in Pyridine-d_5[a]

sugar unit	carbon	**1**	**2**	**3**	**3**[b]	**4**
arabinose	1′	104.35	104.81	102.16	102.44	104.40
	2′	76.07	76.19	75.69	76.01	75.34
	3′	74.85	73.84	74.15	74.08	75.25
	4′	80.38	79.46	79.72	79.50	80.58
	5′	65.42	64.37	64.90	65.00	65.70
rhamnose	1″	101.50	101.61	101.49	101.45	101.06
	2″	72.11	72.28	72.29	72.13	71.68
	3″	72.15	72.14	72.35	72.01	82.54
	4″	73.85	73.84	74.15	73.98	72.79
	5″	69.57	69.70	69.61	69.84	69.28
	6″	18.57	18.50	18.65	17.99	18.36
glucose	1‴	106.60	106.20	106.44	106.03	106.67
	2‴	75.28	75.31	75.45	75.28	75.34
	3‴	78.25	78.31	78.50	77.80	78.28
	4‴	71.02	71.12	71.23	71.28	70.94
	5‴	78.64	78.59	78.79	78.03	78.55
	6‴	62.29	62.36	62.52	62.62	62.23
terminal arabinose	1⁗					106.83
	2⁗					72.61
	3⁗					74.01
	4⁗					69.01
	5⁗					65.40
aglycon	3					81.03
	23					63.73

[a] Spectra were acquired at room temperature at 75 or 90 MHz. Chemical shifts are referenced on solvent peaks recorded in pyridine-d_5 (135.5 ppm). Spectra were recorded in MeOH-d_4 (49 ppm).

Table 3. Molluscicidal Activity of Saponins **1**−**4** Against *B. alexandrina*[a]

compound	24-h exposure		24-h exposure + 24-h recovery		% kill after 48 h	
	I	II	I	II	I	II
1	10/10	9/10	10/10	9/10	100	90
2	10/10	10/10	10/10	10/10	100	100
3	10/10	0/10	10/10	0/10	100	0
4	5/10	9/10	7/10	10/10	70	100
H_2O (control)	0/10	0/10	0/10	0/10	0	0
2% aqueous EtOH (control)	0/10	0/10	0/10	0/10	0	0

[a] The activities are expressed as the number of dead snails/total snails treated at (I) 20 ppm and (II) 10 ppm of the test compund in 2% aqueous EtOH.

Table 4. Antifungal Activity of Saponins **1**, **3**, and **4**[a]

compound	*C. albicans*	*C. neoformans*	*A. fumigatus*
1	≤16	≤8	≥250
3	≤16	≤8	≥125
4	≥125	≤8	≥250

[a] The activities are expressed as the minimum inhibitory concentration (MIC), which is the lowest concentration of saponin resulting in complete inhibition of growth of fungus. MICs are in μg/mL. Compound **2** was not tested in this assay.

Experimental Section

General Experimental Procedures. The analytical equipments, including the chromatographic and spectra techniques used in this investigation, have been described previously.[17] The TOCSY NMR spectrum was recorded on a General Electric GN Omega 500 MHz instrument. Selective INEPT NMR experiments were performed using routine pulse sequences with a Nicolet NT-360 instrument. The average long-range proton-carbon coupling constants are shown with a range of values in brackets for (a) sugars, $^3J_{H1OAr}$ = 7 Hz (6−8 Hz), $^3J_{H1OC}$ = 4 Hz (4−6 Hz), where H1 is the anomeric proton and Ar is aryl group, and (b) the genin and the other sugar protons, ^2J, $^3J_{HCOAr}$ = 7 Hz (6−8 Hz), ^2J, $^3J_{HCOC}$ = 5 Hz (4−6 Hz). Sugar analysis by GC-MS was performed according to the procedure of Hussain *et al.*[18]

Plant Material. *S. salzmanniana* Schlecht., known locally as "Cipo-Cururu", was collected from the wet forest of Santa Rita, Paraiba, Brazil, in April 1991, by one of us (R. M.) A voucher specimen, No. B1017, was authenticated by Mrs. Maria de F. Agna, Laboratorio de Technologia Farmaceutica, Universidade Federal da Paraiba, Joao Pessoa, Brazil, and deposited in the John G. Searle Herbarium, Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The MeOH extract of the powdered stems of *S. salzmanniana* (10 kg) was evaporated *in vacuo* to yield 16% dry wt of residue. An aqueous alcoholic suspension (MeOH−H_2O 1:2 v/v) of the residue was extracted three times with $CHCl_3$ (2 L). The combined $CHCl_3$ extract was dried as above, and the residue was submitted to Si gel column chromatography. The column was eluted initially with $CHCl_3$, followed by addition of MeOH in 5−10% increments. Fractions were collected in 500-mL aliquots and pooled according to their TLC profile in $CHCl_3$−MeOH−H_2O (7:3:1) (organic phase). Fractions with R_f values between 0.04 and 0.14 were pooled and rechromatographed by countercurrent partition chromatography in $CHCl_3$−MeOH−H_2O (7:3:1) where the organic phase was applied as the mobile phase. Final purification of the fractions containing saponins **1**−**4** was achieved by Si gel column chromatography in EtOAc−MeOH−H_2O (18:7:10) (upper phase) or preparative TLC in the same solvent system. The fractions were monitored by TLC, and the spots were visualized by spraying with 10% H_2SO_4, then heated for 2 min at 300 °C. The bands in the preparative TLC separation were visualized by spraying with water (white on a dark background).

Pulsatilla saponin D (3-*O*-[[β-D-glucopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]hederagenin) (1): R_f = 0.09, $CHCl_3$−MeOH−H_2O (7:3:1) organic layer, $[\alpha]_D$ + 6.62° (*c* 0.136, EtOH); HRFABMS *m/z* $[M + H]^+$ 913.5158 (calcd 913.5161 for $C_{47}H_{77}O_{17}$); FABMS *m/z* 912 $[M]^-$ (100), 766 $[M - rha]^-$ (19), 750 $[M - glc]^-$ (31), 603 $[M - rha - glc]^-$ (17.8), 470 $[M - glc - rha - ara]^-$ (32). **1** Acetate: CIMS *m/z* 331 $[glcAc_4]$ (81), 273 $[rhaAc_3]$ (100), 169 (20), 111 (10); hederagenin, EIMS (70 eV) *m/z* 472 $[M]^+$ (0.5), 455 $[M - H_2O]^+$ (1), 248 (100), 203 (75), 189 (15); ^{13}C-NMR and ^{1}H-NMR data, see Tables 1 and 2.

3-*O*-[[β-D-Glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]oleanolic acid (2): R_f = 0.13, $CHCl_3$−MeOH−H_2O (7:3:1), $[\alpha]_D$ −6.11° (*c* 0.135, EtOH); HRFABMS *m/z* 919.5030 $[M + Na]^+$ (calcd 919.5031 for $C_{47}H_{76}O_{16}$·Na); FABMS *m/z* 896 $[M]^-$ (62), 895 $[M - H]^-$ (100), 749 $[M - rha]^-$ (23), 732 $[M - glc]^-$ (49), 617 $[M + H - rha - ara]^-$ (15); FABMS *m/z* 920 $[M + H + Na]^+$ (52), 919 $[M + Na]^+$ (100), 757 [M+Na − glc]$^+$ (3), 455 [oleanolic acid − H]$^+$, 439 [oleanolic acid − H_2O]$^+$ (12), 207 (50), 115 (100). **2** Acetate: positive FABMS *m/z* 1272 $[M + H + K]^+$ (22), 331 $[glcAc_4]^+$ (32), 273 $[rhaAc_4]^+$ (36), 192 (54), 184 (45), 169 (88), 111 (100), 109 (71). ^{13}C-NMR and ^{1}H-NMR data, see Tables 1 and 2.

Salzmannianoside A (3-*O*-[[β-D-pyranosyl(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]gypsogenin) (3): R_f = 0.11, $CHCl_3$−MeOH−H_2O (7:3:1), HRFABMS *m/z* 933.4813 (calcd 933.4824 for $C_{47}H_{74}O_{17}$·Na); positive FABMS *m/z* 1005 [M + 3H + glycerol]$^+$ (12), 1004 [M + 2H + glycerol]$^+$ (16), 911 $[M + H]^+$ (100), 748 $[M + H - glc]^+$ (28), 601 [M + H − glc − Rh]$^+$ (13), 471 [hederagenin + H]$^+$, 453 [hederagenin + H − H_2O]$^+$ (16), 407 [hederagenin − H_2O − COOH]$^+$ (10), 189 (42), 147 (100), 115 (70). **3** Acetate: CIMS *m/z* 331 $[glcAc_4]^+$ (7), 273 $[rhaAc_3]^+$ (14), 169 $[C_6H_6O_2Ac]^+$ (52), 111 $[C_6H_6O_5]^+$ (29), 101 (90), 85 (100); FABMS *m/z* 909 $[M - H]^-$ (100), ^{13}C-NMR and ^{1}H-NMR in Tables 1 and 2.

Salzmannianoside B (3-*O*-[[β-D-glucopyranosyl-(1→4)]-[α-L-arabinopyranosyl-(1→3)-α-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl]hederagenin) (4): R_f = 0.06, $CHCl_3$−MeOH−H_2O (7:3:1), HRFABMS *m/z* 1067.5409 $[M + Na]^+$ (calcd 1067.5403 for $C_{52}H_{84}O_{21}$·Na). FABMS *m/z* 1043 $[M - 1]^-$ (100); FABMS *m/z* 1045 $[M + H]^+$ (100), 914 [M + H − ara]$^+$, 883 [M + H − glc]$^+$, 751 [M + H − ara − glc]$^+$. **4** Acetate: FABMS *m/z* 331 $[glcAc_4]^+$ (40), 259 $[araAc_3]^+$ (62), 187 (73), 171 (43), 169 $[C_6H_6O_2Ac]^+$ (97), 157 (80), 139 (100), 127 $[C_6H_7O_3]^+$ (43), 111 $[C_6H_7O_5]^+$ (73), 109 $[C_6H_5O_2]^+$ (69); ^{13}C-NMR and ^{1}H-NMR data, see Tables 1 and 2.

Molluscicidal Assay. Molluscicidal activity was tested against *B. alexandrina* according to established procedures.[19,20] Tests were done in duplicate with snails ranging from 8 to 14 mm in diameter. Ten snails were used for each test in 50 mL of aged tap H_2O maintained between 20 and 21 °C. In a typical experiment, snails were immersed in aqueous solutions containing 20 ppm of the test compound and 2% EtOH for 24 h. Control snails were tested in identical solutions without the test compounds and in plain H_2O. Surviving snails were washed in aged tap H_2O and allowed to recover for an additional 24 h.

Antifungal Assay. The saponins were tested for antifungal activity according to a revised microplate broth assay developed by one of us (G.M.). All tests were performed in sterile 96-well plastic plates using Sabouraud dextrose broth (SAB broth, Difco, Detroit, MI). The log phase inoculum was obtained for *C. albicans* and *A. fumigatus* by incubating the yeasts in broth for 4−6 h at 35 °C with rotation at 150 rpm. A conidial suspension of *A. fumigatus* was obtained from 96-h growth on Sabouraud dextrose agar plates. Compounds were dissolved in either H_2O, DMSO, EtOH, or an appropriate combination of these solvents. Solutions of each test compound were further diluted with Sabouraud dextrose broth to yield a stock solution of 500 μg/mL. Each sample solution was diluted serially in the microtiter plates using a 75-μL volume. Test wells were inoculated with each of the test fungi by adding 75 μL of the prepared inoculum to all wells such that the final concentration of fungi was 2 × 103 cfu of *C. albicans*, 2 × 104 cfu of *C. neoformans*, and 1 × 103 cfu of *A. fumigatus*. The plates were incubated for 24 h at 35 °C for *Cr. albicans* and 48 h for *C. neoformans* and *A. fumigatus*. The minimum inhibitory concentration (MIC) was determined visually. MIC is defined as the lowest concentration of test compound resulting in the complete inhibition of growth. Amphotericin B (Deerfield, IL) was used as a positive control.

Acknowledgment. We acknowledge the Nuclear Magnetic Resonance and Mass Spectrometry Laboratories of the Research Resources Center, University of Illinois at Chicago, for providing assistance and the spectroscopic equipment used in this study. We thank Mr. E. Robbins of Research Resources Center for recording the CIMS and Dr. K. Zaw and Mr. R. Dvorak of the Department of Medicinal Chemistry and Pharmacognosy for recording the TOCSY and FABMS), respectively. We thank Dr. Mung Sook Chung for performing the GC−MS experiments and Drs. Nam-In Baek and Roberto Gil for helpful discussions.

References and Notes

(1) D'Arcy, P. F., Harron, D. W. G. *Pharm. Int*. **1983**, *4*, 16−20.
(2) Webb, G. In *Plant Molluscicides*, Mott, K. E., Ed. John Wiley and Sons: Chichester, 1987; pp 1−26.
(3) Hostettmann, K. *Naturwissenschften* **1984**, *71*, 247−251.
(4) Farnsworth, N. R.; Henderson, T. O.; Soejarto, D. D. In *Plant Molluscicides*, Mott, K. E., Ed. John Wiley and Sons: Chichester, 1983; pp 131−204.
(5) Henderson, T. O.; Farnsworth, N. R.; Myers, T. C. In Plant Molluscicides Mott, K. E., Ed. John Wiley and Sons: Chichester, 1983; pp 109−130.
(6) Kloos, H.; McCullough, F. S. *Planta Med*. **1982**, *46*, 195−209.
(7) Duncan, J.; Lemma, A. *Ethiopian Med. J*. **1976**, *14*, 3−15.
(8) Mozley, A. *Trans. Royal Soc. Edinburgh* **1939**, *59*, 687−689.
(9) Hamburger, M.; Slacanin, I.; Hostettmann, K.; Wahjo, D.; Sutarjadi. *Phytochem. Anal*. **1992**, *3*, 231−237.
(10) Joshi, B. S.; Moore, K. M.; Pelletier, S. W.; Puar, M. S.; Pramanik, B. N. *J. Nat. Prod*. **1992**, *55*, 1468−1476.
(11) Wolfender, J. L.; Maillard, M.; Marston, A.; Hostettmann, K. *Phytochem. Anal*. **1992**, *3*, 193−214.
(12) Budzikiewicz, H.; Djerassi, C.; Williams, D. H. *Structure Elucidation of Natural Products by Mass Spectrometry*, Holden-Day: San Francisco, 1964; Vol. II: Steroids, Terpenoids, Sugars and Miscellaneous Classes, Chapter 27, pp 203−238.
(13) Kang, S. S. *Arch. Pharmacal. Res*. **1989**, *21*, 42−47.
(14) Mineo, S.; Kenichi, S.; Naokata, M.; Kizu, H.; Tsuyoshi, T. *Chem. Pharm. Bull*. **1978**, *26*, 1666−1671.
(15) Schenkel, E.-P.; Werener, W.; Schulte, K. E. *Planta Medica* **1991**, *57*, 463−467.
(16) Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307−3330.
(17) Ekabo, O. A.; Farnsworth, N. R.; Santisuk, T.; Reutrakul, V. *J. Nat. Prod*. **1993**, *56*, 699−707.
(18) Hussain, R.; Lin, Y.-M.; Poveda, L. J.; Bordas, E.; Chung, M. S.; Pezzuto, J. M.; Soejarto, D. D.; Kinghorn, A. D. *J. Ethnopharmacol*. **1990**, *28*, 103−115.
(19) Mott, K. E. In *Plant Molluscicides*, Mott, K. E. Ed.; John Wiley and Sons: Chichester, 1987; pp 321−323.
(20) Ngassapa, O. D.; Farnsworth, N. R.; Henderson, T. O.; Beecher, C. W. W.; Boye, G. L. *Bull. Chem. Soc. Ethiopia* **1989**, *3*, 91−96.

NP960208R