Notes

New Triterpene Glycosides from the Stems of *Anomospermum grandifolium*

Alberto Plaza,[†] Marina Cinco,[‡] Aurelia Tubaro,[§] Cosimo Pizza,*[,†] and Sonia Piacente[†]

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy, Department of Biomedical Sciences, Universitá degli Studi di Trieste, Via A. Fleming 22, 34127 Trieste, Italy, and DEMREP, Universitá degli Studi di Trieste, Via A. Valerio 6, 34127 Trieste, Italy

Received June 24, 2003

Two new dammarane saponins identified as jujubogenin 3-*O*-α-L-arabinofuranosyl(1→2)-[β-D-glucopyranosyl(1→6) β-D-glucopyranosyl(1→3)]-α-L-arabinopyranoside (**2**) and jujubogenin 3-*O*-α-L-arabinofuranosyl-(1→2)-{6-*O*-[3-hydroxy-3-methylglutaryl]-β-D-glucopyranosyl(1→3)}-α-L-arabinopyranoside (**3**) and a new lupane saponin, 3β-hydroxylup-20(29)-en-27,28-dioic acid 28-*O*-β-D-glucopyranosyl(1→2)-[β-D-xylopyranosyl(1→3)]-β-D-xylopyranosyl(1→2)-β-D-glucopyranoside ester (**5**), along with the known jujubogenin 3-*O*-α-L-arabinofuranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-α-L-arabinopyranoside (**1**) and 3β-hydroxylup-20(29)-ene-27,28-dioic acid (**4**), were isolated from the methanol extract of the stems of *Anomospermum grandifolium*. The structures of the new compounds were established by spectral analysis. Antimicrobial activity screening of compounds **1**−**3** revealed antifungal properties against *C. albicans* ATCC 3153 for compounds **2** and **3**. The antibacterial and antifungal activities of the petroleum ether, chloroform, and methanol extracts of *A. grandifolium* stems were also evaluated.

In our ongoing research for new bioactive compounds from medicinal plants of the Peruvian rainforest, the stems of "Icu" (*Anomospermum grandifolium* Eichler; Menispermaceae) have been studied. This liana grows in the Departments of Huánuco, Madre de Dios, San Martín and Ucayali in the Peruvian rainforest at altitudes between 0 and 1000 m above sea level, where it is traditionally used as an ingredient of curare.[1] Previous studies on its leaves and/or stems have reported the presence of alkaloids, and the extracts of the stems have also shown a curare-like action.[2,3] Because other species of South American Menispermaceae have shown pharmacological activities,[4,5] a phytochemical study has been performed in order to isolate the secondary metabolites of the stems. Two new jujubogenin glycosides (**2** and **3**) and one new lupane-type glycoside (**5**) were isolated, along with two known saponins (**1** and **4**). Since jujubogenin glycosides have been reported to possess antifungal and antibacterial activity as well as antisweet activity,[6−10] the antifungal and antimicrobial activities of the three jujubogenin glycosides (**1**−**3**) were evaluated, as well as those of the petroleum ether, chloroform, and methanol extracts from *A. grandifolium* stems.

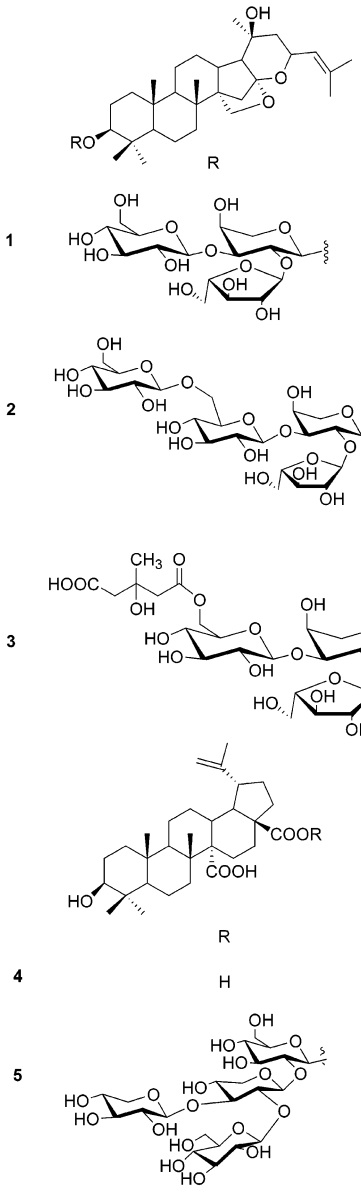
The dried stems of *A. grandifolium* were subjected to sequential extraction with petroleum ether, chloroform, and methanol. The methanol extract was fractionated on Sephadex LH-20. Repeated column chromatography of the fractions containing triterpenoid glycosides on reversed-phase HPLC or droplet countercurrent chromatography (DCCC) yielded three new compounds, **2**, **3**, and **5**, along with two known compounds, **1** and **4**.

Compound **1**, the major saponin in this plant, showed a quasimolecular ion peak in the negative ESIMS at *m*/*z* 897 [M − H]$^-$. It was identified as jujubogenin 3-*O*-α-L-arabinofuranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-α-L-arabinopyranoside by comparison of its ^{1}H and ^{13}C NMR spectra with those reported by Li et al. for the same compound from *Colubrina retusa*.[6]

Compound **2** showed a quasimolecular ion peak at *m*/*z* 1059 [M − H]$^-$ in the negative ESIMS. In conjunction with the analysis of the ^{13}C NMR and HSQC spectrum, its molecular formula was deduced by HRESIMS to be $C_{52}H_{84}O_{22}$. The ^{1}H and ^{13}C NMR chemical shifts of the aglycon moieties of **1** and **2** were almost superimposable. Thus, the genin structure was assigned as jujubogenin on the basis of ^{1}H and ^{13}C NMR spectra and the correlations observed in the ^{1}H−^{1}H DQF-COSY, HSQC, and HMBC experiments (see Experimental Section). Resonances of the anomeric protons were observed in the ^{1}H NMR spectrum (Table 1) at δ 4.37 (d, J = 7.5 Hz), 4.43 (d, J = 7.0 Hz), 4.55 (d, J = 7.5 Hz), and 5.33 (d, J = 1.3 Hz). The chemical shifts of all the individual protons of the four sugar units were ascertained from a combination of 1D TOCSY and ^{1}H−^{1}H DQF-COSY spectral analysis, and the ^{13}C NMR chemical shifts (Table 2) of their attached carbons could be assigned unambiguously from the HSQC spectrum. These data showed that **2** differed from **1** only in the occurrence of an additional β-glucose unit. The presence of this sugar on the inner glucose C-6‴ (δ 70.1) in **2** shifted this carbon signal downfield when compared with compound **1**, in which it resonated at δ 62.1. Finally, direct connectivity information was obtained from the HMBC spectrum, which showed a correlation between H-1⁗ of the terminal β-glucose at δ 4.37 (d, J = 7.5 Hz) and C-6‴ (δ 70.1) of the inner glucose. The configurations of the sugar units were assigned after hydrolysis of **2** with 1 N HCl. The hydrolysate was trimethylsilated, and GC retention times of each sugar were compared with those of the authentic samples prepared in the same manner. In this way the sugar units of **2** were determined to be L-arabinose and D-glucose in the ratio 1:1. On the basis of all of this

* To whom correspondence should be addressed. Tel: ++39089962813. Fax: ++39089962828. E-mail: pizza@unisa.it.
[†] Università degli Studi di Salerno.
[‡] Department of Biomedical Sciences, Università degli Studi di Trieste.
[§] DEMREP, Università degli Studi di Trieste.

10.1021/np030283j CCC: $25.00
Published on Web 11/13/2003



evidence, the structure of the new compound **2** was established as jujubogenin 3-*O*-α-L-arabinofuranosyl(1→2)-[*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl(1→3)]-α-L-arabinopyranoside.

Compound **3** showed a quasimolecular ion peak at *m*/*z* 1041 $[M - H]^-$ and significant fragment ion peaks at *m*/*z* 997 $[M - H - 44]^-$ and *m*/*z* 897 $[M - H - 144]^-$ in the negative ESIMS. In conjunction with the analysis of the ^{13}C NMR and HSQC spectrum, its molecular formula was deduced by HRESIMS to be $C_{52}H_{82}O_{21}$. It displayed a ^1H NMR spectrum similar to **1** with the exception of additional signals at *δ* 1.33 (s), 2.35 (d, *J* = 15.5 Hz), 2.56 (d, *J* = 15.5 Hz), 2.57 (d, *J* = 15.5 Hz), and 2.63 (d, *J* = 15.5 Hz). Detailed ^{13}C NMR spectral analysis led to the identification of dicrotalic acid (3-hydroxy-3-methylglutaric acid) (Tables 1 and 2).[11] In the ESIMS, the $[M - H - 144]^-$ ion at *m*/*z* 897 was compatible with the derived structure. The linkage position of the dicrotalic acid unit to the sugar moiety was determined by 2D NMR spectra in the following manner. It was observed that the signals of H_2-6 of glucose were significantly shifted to downfield values of *δ* 4.17 (dd, *J* = 4.5, 12.0 Hz) and 4.50 (dd, *J* = 2.5, 12.0 Hz) when compared with compound **1**, in which they resonated at *δ* 3.72 (dd, *J* = 4.5, 12.0 Hz) and 3.88 (dd, 2.5, 12.0 Hz), respectively. This indicated that the dicrotalic acid was linked at the C-6‴ position of the glucose. Direct connectivity information was obtained from the HMBC spectrum, which showed a correlation between the proton signal of H_2-6‴ of glucose at *δ* 4.17 (dd, *J* = 4.5, 12.0 Hz) and the carboxylic carbon signal of the dicrotalic acid unit at *δ* 177.0. Also in this case, the D configuration of the glucose unit and the L configuration of the arabinose units were determined by acid hydrolysis of **3** followed by GC analysis. Thus, the new compound **3** was identified as jujubogenin 3-*O*-α-L-arabinofuranosyl(1→2)-{6-*O*-[3-hydroxy-3-methylglutaryl]-*β*-D-glucopyranosyl(1→3)}-α-L-arabinopyranoside.

Compound **4** showed a quasimolecular ion peak at *m*/*z* 485 $[M - H]^-$ in the negative ESIMS. Its ^{13}C NMR spectrum showed 30 signals corresponding to five methyls, 11 methylenes, six methines, and eight quaternary carbons, which indicated this compound is a lupane-type triterpene.[12] A combination of 1D and 2D NMR techniques led to the assignment of two carboxylic functions in C-27 and C-28 and the identification of compound **4** as the 3*β*-hydroxylup-20(29)-ene-27,28-dioic acid previously isolated by Lee.[13]

Compound **5** showed a quasimolecular ion peak at *m*/*z* 1073 $[M - H]^-$, and the molecular formula $C_{52}H_{82}O_{23}$ was determined by HRESIMS. Furthermore, fragment ion peaks at *m*/*z* 911 $[M - H - 162]^-$, 779 $[M - H - 162 - 132]^-$, 647 $[M - H - 162 - 132 - 132]^-$, and 485 $[M - H - 162 - 132 - 132 - 162]^-$ in the negative ESIMS indicated the loss of two pentose units (132 mass units) and two hexose units (162 mass units). It was apparent from the ^1H and ^{13}C NMR data of **5** that this compound is based on the same aglycon as **4** (see Experimental Section). Additionally for **5**, resonances of anomeric protons were observed in the ^1H NMR spectra at *δ* 4.59 (d, *J* = 7.5 Hz), 4.78 (d, *J* = 7.5 Hz), 4.82 (d, *J* = 7.9 Hz), and 5.59 (d, *J* = 7.5 Hz). 1D-TOCSY, DQF-COSY, and HSQC NMR experiments showed the presence of two *β*-xylopyranosyl units (*δ* 4.59 and 4.78) and two *β*-glucopyranosyl units (*δ* 4.82 and 5.59) (Table 1). The HSQC spectrum also showed glycosylation shifts for C-2′ (*δ* 81.3) of the glucopyranosyl unit with the anomeric proton at *δ* 5.59 and C-2″ (*δ* 81.6) and C-3″ (*δ* 86.3) of the xylopyranosyl unit with the anomeric proton at *δ* 4.78. An unambiguous determination of the sequence and linkage sites was obtained from the HMBC correlations. Key correlation peaks were observed in the HMBC spectrum of **5** between H-1′ of the glucopyranosyl unit at *δ* 5.59 and the carboxylic function of the aglycon at C-28 (*δ* 178.6), H-1″ of the xylopyranosyl unit at *δ* 4.78 and C-2′ (*δ* 81.3) of the glucopyranosyl unit, H-1‴ of the terminal glucopyranosyl unit at *δ* 4.82 and C-2″ (*δ* 81.6) of the inner xylopyranosyl unit, and finally between H-1⁗ of the terminal xylopyranosyl unit at *δ* 4.59 and C-3″ (*δ* 86.3) of the inner xylanopyranosyl unit. The configurations of the sugar units were assigned after hydrolysis of **5** with 1 N HCl. The hydrolysate was trimethylsilated, and GC retention times of each sugar were compared with those of the authentic samples prepared in the same manner. In this way, the sugar units of **5** were determined to be D-xylose and D-glucose in the ratio 1:1. On the basis of this evidence, **5** was established as the new compound 3*β*-hydroxylup-20(29)-ene-27,28-dioic acid 28-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-xylopyranosyl(1→3)]-*β*-D-xylopyranosyl(1→2)-*β*-D-glucopyranoside ester.

Since jujubogenin glycosides have been reported to possess antifungal and antimycobacterial activity,[6,7] compounds **1**−**3** were tested against *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa*

Table 1. ^{1}H NMR Data of the Sugar Portions of Compounds **2**, **3**, and **5** in CD_3OD[a]

position	**2** δ_H (J in Hz)	position	**3** δ_H (J in Hz)	position	**5** δ_H (J in Hz)
	Ara (*p*)		Ara (*p*)		GlcI
1′	4.43 d (7.0)	1′	4.42 d (5.7)	1′	5.59 d (7.5)
2′	3.76 dd (7.0, 8.2)	2′	3.76 dd (5.7, 8.2)	2′	3.58 dd (7.5, 9.0)
3′	3.82 dd (3.0, 8.2)	3′	3.77 dd (3.0, 8.2)	3′	3.75 t (9.0)
4′	4.10 m	4′	4.05 m	4′	3.47 t (9.0)
5′	3.59 dd (3.0, 12.0)	5′	3.56 dd (3.0, 12.0)	5′	3.40 ddd (2.5, 4.5, 9.0)
	3.87 dd (2.0, 12.0)		3.88 dd (2.0, 12.0)		
				6′	3.72 dd (4.5, 12.0)
					3.85 dd (2.5, 12.0)
	Ara (*f*)		Ara (*f*)		XylI
1″	5.33 d (1.3)	1″	5.33 d (1.3)	1″	4.78 d (7.5)
2″	4.11 d (3.3, 1.3)	2″	4.13 d (3.3, 1.3)	2″	3.68 dd (7.5, 9.0)
3″	3.94 d (5.8, 3.3)	3″	3.95 d (5.8, 3.3)	3″	3.66 t (9.0)
4″	4.00 ddd (5.8, 5.8, 3.4)	4″	4.01 ddd (5.8, 5.8, 3.4)	4″	3.59 ddd (4.5, 9.0, 11.0)
5″	3.63 d (11.7, 5.8)	5″	3.62 d (11.7, 5.8)	5″	3.22 t (11.0)
	3.72 d (11.7, 3.4)		3.72 d (11.7, 3.4)		3.96 dd (4.5, 11.0)
	GlcI		Glc		GlcII
1‴	4.55 d (7.5)	1‴	4.54 d (7.5)	1‴	4.82 d (7.9)
2‴	3.32 dd (7.5, 9.0)	2‴	3.31 dd (7.5, 9.0)	2‴	3.23 dd (7.5, 9.0)
3‴	3.35 t (9.0)	3‴	3.34 t (9.0)	3‴	3.41 t (9.0)
4‴	3.36 t (9.0)	4‴	3.37 t (9.0)	4‴	3.36 t (9.0)
5‴	3.55 ddd (2.5, 4.5, 9.0)	5‴	3.51 ddd (2.5, 4.5, 9.0)	5‴	3.31 ddd (2.5, 4.5, 9.0)
6‴	3.76 dd (4.5, 12.0)	6‴	4.17 dd (4.5, 12.0)	6‴	3.39 dd (4.5, 12.0)
	4.55 dd (2.5, 12.0)		4.50 dd (2.5, 12.0)		3.78 dd (2.5, 12.0)
	GlcII		Dicrotalic acid		XylII
1⁗	4.37 d (7.5)	1⁗		1⁗	4.59 d (7.5)
2⁗	3.21 dd (7.5, 9.0)	2⁗	2.57 d (15.5)	2⁗	3.31 dd (7.5, 9.0)
			2.63 d (15.5)		
3⁗	3.36 t (9.0)	3⁗		3⁗	3.36 t (9.0)
4⁗	3.30 t (9.0)	4⁗	2.56 d (15.5)	4⁗	3.56 ddd (4.5, 9.0, 11.0)
			2.35 d (15.5)		
5⁗	3.35 ddd (2.5, 4.5, 9.0)	5⁗		5⁗	3.28 t (11.0)
					3.96 dd (4.5, 11.0)
6⁗	3.87 dd (4.5, 12.0)	6⁗	1.33 s		
	3.68 dd (2.5, 12.0)				

[a] Assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.

Table 2. ^{13}C NMR Data of the Sugar Portions of Compounds **2**, **3**, and **5** in CD_3OD[a]

position	**2** δ_C	position	**3** δ_C	position	**5** δ_C
	Ara (*p*)		Ara (*p*)		GlcI
1′	105.2	1′	105.6	1′	93.4
2′	77.2	2′	76.9	2′	81.3
3′	83.6	3′	83.3	3′	77.8
4′	69.0	4′	69.0	4′	70.4
5′	65.6	5′	65.7	5′	77.4
				6′	62.1
	Ara (*f*)		Ara (*f*)		XylI
1″	110.3	1″	110.0	1″	104.6
2″	83.8	2″	83.5	2″	81.6
3″	77.6	3″	77.5	3″	86.3
4″	84.7	4″	84.4	4″	69.7
5″	62.0	5″	61.8	5″	65.9
	GlcI		Glc		GlcII
1‴	104.4	1‴	104.3	1‴	104.6
2‴	75.0	2‴	74.7	2‴	75.6
3‴	77.9	3‴	77.2	3‴	77.3
4‴	71.5	4‴	71.0	4‴	71.3
5‴	76.7	5‴	75.3	5‴	77.9
6‴	70.1	6‴	63.8	6‴	62.4
	GlcII		Dicrotalic acid		XylII
1⁗	105.9	1⁗	177.0	1⁗	105.0
2⁗	75.1	2⁗	47.0	2⁗	74.9
3⁗	77.9	3⁗	70.8	3⁗	78.0
4⁗	71.6	4⁗	47.4	4⁗	71.4
5⁗	78.0	5⁗	180.0	5⁗	67.1
6⁗	62.8	6⁗	27.6		

[a] Assignments were confirmed by HSQC and HMBC experiments.

ATCC 27853, and *C. albicans* ATCC 3153. The three compounds showed no activity on the four bacterial strains at the highest tested concentration (250 μg/mL for compounds **2** and **3** and 120 μg/mL for compound **1**). However, compounds **2** and **3** showed some activity against the fungus *C. albicans* ATCC 3153, with a MIC value of 250 μg/mL.

The three crude extracts of *A. grandifolium* (petroleum ether, chloroform, and methanol) were tested on the four bacterial strains. As shown in Table S1, the three extracts were quite active against the Gram-positive bacteria, with MIC values ranging from 200 μg/mL for the methanol extract on *E. faecalis* ATCC 29212 to 25 μg/mL for the chloroform extract on both *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212. The extracts were almost inactive against the tested Gram-negative bacteria, with the exception of *E. coli* ATCC 25922, for which a MIC of 200 μg/mL was recorded for the chloroform extract. The same MIC value was recorded for the chloroform extract against *C. albicans* ATCC 3153 (Table S1).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 243 B polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. IR measurements were obtained on a Bruker IFS-48 spectrometer. NMR spectra in CD_3OD were obtained using a Bruker DRX-600 spectrometer, operating at 599.19 MHz for ^{1}H and 150.86 MHz for ^{13}C. 2D experiments, ^{1}H−^{1}H DQF-COSY (double-filtered direct chemical shift correlation spectroscopy) and inverse-detected ^{1}H−^{13}C HSQC (heteronuclear single quantum coherence), and HMBC (heteronuclear multiple bond connectivity) spectra, were performed using UXNMR software. 1D-TOCSY selective excitation spectra were acquired using waveform generator-based GAUSS-shaped pulses, with mixing times ranging from 100 to 120 ms and with a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse. Exact masses were measured by a Q-Star Pulsar (Applied Biosystems, Foster City, CA) triple-quadrupole orthogonal time-of-flight (TOF) mass spectrometer. ESIMS were performed on a Finnigan LC-Q Deca Ion Trap mass spectrometer scanned from 150 to 1200 Da. The mass spectral data were acquired

and processed using Xcalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump at a flow rate of 3 μL/min. The capillary voltage was 5 V, the spray voltage 5 kV, and the tube lens offset 50 V. The capillary temperature was 220 °C. Droplet countercurrent chromatography (DCCC) was performed on an apparatus manufactured by Büchi, equipped with 300 tubes. Column chromatography was performed over Sephadex LH-20 (Pharmacia), and HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters μ-Bondapak RP18 column, and a U6K injector. TLC was performed on silica gel F254 (Merck) plates, and reagent grade chemicals (Carlo Erba) were used throughout.

Plant Material. *Anomospermum grandifolium* Eichler (Menispermaceae) stems were collected in Ucayali, Perú, in August 2001. A voucher specimen of this plant (JAC 13659) is deposited at the Herbarium of the Museum of Natural History of the Universidad Nacional Mayor de San Marcos (Lima, Perú).

Extraction and Isolation. Dried stems (200 g) were extracted at room temperature using solvents of increasing polarity, namely, petroleum ether (0.24 g), chloroform (1.49 g), and methanol (8.21 g). Part of the methanol extract (2.3 g) was fractionated initially on a Sephadex LH-20 column (100 × 5.0 cm), using MeOH as mobile phase, and 113 fractions (8 mL each) were obtained. Fractions 47−49 (18.5 mg) contained compound **4**. Fractions 20−22 were chromatographed by HPLC on a Waters (μ-Bondapack RP-18) column (30 cm × 7.8 mm i.d.) using MeOH−H_2O (57:43) as mobile phase (flow rate 2.5 mL/h), to yield compounds **1** (8.0 mg, t_R = 51 min) and **2** (4.9 mg, t_R = 37.8 min). Fractions 27−28 were chromatographed by HPLC on a Waters (μ-Bondapack RP-18) column (30 cm × 7.6 mm i.d.) using MeOH−H_2O (27:23) as eluent (flow rate 2.5 mL/h), to afford compound **5** (3.6 mg, t_R = 40.8 min). Fractions 7−17 (930 mg) containing triterpenoid glycosides were further purified on DCCC in the ascending mode of operation (flow rate 12 mL/h) with the following solvent system: $CHCl_3$−MeOH−H_2O−*n*-PrOH (5:6:4:1). Altogether, 343 fractions (4 mL each) were obtained. Fractions 246−271 (28.4 mg) were chromatographed by RP-HPLC, using MeOH−H_2O (1:1) as mobile phase to yield compound **4** (2.9 mg, t_R = 22.5 min).

Compound 2: amorphous white powder (MeOH); $[\alpha]^{22}_D$ −12° (*c* 0.05 MeOH); IR (KBr) ν_{max} 3250, 2940, 1462, 1270 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) aglycon moiety δ 5.21 (1H, d, *J* = 7.9 Hz, H-24), 4.67 (1H, ddd, *J* = 10.0, 7.9, 2.5 Hz, H-23), 4.03 (1H, d, *J* = 12.5 Hz, H-30a), 3.94 (1H, d, *J* = 12.5 Hz, H-30b), 3.13 (1H, dd, *J* = 11.0, 4.5 Hz, H-3), 1.73 (3H, s, Me-26), 1.70 (3H, s, Me-27), 1.17 (6H, s, Me-21, Me-18), 1.08 (3H, s, Me-29), 0.91 (3H, s, Me-19), 0.89 (3H, s, Me-28); ^{13}C NMR (CD_3OD, 150 MHz) aglycon moiety δ 39.8 (C-1), 27.1 (C-2), 90.3 (C-3), 40.4 (C-4), 57.5 (C-5), 18.8 (C-6), 36.9 (C-7), 37.5 (C-8), 53.9 (C-9), 37.9 (C-10), 22.3 (C-11), 28.8 (C-12), 37.2 (C-13), 54.0 (C-14), 36.3 (C-15), 110.3 (C-16), 54.5 (C-17), 18.9 (C-18), 16.6 (C-19), 69.1 (C-20), 29.5 (C-21), 45.3 (C-22), 69.6 (C-23), 126.3 (C-24), 137.5 (C-25), 25.5 (C-26), 18.2 (C-27), 16.6 (C-28), 28.2 (C-29), 66.7 (C-30); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) sugar moiety, see Tables 1 and 2; ESIMS *m*/*z* 1059 $[M - H]^-$, 879 $[M - H - 162]^-$, and 717 $[M - H - 162 - 162]^-$; HRESIMS *m*/*z* $[M + H]^+$ calcd for $C_{52}H_{84}O_{22}$ 1061.5533, found 1061.5579.

Compound 3: amorphous white powder (MeOH); $[\alpha]^{22}_D$ −22° (*c* 0.05, MeOH); IR (KBr) ν_{max} 3225, 2963, 1720, 1690, 1470, 1260; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) aglycon moiety data superimposable with **2**; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), sugar moiety see Tables 1 and 2; ESIMS *m*/*z* 1041 $[M - H]^-$, 997 $[M - H - 44]^-$, 897 $[M - H - 144]^-$, 735 $[M - H - 144 - 162]^-$; HRESIMS *m*/*z* $[M + H]^+$ calcd for $C_{52}H_{82}O_{21}$ 1043.5427, found 1043.5495.

Compound 5: amorphous white powder (MeOH); $[\alpha]^{22}_D$ +54° (*c* 0.05, MeOH); IR (KBr) ν_{max} 3387, 2940, 1742, 1061 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) aglycon moiety δ 4.71 (1H, s, H-29), 4.59 (1H, s, H-29), 3.09 (2H, m, H-3, H-19), 1.69 (3H, s, Me-30), 1.02 (3H, s, Me-26), 0.91 (3H, s, Me-23), 0.86 (3H, s, Me-25), 0.73 (3H, s, Me-24); ^{13}C NMR (CD_3OD, 150 MHz) aglycon moiety δ 39.5 (C-1), 27.6 (C-2), 79.6 (C-3), 39.1 (C-4), 56.4 (C-5), 17.1 (C-6), 51.8 (C-7), 40.7 (C-8), 38.7 (C-9), 18.8 (C-10), 21.1 (C-11), 28.1 (C-12), 40.9 (C-13), 60.4 (C-14), 26.7 (C-15), 35.0 (C-16), 56.7 (C-17), 52.3 (C-18), 48.0 (C-19), 152.2 (C-20), 30.6 (C-21), 37.8 (C-22), 28.3 (C-23), 16.1 (C-24), 17.1 (C-25), 18.0 (C-26), 179.3 (C-27), 178.6 (C-28), 110.3 (C-29), 19.4 (C-30); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), sugar moiety see Tables 1 and 2; ESIMS *m*/*z* 1073 $[M - H]^-$, 911 $[M - H - 162]^-$, 779 $[M - H - 162 - 132]^-$, 647 $[M - H - 162 - 132 - 132]^-$, 485 $[M - H - 162 - 132 - 132 - 162]^-$; HRESIMS *m*/*z* $[M + H]^+$ calcd for $C_{52}H_{82}O_{23}$ 1075.5325, found 1075.5367.

Acid Hydrolysis of Saponins 2, 3, and 5. A solution (0.8 mg each) of saponins in 1 N HCl (0.25 mL) was stirred at 80 °C for 4 h. After cooling, the solution was concentrated by blowing with N_2. The residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.1 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution with a stream of N_2, the residue was separated by water and CH_2Cl_2 (1 mL, 1:1 v/v). The CH_2Cl_2 layer was analyzed by GC using an l-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. Peaks of the hydrolysate of **2** were detected at 8.91 and 9.82 min (L-arabinose) and 14.72 min (D-glucose). Peaks of the hydrolysate of **3** were detected at 8.89 and 9.80 min (L-arabinose) and 14.74 min (D-glucose). Peaks of the hydrolysate of **5** were detected at 10.96 and 11.98 min (D-xylose) and 14.74 min (D-glucose). Retention times for authentic samples after being treated simultaneously with 1-(trimethylsilyl)imidazole in pyridine were detected at 8.90 and 9.78 min (L-arabinose), 10.99 and 12.1 min (D-xylose), and 14.76 min (D-glucose).

Antimicrobial Activity. Extracts were tested against the reference strains for their inhibitory activity, using a common broth microdilution method in 96 multiwell microtiter plates, in duplicate, as reported by Koneman[14] and recommended by the National Committee for Clinical Laboratory Standards.[15]

The antimicrobial activity of the extracts was tested against four aerobic reference bacterial strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212) as well as against the fungus *Candida albicans* ATCC3153, as previously reported.[16] For susceptibility testing, in a first step 50 μL of Mueller Hinton broth was distributed from the second to the twelfth test tubes. Sabouraud broth was employed for *C. albicans*. The final concentration of the extracts adopted to evaluate the antibacterial activity was included from 1 to 0.025 mg/mL.

Due to small quantities available, the activities of the pure compounds were evaluated at different dilution intervals. Plates were incubated for 18 h at 37 °C, and then they were examined from below with a reflective viewer and the lowest concentration of each extract showing growth was taken as its minimal inhibitory concentration (MIC). A blank control was taken using DMSO alone (100 μL/mL) added to the series of tubes, and the MIC was evaluated as described above. No growth inhibition was observed at DMSO concentrations less than or equal to 25 μg/mL.

Ampicillin was used as positive control and was evaluated up to 1 μg/mL against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, exhibiting MIC values of 0.5−2 and 2−8 μg/mL, respectively. For *C. albicans* ATCC3153, the control antibiotic was ketoconazole, and its MIC ranged between 0.1 and 2 μg/mL. The experimental conditions, such as the test medium and the number of bacteria or *C. albicans* determined by CFU/mL, were appropriate to reproduce inhibitory data of these known antibiotics, as recommended by NCCLS.[15]

Supporting Information Available: Table S1, summarizing the antimicrobial activity of *A. grandifolium* extracts. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Brack, A. *Diccionario Enciclopedico de Plantas Utiles del Peru*; Programma de las Naciones Unidas para el Desarrollo y Centro de Estudios Regionales Andinos Bartolome de las Casas: Cusco, 1999; p 40.
(2) Da Rocha, A.; Luz, A.; Da Silva, M. *Acta Amazonica* **1984**, *14*, 244−254.
(3) King, H. *J. Chem. Soc.* **1948**, 1945−1949.
(4) Menachery, M. In *Alkaloids: Chemical and Biological Prospectives*; Pelletier, S. W., Ed.; Pergamon Press: Oxford, U.K., 1996; Vol. 11, Chapter 3, pp 269−302.
(5) Ciccia, G.; Coussio, J.; Mongelli, E. *J. Ethnopharmacol.* **2000**, *72*, 185−189.
(6) Li, X.-C.; ElSholy, H. N.; Nimrod, A. C.; Clark, A. M. *J. Nat. Prod.* **1999**, *62*, 674−677.
(7) ElSohly, H. N.; Danner, S.; Li, X.-C.; Nimrod, A. C.; Clark, A. M. *J. Nat. Prod.* **1999**, *62*, 1341−1342.
(8) Suttrisri, R.; Lee, I.-S.; Kinghorn, A. D. *J. Ethnopharmacol.* **1995**, *47*, 9−26.
(9) Yoshikawa, K.; Shimono, N.; Arihara, S. *Tetrahedron Lett.* **1991**, *48*, 7059−7062.
(10) Kim, N. C.; Kinghorn, A. D. In *Studies in Natural Products Chemistry: Bioactive Natural Products (Part H)*; A.-ur-Rahman, Ed.; Elsevier Science Publishers: Amsterdam, 2002; Vol. 27, pp 3−57.
(11) Basnet, P.; Kadota, S.; Hase, K.; Namba, T. *Chem Pharm. Bull.* **1995**, *43*, 1558−1564.
(12) Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517−1575.
(13) Lee, C. *J. Chin. Chem. Soc.* **1998**, *45*, 303−306.
(14) Koneman, E. W. In *Testo Atlante di Microbiologia Diagnostica*, Ed.: A. Delfino: Rome, 1995; Vol. 1, pp 550−605.
(15) Document M100-S11; National Committee for Clinical Laboratory Standards: Wayne, PA, 2001.
(16) Camporese, A.; Balick, M. J.; Arvigo, R.; Esposito, R. G.; Morsellino, N.; De Simone, F.; Tubaro, A. *J. Ethnopharmacol.* **2003**, *87*, 103−107.

NP030283J