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Antiproliferative Oleanane Saponins from *Polyscias guilfoylei*

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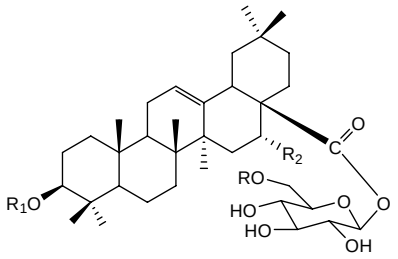
Three new oleanane saponins (**1-3**), together with four known ones (**4-7**), were isolated from the aerial parts of *Polyscias guilfoylei*. Their structures were elucidated by 1D and 2D NMR experiments, including 1D TOCSY, DQF-COSY, ROESY, HSQC, and HMBC spectroscopy, as well as ESIMS analysis. The antiproliferative activity of all compounds was evaluated using three murine and human cancer cell lines; J774.A1, HEK-293, and WEHI-164. All the compounds were inactive except for 3β-*O*-[β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-echinocystic acid 28-[*O*-β-D-glucopyranosyl-(1→6)*O*-β-D-glucopyranosyl] ester (**3**), which was active against all the cell lines.

Keywords: *Polyscias guilfoylei*, Arialiaceae, triterpenes, glycosides, NMR, antiproliferative activity.

Chemical and pharmacological investigations have indicated that triterpenoid saponins of the family Araliaceae are important bioactive components, with various biological activities [1-3]. As part of our investigation on new bioactive compounds from Araliaceae plants growing at the Botanical Garden of Palermo [1-3], we have studied the aerial parts of *Polyscias guilfoylei* Seem (Araliaceae), a plant never investigated before [4]. Three new oleanane saponins (**1-3**) were isolated from the *n*-BuOH extract of *P. guilfoylei*, along with four known saponins (**4-7**). Since triterpenoid saponins have been reported to possess cytotoxic activity [2-5], the antiproliferative activities of **1-7** were evaluated in the cell lines J774.A1, HEK-293, and WEHI-164 [5].



	R	R_1	R_2
1	Glc_I	Rha-(1→4)-Glc_{II}-(1→2)-Ara	H
2	Glc_I	Ara	H
3	Glc_I	Glc_{II}-(1→2)-Ara	OH
4	Rha-(1→4)-Glc_I	Ara	H
5	Glc_I	Glc-(1→2)-Ara	H
6	Rha-(1→4)-Glc_I	Rha-(1→2)-Ara	H
7	Glc	Glc-(1→3)-Ara	H

Compound **1** was assigned a molecular formula $C_{59}H_{96}O_{26}$, as determined by positive ESIMS (*m/z* 1243 $[M+Na]^+$). Tandem mass spectra showed fragments at *m/z* 1097 $[M-146+Na]^+$, 935 $[M-(146+162)+Na]^+$, and a predominant peak at *m/z* 773 $[M-(146+162+162)+Na]^+$, due to the loss of one deoxyhexose and two hexose moieties, respectively. However, the most abundant species was observed at *m/z* 919 $[M-(162+162)+Na]^+$ and was produced by the loss of the whole esterified sugar chain. Finally, a fragment ion was detected at *m/z* 463, corresponding to the sodium-cationized etherified sugar portion of compound **1**, followed by fragment peaks at *m/z* 317 and 331, generated by the elimination of one pentose and one deoxyhexose residue, respectively [6]. These results suggested that **1** had an esterified sugar chain composed of two hexose units. The ^{13}C NMR spectrum of **1** displayed 59 carbon resonances, of which 30 were assigned to the aglycone and 29 to the sugar moiety. The ^{13}C NMR spectrum (Tables 1 and 2) suggested a triterpenoid glycoside structure. The spectrum of the

Table 1: ^{13}C NMR data for glycosyl moieties of compounds **1-3** (CD_3OD, 600 MHz)[a]

	1	**2**	**3**
position	δ_C	δ_C	δ_C
GlcC28-1	95.7	95.8	95.7
2	76.0	74.5	75.0
3	77.5	77.9	78.0
4	70.2	70.4	70.7
5	77.1	77.3	76.9
6	69.0	69.0	69.2
GlcI 1	104.3	104.3	104.0
2	73.5	74.8	75.0
3	78.0	78.3	78.3
4	70.4	71.2	70.8
5	77.9	78.0	77.8
6	62.2	62.5	62.0
Ara 1	105.4	106.6	105.4
2	76.8	73.4	77.0
3	72.1	75.0	72.2
4	69.7	69.7	69.8
5	64.5	65.2	64.5
GlcII 1	103.7		103.7
2	75.0		75.0
3	77.0		78.0
4	**79.5**		71.0
5	77.0		77.8
6	61.8		61.6
Rha 1	102.4		
2	72.0		
3	71.7		
4	73.4		
5	70.2		
6	18.3		

[a] chemical shifts are given in δ ppm; assignments were confirmed by COSY, TOCSY, HSQC, and HMBC experiments.

Table 2: ^{1}H NMR data for glycosyl moieties of compounds **1-3** (CD_3OD, 600 MHz)[a]

	1	**2**	**3**
position	δ_H	δ_H	δ_H
GlcC28-1	5.38 d (7.5)	5.36	5.37 d (7.5)
2	3.38 dd (9.5, 7.5)	3.36	3.38 dd (9.5, 7.5)
3	3.45 t (9.5)	3.45	3.45 t (9.5)
4	3.42 t (9.5)	3.40	3.40 t (9.5)
5	3.60 m	3.57	3.58 m
6a	3.86 dd (12.0, 5.0)	3.85	3.86 dd (12.0, 5.0)
6b	4.14 dd (12.0, 3.0)	4.12	4.12 dd (12.0, 3.0)
GlcI 1	4.50 d (7.6)	4.49	4.50 d (7.6)
2	3.27 dd (9.5, 7.6)	3.28	3.22 dd (9.5, 7.6)
3	3.40 t (9.5)	3.42	3.40 t (9.5)
4	3.28 t (9.5)	3.30	3.27 t (9.5)
5	3.40 m	3.40	3.38 m
6a	3.70 dd (12.0, 5.0)	3.68	3.65 dd (12.0, 5.0)
6b	3.84 dd (12.0, 3.0)	3.84	3.82 dd (12.0, 3.0)
Ara 1	4.40 d (6.5)	4.38	4.48 d (6.0)
2	3.85dd (6.0, 9.0)	3.84	3.91dd (6.0, 9.0)
3	3.80 t (9.0, 3.0)	3.82	3.80 t (9.0, 3.0)
4	3.96 m	3.98	4.03 m
5a	4.00 dd (12.0, 2.0)	4.00	4.00 dd (12.0, 2.0)
5b	3.60 dd (12.0, 3.5)	3.62	3.60 dd (12.0, 3.5)
GlcII 1	4.48 d (7.5)		4.47 d (7.6)
2	3.22 dd (9.5, 7.6)		3.21 dd (9.5, 7.6)
3	3.54 t (9.5)		3.53 t (9.5)
4	3.62 t (9.5)		3.62 t (9.5)
5	3.30 m		3.30 m
6a	3.73 dd (12.0, 5.0)		3.73 dd (12.0, 5.0)
6b	3.84 dd (12.0, 3.0)		3.84 dd (12.0, 3.0)
Rha 1	4.85 d (1.6)		
2	3.90 dd (3.0, 1.6)		
3	3.71 dd (9.0, 3.0)		
4	3.45 t (9.0)		
5	4.00 m		
6	1.27 d (6.2)		

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in δ ppm); assignments were confirmed by COSY, TOCSY, HSQC, and HMBC experiments

aglycone portion exhibited resonances assigned to seven tertiary methyl, two sp^2-hybridized, one hydroxymethine (δ 91.0), and one carboxylic (δ 178.2) carbons. The combined NMR data indicated that the aglycone of **1** was oleanolic acid [7]. In compound **1**, C-28 appeared at δ 178.0 in the ^{13}C NMR spectrum and H-18 appeared at δ 2.92 (dd, J = 12.0, and 4.0 Hz) in the ^{1}H NMR spectrum, indicating that the carboxyl group was glycosylated. Attachment of another glycoside chain at C-3 was suggested by the significant downfield shift observed for this carbon in **1** relative to the corresponding signal in oleanolic acid derivatives [7]. Assignments of all NMR signals of the aglycone portion were ascertained from a combination of 1D TOCSY, DQF-COSY, and HSQC experiments. The sugar portion of **1** exhibited five anomeric proton resonances (δ 5.38, d, J = 7.5 Hz; 4.85, d, J = 1.6 Hz; 4.50, d, J = 7.6 Hz; 4.48, d, J = 7.5 Hz; 4.40, d, J = 6.5 Hz) and one methyl doublet (δ 1.27, d, J = 6.2 Hz) in the ^{1}H NMR spectrum (Table 2). The 1D TOCSY and 2D NMR experiments indicated that three β-glucopyranose, one α-arabinopyranose, and one α-rhamnopyranose moieties were present (Tables 1 and 2). The configurations of the sugar units were assigned after hydrolysis of **1** with 1N HCl. The hydrolyzate was trimethylsilated, and GC retention times of each sugar were compared with those of authentic sugar samples prepared in the same manner. The absence of any ^{13}C NMR glycosidation shift for the α-L-rhamnopyranosyl, and one of the β-glucopyranosyl moieties indicated that these sugars were terminal units. Glycosidation shifts were observed for C-$6_{glc\text{-}C28}$ (δ 69.0), C-2_{ara} (δ 76.8), and C-4_{glcII} (δ 79.5) (Table 1). The chemical shifts of H-1_{glc} (δ 5.38) and C-1_{glc} (δ 95.7) indicated that this sugar unit was involved in an ester linkage with the C-28 carboxylic group. Direct evidence for the sugar sequence and their linkage sites to the aglycone was derived from the HMBC experiment that showed unequivocal correlations between resonances at δ 4.40 and δ 91.0 (H-1_{ara}—C-3) indicating that arabinose was linked to C-3 of the aglycone; a cross peak between δ 4.48 and δ 76.8 (H-1_{glcII}—C-2_{ara}) indicated that glucose II was the second unit, and a cross peak between δ 4.85 and δ 79.5 (H-1_{rha}—C-4_{glcII}) indicated that rhamnose was the terminal unit of the trisaccharide chain at C-3. Similarly, the sequence of the disaccharide chain at C-28 was indicated by the cross peaks between C-6_{glc} (δ 69.0) and H-1_{glcI} (δ 4.50). Thus, compound **1** was

identified as 3β-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-olean-12-en-28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Compound **2** was obtained as an amorphous powder with the molecular formula $C_{47}H_{76}O_{17}$, as deduced from the ESIMS spectrum and confirmed by ^{13}C NMR and ^{13}C DEPT data. The MS/MS spectrum of compound **2** showed a prominent fragment at *m/z* 611 $[M-(162+162)+Na)]^+$. The spectroscopic data of the aglycone moiety of **2** were identical to those of **1**. The proton coupling network within each sugar residue was established, using a combination of 1D TOCSY, DQF-COSY, and HSQC experiments. Once again, direct evidence for the sugar sequence and the linkage sites was derived from the HSQC and HMBC data. Comparison of NMR data of the sugar moieties (Tables 1 and 2) of **2** with those of **1** indicated that **2** differed from **1** only by the absence of a rhamnopyranosyl and glucopyranosyl moieties on the C-3 sugar chain. The structures of the sugar units were determined as reported for compound **1**. Thus, compound **2** was defined as 3β-*O*-α-L-arabinopyranosyl-olean-12-en-28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Compound **3** (molecular formula $C_{53}H_{86}O_{23}$) showed a quasimolecular ion peak at *m/z* 1113 $[M+Na]^+$ in the positive ESIMS. The ^{13}C and ^{13}C DEPT spectra showed 53 resonances, of which 30 were assigned to the aglycone and 23 to the sugar portion. The ^{13}C NMR spectra showed, for the aglycone moiety, signals that could be correlated unambiguously to the corresponding proton chemical shifts from the HSQC experiment, leading to the identification of the aglycone as echinocystic acid [8]. Analysis of the NMR data of the sugar chains (Tables 1 and 2) of compound **3** and comparison with those of **1** revealed **3** to differ from **1** only in the sugar chain at C-3. The structure of the sugar chain at C-3 was deduced using 1D TOCSY and COSY experiments, leading to the identification of β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside. Thus, compound **3** was defined as 3β-*O*-[β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-echinocystic acid 28-[*O*-β-D-glucopyranosyl-(1→6)*O*-β-D-glucopyranosyl] ester.

The four known triterpene glycosides were identified by detailed NMR and MS analyses and comparison with literature data. as 3β-*O*-α-L-arabinopyranosyl-olean-12-en-28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**4**) [9], 3β-*O*-α-L-arabinopyranosyl -(1→2)-β-D-glucopyranosyl-olean-12-en-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyrano-side (**5**) [10], and 3β-*O*-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranosyl-olean-12-en-28-*O*-α-L-rham-nopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**6**) [11], and 3β-*O*-α-L-arabino-pyranosyl-(1→3)-β-D-glucopyranosyl-olean-12-en-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyrano-side (**7**) [12].

The antiproliferative activity of compounds **1-7** was evaluated against the J774.A1, WEHI-164, and HEK-293 cell lines [13]. Compounds **1-2** and **4-7** were inactive. Compound **3**, having echinocystic acid as the aglycone, showed activity against all cell lines with an IC_{50} of 0.19 ± 0.001 μM for J774. A.1, 0.35 ± 0.003 for HEK-293, and 0.64± 0.045 for WEHI-164, respectively.

Experimental

General experimental procedures: Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. Elemental analysis was obtained using a Carlo Erba 1106 elemental analyzer. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. 2D NMR spectra were acquired in CD_3OD in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (Time Proportional Phase Increment) used to achieve frequency discrimination in the ω_1 dimension. Standard pulse sequence and phase cycling were used for DQF-COSY, TOCSY, HSQC, and HMBC experiments. The NMR data were processed on a Silicon Graphics Indigo2 Workstation using UXNMR software. ESIMS and MS/MS spectra (positive and negative mode) were obtained from an LCQ Advantage ThermoFinnigan spectrometer, equipped with Xcalibur software. Column chromatography was performed on Sephadex LH-20. HPLC separations were conducted using a Shimadzu LC-8A series pumping system equipped with a Waters R401 refractive index detector and Shimadzu injector on a C_{18} μ-Bondapak column (30 cm x 7.8 mm, flow rate 2.0 mL min^{-1}). GC analyses were performed using a Dani GC 1000 instrument on a L-CP-Chirasil-Val column (0.32 mm x 25 m).

Plant material: The aerial parts of *P. guilfoylei* were collected in Palermo, Italy, during April 2005 and

were identified by Prof. Giuseppe Venturella of the Dipartimento di Scienze Botaniche, University of Palermo, Italy, where a voucher specimen is deposited.

Extraction and isolation: The dried powdered *P. guilfoylei* aerial parts (250 g) were defatted with *n*-hexane, and then extracted with MeOH to give 14.6 g of extract. The MeOH extract was partitioned between *n*-BuOH and AcOEt, to afford a *n*-BuOH soluble portion (3.5 g) and an AcOEt portion (5 g). A portion of the *n*-BuOH residue (2.0 g) was separated on a Sephadex LH-20 column, using MeOH as eluent. Fractions were collected, analyzed by TLC (silica 60 F_{254} gel-coated glass sheets with *n*-BuOH-HOAc-H_2O (60:15:25) and $CHCl_3$-MeOH-H_2O (40:9:1)), and grouped to obtain six fractions (1-6). Fraction 2 (123 mg) was chromatographed using RP-HPLC (MeOH-H_2O (1:1) to yield compounds **6** (4.5 mg), **5** (9 mg), and **1** (7 mg). Fractions 3 (80 mg) and 5 (30 mg) were subjected to RP-HPLC with MeOH-H_2O (3:2) to yield compounds **7** (4.5 mg) and **4** (3 mg) from fraction 3, and **2** (8 mg) from fraction 5, respectively. Fraction 6 (135 mg) was purified by preliminary SPE, followed by RP-HPLC with MeOH-H_2O (1:1) to afford compounds **1** (3.0 mg) and **3** (11.0 mg).

Compound 1

$[\alpha]_D$: +3.10 (*c* 1.00, MeOH).
NMR data of the aglycone see [1-2].
^{1}H NMR and ^{13}C NMR: Tables 1 and 2.
ESIMS: *m/z* 1243 $[M+Na]^+$ *m/z* 1097 $[M-146+Na]^+$, *m/z* 935 $[M-146-162+Na]^+$, *m/z* 919 $[M-162-162+Na]^+$, *m/z* 773 $[M-146-162-162+Na]^+$.
Elemental analysis: C, 58.02; H, 7.92, calcd for $C_{59}H_{96}O_{26;}$ C, 58.05; H, 7.93.

Compound 2

$[\alpha]_D$: +35.2 (*c* 1.00, MeOH).
NMR data of the aglycone see [1-2].
^{1}H NMR and ^{13}C NMR: Tables 1 and 2.
ESIMS: *m/z* 935 $[M+Na]^+$ *m/z* 803$[M-132+Na]^+$, *m/z* 641$[M-132-162+Na]^+$, *m/z* 611 $[M-162-162+Na]^+$,
Elemental analysis: C, 61.82; H, 8.39; calcd for $C_{47}H_{76}O_{17}$; C, 61.86; H, 8.38.

Compound 3

$[\alpha]_D$: +40 (*c* 1.00, MeOH).
NMR data of the aglycone see [8]
^{1}H NMR and ^{13}C NMR: Tables 1 and 2.
ESIMS: *m/z* 1113$[M+Na]^+$, *m/z* 951$[M-162+Na]^+$, *m/z* 789$[M-162-162+Na]^+$,
Elemental analysis: C, 58.33; H, 7.94; calcd for $C_{53}H_{86}O_{23;}$ C, 58.36; H, 7.96.

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