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Cytotoxic Saponins from *Schefflera rotundifolia*

Abstract

Eight new oleanane and lupane saponins (**1 – 8**) as well as two new benzyl glycosides (**9** and **10**) were isolated from the aerial parts of *Schefflera rotundifolia* (Ten) Frodin (Araliaceae) together with two known benzyl glycosides. Their structures were established using 1D- and 2D-NMR spectroscopy and mass spectrometry. The antiproliferative activity of all compounds was evaluated using three continuous murine and human culture cell lines J774.A1, HEK-293, and WEHI-164. Compounds **7** and **8**, having betulinic acid as aglycone, were the most active constituents.

Key words
Schefflera rotundifolia · Araliaceae · triterpene saponins · benzyl glycosides · antiproliferative activity

Introduction

In our ongoing search for new bioactive compounds from Araliaceae plants growing in the Botanical Garden of Palermo [1], [2] the aerial parts of *Schefflera rotundifolia* (Ten) Frodin, a plant never before investigated, have been studied. Because plants of *Schefflera* genus have been used as folk remedies for the treatment of pain, rheumatic arthritis, fracture, sprains, and lumbago in Asian countries [3], a phytochemical study of the aerial parts of *S. rotundifolia* has been performed. Eight new oleanane and lupane saponins (**1 – 8**) as well as two new benzyl glycosides (**9** and **10**) were isolated (Fig. **1**), along with two known benzyl glycosides. Since triterpenoid saponins have been reported to possess cytotoxic activity [2], [4], the antiproliferative activities of all compounds (cell lines J774.A1 HEK-293, WEHI-164) were evaluated.

Materials and Methods

General

The instrumentation used in this work is described in our previous paper [2]. GC analyses were performed using a Dani GC 1000 instrument.

Plant material

The aerial parts of *S. rotundifolia* (Ten) Frodin were obtained in April 1997 from plants cultivated in the Botanical Garden of Palermo, Italy, where a voucher specimen was deposited (No. 158).

Extraction and isolation

Dried aerial parts of *S. rotundifolia* (250 g) were defatted with petroleum ether then extracted with 80% EtOH to give 12 g of residue. The ethanolic extract was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH-soluble portion (5.0 g). Part of the *n*-butanol residue (2.0 g) was chromatographed over a Sephadex

Dedicated to Professor Adolf Nahrstedt on the occasion of his retirement as Editor-in-Chief of Planta Medica

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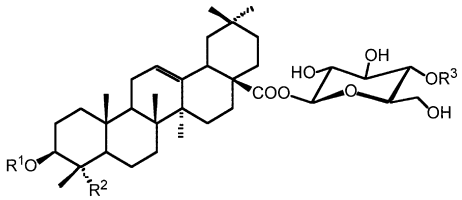
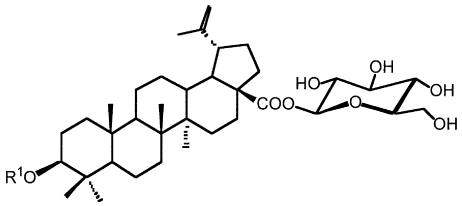


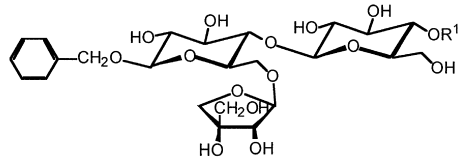
	R^1	R^2	R^3
1	Glc(1→3)Rha(1→2)Ara	CH_2OH	Glc
2	Glc(1→3)Rha(1→2)Ara	CH_2OH	H
3	Rha(1→2)Ara	CH_2OH	Glc
4	Rha(1→3)Rha(1→2)Ara	CH_3	Glc
5	Rha(1→2)Ara	CH_3	Glc
6	Xyl	CH_3	Rha(1→4)Glc

Glc = β-D-glucopyranosyl
Rha = α-L-rhamnopyranosyl
Ara = α-L-arabinopyranosyl



	R^1
7	Rha(1→2)Ara
8	Ara

Rha = α-L-rhamnopyranosyl
Ara = α-L-arabinopyranosyl



	R^1
9	Glc
10	H

Glc = β-D-glucopyranosyl

Fig. **1** Structures of compounds **1 – 10**.

LH-20 column (100 × 5 cm) with MeOH as eluent (600 mL). A total of 75 fractions were collected (8 mL each) and combined by TLC results on silica 60 F_{254} gel-coated glass sheets with *n*-BuOH-AcOH-H_2O (60 : 15 : 25) and $CHCl_3$-MeOH-H_2O (40 : 9 : 1), to give 11 pooled fractions (A – K): tubes 1 – 17 = fr A, tubes 18 – 23 = fr B, tubes 24 – 26 = fr C, tubes 27 – 31 = fr D, tubes 32 – 35 = fr E, tubes 36 – 38 = fr F, tubes 39 – 49 = fr G, tubes 50 – 55 = fr H, tubes 56 – 66 = fr I, tubes 67 – 69 = fr J, tubes 70 – 75 = fr K. Fraction B (207 mg) was submitted to separation by RP-HPLC on a C-18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) with MeOH-H_2O (2 : 3) as eluent to yield pure compounds **9** (5.0 mg, t_R = 10 min) and **10** (3.0 mg, t_R = 12 min). Fraction C (352 mg) was purified by RP-HPLC on a C-18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) with MeOH-H_2O (1 : 1) as eluent to give compounds **1** (10.0 mg, t_R = 8 min), **2** (8.0 mg, t_R = 12 min), **4** (2.0 mg, t_R = 13 min), and **3** (3.5 mg, t_R = 16 min). Fraction D (260 mg) was chromatographed over RP-HPLC on a C-18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) with MeOH-H_2O (1 : 1) as eluent to yield icariside F2 (15.0 mg, t_R = 7 min) and compounds **4** (1.5 mg, t_R = 13 min), **3** (3.0 mg, t_R = 16 min), and **5** (6.3 mg, t_R = 17 min). Similarly, fraction E (60 mg) was submitted to separation by RP-HPLC on a C-18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) with MeOH-H_2O (3 : 2) as eluent to yield benzyl β-D-glucopyranoside (12.0 mg, t_R = 7 min) and compound **6** (4.5 mg, t_R = 9 min). Finally, fraction G (59 mg) was chromatographed over RP-HPLC on a C-18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 3.0 mL/min) with MeOH-H_2O (3 : 2) as eluent to give compounds **7** (2.7 mg, t_R = 10 min) and **8** (5.4 mg, t_R = 13 min).

Isolates

Compound **1**, *3β-O-(β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-hederagenin-28-O-(β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl) ester:* white powder, $[\alpha]_D^{25}$: + 29°, (c 1, MeOH); FABMS: *m/z* = 1235 $[M-H]^-$, 911 [(M – H) -(162 + 162)]$^-$, 765 [(M – H) - (162 + 162+ 146)]$^-$, 603 [(M – H) - (162 + 162 + 146 + 162)]$^-$; elemental analysis: found: C 57.20%, H 7.83%; calcd. for $C_{59}H_{96}O_{27}$: C 57.27%, H 7.82%; for NMR data of the aglycone moiety, see Shao et al. [5]; ^{1}H- and ^{13}C-NMR data of the sugar moiety: see Table **1**.

Compound **2**, *3β-O-(β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-hederagenin-28-O-β-D-glucopyranosyl ester*: white powder, $[\alpha]_D^{25}$: + 47°, (*c* 1, MeOH); FABMS: *m/z* = 1073 $[M-H]^-$, 765 [(M – H) - (162 + 146)]$^-$, 603 [(M – H) - (162 + 146 + 162)]$^-$, 471 [(M – H) - (162 + 146 + 162 + 132)]$^-$; elemental analysis: found: C 59.18%, H 8.07%; calcd. for $C_{53}H_{86}O_{22}$: C 59.20%, H 8.06%; for NMR data of the aglycone moiety, see Shao et al. [5]; ^{1}H- and ^{13}C-NMR data of the sugar moiety: see Table **1**.

Compound **3**, *3β-O-(α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-hederagenin-28-O-(β-D-*glucopyranosyl-(1→4)-*β-D-glucopyranosyl) ester:* white powder, $[\alpha]_D^{25}$: + 58°, (*c* 1, MeOH); FABMS: *m/z* = 1073 $[M-H]^-$, 765 [(M – H) - (162 + 146)]$^-$, 603 [(M – H) - (162 + 146 + 162)]$^-$; elemental analysis: found: C 59.22%, H 8.05%; calcd. for $C_{53}H_{86}O_{22}$: C 59.20%, H 8.06%; for NMR data of the aglycone moiety, see Shao et al. [5]; ^{1}H- and ^{13}C-NMR data of the sugar moiety: see Table **1**.

Compound **4**, *3β-O-(α-L-*rhamnopyranosyl-(1→3)-*α-L-rhamnopyranosyl*-(1→2)-*α-L-arabinopyranosyl*)-olean-12-ene-28-*O-(β-D-glucopyranosyl*-(1→4)-*β-D-glucopyranosyl) ester:* white powder, $[\alpha]_D^{25}$: + 22°, (c 1, MeOH); FABMS: *m/z* = 1203 $[M-H]^-$, 911 [(M – H) - (146 + 146)]$^-$, 749 [(M – H) - (146 + 146 + 162)]$^-$; elemental analysis: found: C 58.73%, H 8.05%; calcd. for $C_{59}H_{96}O_{25}$: C 58.79%, H 8.03%; for NMR data of the aglycone moiety, see Miyase et al. [6]; ^{1}H- and ^{13}C-NMR data of the sugar moiety: see Table **2**.

Compound **5**, *3β-D-O-(α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-olean-12-ene-28-O-(β-D-glucopyranosyl*-(1→4)-*β-D-glucopyranosyl) ester*: white powder, $[\alpha]_D^{25}$: + 37°, (*c* 1, MeOH); FABMS: *m/z* = 1057 $[M-H]^-$, 749 [(M – H) - (162 + 146)]$^-$; elemental analysis: found: C 60.08%, H 8.20%; calcd. for $C_{53}H_{86}O_{21}$: C 60.10%, H 8.18%; for NMR data of the aglycone moiety, see Miyase et al. [6]; ^{1}H- and ^{13}C-NMR data of the sugar moiety are superimposable on those reported for compound **3**.

Compound **6**, *3β-D-O-(β-D-xylopyranosyl)-olean-12-ene-28-O-(α-*L-rhamnopyranosyl-(1→4)-*β-D-glucopyranosyl*-(1→4)-*β-D-glucopyranosyl) ester*: white powder, $[\alpha]_D^{25}$: + 68° (*c* 1, MeOH); FABMS: *m/z* = 1057 $[M-H]^-$, 911 [(M – H) - 146]$^-$, 749 [(M – H) - (146

Table **1** ^{1}H- and ^{13}C-NMR data (δ value, J in Hz) for the oligosaccharide moieties of compounds **1 – 3** in CD_3OD, 300 K[a]

	No	1		2		3	
		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Ara C-3	1	4.31 d (6.8)	106.7	4.32 d (6.8)	107.9	4.45 d (6.8)	105.2
	2	3.90 dd (8.5, 6.8)	76.5	3.90 dd (8.5, 6.8)	76.4	3.90 dd (8.5, 6.8)	76.1
	3	3.79 dd (8.5, 3.0)	71.9	3.79 dd (8.5, 3.0)	72.1	3.65 dd (8.5, 3.0)	72.0
	4	4.05 m	69.0	4.04 m	69.4	4.02 m	69.4
	5a	3.88 dd (12.0, 2.0)	67.0	3.90 dd (12.0, 2.0)	66.4	3.90 dd (12.0, 2.0)	66.5
	5b	3.58 dd (12.0, 3.0)		3.58 dd (12.0, 3.0)		3.60 dd (12.0, 3.0)	
Rha	1	4.88 d (1.5)	102.6	4.90 d (1.8)	102.7	4.90 d (1.5)	103.0
	2	4.20 dd (3.0, 1.5)	70.8	4.18 dd (3.0, 1.8)	70.6	3.87 dd (3.0, 1.5)	72.2
	3	3.85 dd (9.0, 3.0)	80.8	3.86 dd (9.0, 3.0)	81.0	3.70 dd (9.0, 3.0)	71.1
	4	3.60 t (9.0)	71.2	3.58 t (9.0)	71.5	3.44 t (9.0)	73.5
	5	3.90 dd (9.0, 6.0)	69.8	3.87 dd (9.0, 6.0)	69.7	4.00 dd (9.0, 6.0)	70.1
	6	1.31 d (6.0)	18.0	1.28 d (6.0)	18.0	1.28 d (6.0)	17.9
Glc I	1	4.71 d (7.5)	105.0	4.70 d (7.5)	104.4		
	2	3.27 dd (9.0, 7.5)	74.6	3.26 dd (9.0, 7.5)	74.5		
	3	3.39 t (9.0)	77.8	3.40 t (9.0)	77.8		
	4	3.25 t (9.0)	71.7	3.26 t (9.0)	71.5		
	5	3.31 m	78.0	3.30 m	78.0		
	6a	3.85 dd (12.0, 3.0)	62.1	3.84 dd (12.0, 3.0)	61.5		
	6b	3.70 dd (12.0, 5.5)		3.68 dd (12.0, 5.0)			
Glc II C-28	1	5.37 d (7.5)	95.4	5.38 d (7.5)	95.5	5.36 d (7.5)	96.0
	2	3.37 dd (9.0, 7.5)	74.0	3.42 dd (9.0, 7.5)	74.6	3.35 dd (9.0, 7.5)	74.0
	3	3.45 t (9.0)	77.7	3.49 t (9.0)	78.1	3.44 t (9.0)	77.7
	4	3.57 t (9.0)	78.8	3.44 t (9.0)	71.0	3.62 t (9.0)	78.8
	5	3.45 m	77.5	3.41 m	78.2	3.45 m	77.5
	6a	3.90 dd (12.0, 2.5)	62.2	3.84 dd (12.0, 3.0)	61.8	3.88 dd (12.0, 3.0)	62.2
	6b	3.69 dd (12.0, 5.0)		3.70 dd (12.0, 5.0)		3.65 dd (12.0, 5.5)	
Glc III	1	4.70 d (7.5)	105.1			4.72 d (7.5)	105.1
	2	3.27 dd (9.0, 7.5)	75.0			3.24 dd (9.0, 7.5)	75.3
	3	3.38 t (9.0)	77.8			3.38 t (9.0)	77.9
	4	3.26 t (9.0)	69.8			3.26 t (9.0)	71.0
	5	3.38 m	78.5			3.40 m	78.1
	6a	3.85 dd (12.0, 3.0)	62.1			3.87 dd (12.0, 3.0)	62.3
	6b	3.65 dd (12.0, 5.0)				3.65 dd (12.0, 5.5)	

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

+ 162)]$^-$; elemental analysis: found: C 60.07%, H 8.20%; calcd. for $C_{53}H_{86}O_{21}$: C 60.10%, H 8.18%; for NMR data of the aglycone moiety, see Miyase et al. [6]; ^{1}H- and ^{13}C-NMR data of the sugar moiety: see Table **2**.

Compound **7**, *3β-D-O-(α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-lup-20(29)-ene-28-O-β-D-glucopyranosyl ester*: white powder, $[\alpha]_D^{25}$: +71°, (*c* 0.1, MeOH); FABMS: *m/z* = 895 [M - H]$^-$, 749 [(M – H) - 146]$^-$, 455 [(M – H) - (146 + 162 + 132)]$^-$; elemental analysis: found: C 62.92%, H 8.52%; calcd. for $C_{47}H_{76}O_{16}$: C 62.93%, H 8.54%; for NMR data of the aglycone moiety, see De Tommasi et al. [1]; ^{1}H- and ^{13}C-NMR data of the sugar moiety: see Table **2**.

Compound **8**, *3β-D-O-(α-L-arabinopyranosyl)-lup-20(29)-ene-28-O-β-D-glucopyranosyl ester*: white powder, $[\alpha]_D^{25}$: +93°, (*c* 0.1, MeOH); FABMS: *m/z* = 749 [M – H]$^-$, 587 [(M – H) - 162]$^-$; elemental analysis: found: C 65.50%, H 8.88%; calcd. for $C_{41}H_{66}O_{12}$: C 65.57%, H 8.86%; for NMR data of the aglycone moiety, see De Tommasi et al. [1]; ^{1}H- and ^{13}C-NMR data of the sugar moiety: see Table **2**.

Compound **9**, *benzyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→6)]-β-D-glucopyranoside*: yellowish powder, $[\alpha]_D^{25}$: –26°, (*c* 0.1, MeOH); FABMS: *m/z* = 725 [M – H]$^-$, 563 [(M – H) - 162]$^-$, 401 [(M – H) - (162 + 162)]$^-$; elemental analysis: found: C 49.60%, H 6.35%; calcd. for $C_{30}H_{46}O_{20}$: C 49.58%, H 6.38%; ^{1}H- and ^{13}C-NMR data: see Table **3**.

Compound **10**, *benzyl β-D-glucopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→6)]-β-D-glucopyranoside*: pale-yellow powder, $[\alpha]_D^{25}$: –58°, (*c* 0.1, MeOH); FABMS: *m/z* = 563 [M – H]$^-$; elemental analysis: found: C 51.05%, H 6.45%; calcd. for $C_{24}H_{36}O_{15}$: C 51.06%, H 6.43%; ^{1}H- and ^{13}C-NMR data: see Table **3**.

The known compounds benzyl β-D-glucopyranoside $[\alpha]_D^{25}$: –43° (*c* 0.1, MeOH) [7] and icariside F2 $[\alpha]_D^{25}$: –84° (*c* 0.1, MeOH) [8] were also isolated. Their structures were identified by comparison of their spectral data (^{1}H-, ^{13}C-NMR, and MS data) with the literature values [7], [8].

Acid hydrolysis of compounds 1 – 10

A solution of compounds **1 – 10** (2.0 mg each) in 1 N HCl (1 mL) was stirred at 80 °C in a stoppered reaction vial for 4 h. After cooling, the solution was evaporated under a stream of N_2. The residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.2 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution, the residue was partitioned between water and $CHCl_3$. The $CHCl_3$ layer was analysed by GC using a 1-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient sys-

Table **2** ^{1}H- and ^{13}C-NMR data (δ value, J in Hz) for the oligosaccharide moieties of compounds **4**, **6**, **7**, and **8** in CD_3OD, 300 K[a]

	No	4		6		7		8	
		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Ara C-3	1	4.33 d (6.8)	108.0			4.38 d (6.8)	107.7	4.50 d (6.8)	105.2
	2	3.86 dd (8.5, 6.8)	76.8			3.85 dd (8.5, 6.8)	76.7	3.50 dd (8.5, 6.8)	72.4
	3	3.81 dd (8.5, 3.0)	71.7			3.82 dd (8.5, 3.0)	72.1	3.66 dd (8.5, 3.0)	75.2
	4	4.02 m	69.1			4.04 m	69.6	4.02 m	70.5
	5a	3.90 dd (12.0, 2.0)	66.0			3.90 dd (12.0, 2.0)	66.0	3.90 dd (12.0, 2.0)	66.0
	5b	3.62 dd (12.0, 3.0)				3.59 dd (12.0, 3.0)		3.60 dd (12.0, 3.0)	
Xyl C-3	1			4.40 d (7.0)	108.5				
	2			3.22 dd (9.0, 7.0)	75.0				
	3			3.42 t (9.0)	77.8				
	4			3.44 m	70.5				
	5a			3.90 t (11.0)	68.1				
	5b			3.15 dd (11.0, 4.0)					
Rha I	1	4.90 d (1.5)	102.6			4.90 d (1.5)	103.0		
	2	4.18 dd (3.0, 1.5)	70.6			3.87 dd (3.0, 1.5)	72.1		
	3	3.84 dd (9.0, 3.0)	81.0			3.68 dd (9.0, 3.0)	71.0		
	4	3.58 t (9.0)	71.4			3.42 t (9.0)	73.2		
	5	3.87 dd (9.0, 6.0)	69.5			3.98 dd (9.0, 6.0)	70.1		
	6	1.29 d (6.0)	18.3			1.28 d (6.0)	18.1		
Rha II	1	4.88 d (1.5)	102.6						
	2	3.87 dd (3.0, 1.5)	72.1						
	3	3.68 dd (9.0, 3.0)	71.0						
	4	3.44 t (9.0)	73.4						
	5	4.00 dd (9.0, 6.0)	70.2						
	6	1.28 d (6.0)	18.0						
Glc I C-28	1	5.36 d (7.5)	96.0	5.35 d (7.5)	96.1	5.40 d (7.5)	95.6	5.40 d (7.5)	95.6
	2	3.37 dd (9.0, 7.5)	74.0	3.36 dd (9.0, 7.5)	74.1	3.42 dd (9.0, 7.5)	74.0	3.42 dd (9.0, 7.5)	74.2
	3	3.38 t (9.0)	77.8	3.42 t (9.0)	77.6	3.49 t (9.0)	78.0	3.49 t (9.0)	77.9
	4	3.61 t (9.0)	78.4	3.60 t (9.0)	78.8	3.39 t (9.0)	70.8	3.39 t (9.0)	71.0
	5	3.42 m	77.2	3.45 m	77.5	3.41 m	78.1	3.41 m	78.1
	6a	3.90 dd (12.0, 2.5)	62.4	3.88 dd (12.0, 3.0)	62.2	3.87 dd (12.0, 3.0)	62.4	3.87 dd (12.0, 3.0)	62.2
	6b	3.67 dd (12.0, 5.0)		3.65 dd (12.0, 5.0)		3.61 dd (12.0, 5.0)		3.61 dd (12.0, 5.0)	
Glc II	1	4.71 d (7.5)	105.2	5.36 d (7.5)	104.3				
	2	3.22 dd (9.0, 7.5)	75.8	3.35 dd (9.0, 7.5)	75.6				
	3	3.32 t (9.0)	77.8	3.41 t (9.0)	77.7				
	4	3.27 t (9.0)	69.9	3.58 t (9.0)	78.9				
	5	3.40 m	78.5	3.42 m	77.2				
	6a	3.86 dd (12.0, 3.0)	61.9	3.90 dd (12.0, 3.0)	61.9				
	6b	3.65 dd (12.0, 5.0)		3.65 dd (12.0, 5.0)					
Rha III	1			4.86 d (1.8)	102.7				
	2			3.84 dd (3.0, 1.8)	72.5				
	3			3.69 dd (9.0, 3.0)	71.1				
	4			3.48 t (9.0)	73.6				
	5			3.98 dd (9.0, 6.5)	70.1				
	6			1.30 d (6.5)	17.8				

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

tem 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 were detected by comparison with retention times of authentic samples of D-apiose, L-arabinose, D-glucose, L-rhamnose, and D-xylose (Sigma Aldrich, Milan, Italy) after treatment with 1-(trimethylsilyl)imidazole in pyridine.

Antiproliferative assay

J774.A1, murine monocyte/macrophage, WEHI-164, murine fibrosarcoma, and HEK-293, human epithelial kidney cells were grown as reported previously [2]. All reagents for cell culture were from Hy-Clone (Euroclone, Paignton Devon, U.K.); MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2*H*-tetrazolium bromide] and 6-mercaptopurine (6-MP) were from Sigma Chemicals (Milan, Italy). J774.Al, WEHI-164, and HEK-293 (3.4×10^4 cells) were plated on 96-well microtiter plates and allowed to adhere at 37 °C in 5 % CO_2 and 95 % air for 2 h. Thereafter, the medium was replaced with 50 μL of fresh medium and a 75 μL aliquot of 1 : 4 serial dilution of each test compound was added and then the cells incubated for 72 h. In some experiments, serial dilutions of 6-MP were added. The cell viability was assessed through an MTT conversion assay [9], [10], [11]. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter. The viability of each cell line in response to treatment with tested compounds and 6-MP [12] was calculated as: % dead cells = 100 - (OD treated/OD control) × 100. Table **4** shows the results obtained expressed as an IC_{50} value (μM), the concentration that inhibited cell growth by 50 % as compared to the control.

Table **3** ^{1}H- and ^{13}C-NMR data (δ value, J in Hz) for the oligosaccharide moieties of compounds **9** and **10** in CD_3OD, 300 K[a]

	No	9		10	
		δ_H	δ_C	δ_H	δ_C
	1		138.0		138.0
	2	7.45 dd (7.5, 1.5)	129.2	7.47 dd (7.5, 1.5)	129.2
	3	7.35 m	129.3	7.35 m	129.3
	4	7.29 t (7.5)	128.0	7.30 t (7.5)	128.0
	5	7.35 m	129.3	7.35 m	129.3
	6	7.45 dd (7.5, 1.5)	129.2	7.47 dd (7.5, 1.5)	129.2
	7a	4.93 d (12.0)	71.4	4.90 d (12.0)	71.4
	7b	4.68 d (12.0)		4.68 d (12.0)	
Glc I	1	4.44 d (7.5)	103.4	4.36 d (7.5)	103.0
	2	3.30 dd (9.0, 7.5)	75.0	3.27 dd (9.0, 7.5)	74.9
	3	3.30 t (9.0)	78.0	3.29 t (9.0)	77.6
	4	3.36 t (9.0)	78.5	3.33 t (9.0)	78.4
	5	3.45 m	76.5	3.43 m	76.7
	6a	4.04 dd (12.0, 3.0)	68.4	4.04 dd (12.0, 3.0)	68.4
	6b	3.66 dd (12.0, 5.0)		3.66 dd (12.0, 5.0)	
Api	1	5.09 d (2.0)	111.0	5.07 d (2.0)	111.0
	2	3.97 d (2.0)	77.9	3.97 d (2.0)	77.9
	3		81.1		81.1
	4a	3.80 d (10.0)	74.8	3.81 d (10.0)	74.8
	4b	3.98 d (10.0)		3.95 d (10.0)	
	5	3.61 br s	65.0	3.62 br s	65.0
Glc II	1	4.36 d (7.5)	103.2	4.38 d (7.5)	103.0
	2	3.37 dd (9.0, 7.5)	75.2	3.29 dd (9.0, 7.5)	75.0
	3	3.46 t (9.0)	77.8	3.36 t (9.0)	77.9
	4	3.59 t (9.0)	78.7	3.34 t (9.0)	71.9
	5	3.45 m	77.6	3.40 m	77.7
	6a	3.90 dd (12.0, 2.5)	62.2	3.92 dd (12.0, 2.5)	62.4
	6b	3.67 dd (12.0, 5.0)		3.61 dd (12.0, 5.0)	
Glc III	1	4.46 d (7.5)	104.2		
	2	3.28 dd (9.0, 7.5)	75.0		
	3	3.35 t (9.0)	77.9		
	4	3.26 t (9.0)	70.8		
	5	3.39 m	78.2		
	6a	3.85 dd (12.0, 3.0)	62.3		
	6b	3.65 dd (12.0, 5.0)			

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

Results and Discussion

Chromatographic separations of the *n*-BuOH extract of *S. rotundifolia* on Sephadex LH-20 and RP-HPLC gave compounds **1 – 10**, icariside F2, and benzyl β-D-glucopyranoside.

Compound **1** had a molecular formula $C_{59}H_{96}O_{27}$, as determined by ^{13}C-, ^{13}C-DEPT NMR data and a quasi-molecular ion peak $[M-H]^-$ at m/z = 1235 in the negative-ion FAB-MS spectrum. The ^{13}C- and ^{13}C-DEPT NMR spectra showed 59 signals, of which 30 were assigned to a triterpenoid moiety and 29 to the saccharide portion. The spectroscopic features, particularly the complete NMR analysis, that revealed the presence of a carboxylic group located at C-28, were completely in agreement with hederagenin as the aglycone of compound **1**[5], [13]. Five anomeric protons were observed in the ^{1}H-NMR spectrum of **1** at δ = 4.31 (d, J = 6.8 Hz), 4.70 (d, J = 7.5 Hz), 4.71 (d, J = 7.5 Hz), 4.88 (d, J = 1.5 Hz), and 5.37 (d, J = 7.5 Hz). The chemical shifts of all the individual protons of the five sugar units was ascertained from a combination of 1D-TOCSY and DQF-COSY spectral analysis, and the ^{13}C-NMR chemical shifts (Table **1**) of their attached carbons could be unambiguously assigned from the HSQC spectrum. In this way, the sugar units were identified as one α-arabinopyranoside, one α-rhamnopyranoside, and three β-glucopyranoside. In the HSQC experiment glycosidation shifts were observed for C-2_{ara} (76.5 ppm), C-3_{rha} (80.8 ppm), and C-4_{glcII} (78.8 ppm). The absence of any glycosidation shifts for two glycopyranosyl units suggested that these sugars were terminal units. The anomeric carbon signal at δ = 95.4 (C-1_{glcII}) and the carbonyl signal at δ = 176.5 revealed the presence of an ester glycosidic linkage between C-28 and a glucopyranosyl moiety [14]. The glycosidic linkages of the sugars was defined by the HMBC experiment: α-arabinose unit was linked at C-3 as shown by the cross-peak between δ = 4.31 (H-1_{ara}) and 83.8 ppm (C-3); other key correlations were observed between H-1_{rha}-C-2_{ara}, H-1_{glcI}-C-3_{rha}, and H-1_{glcIII}-C-4_{glcII}. The configuration of the sugar units was assigned after hydrolysis of **1** with 1 N HCl. The hydrolysate was trimethylsilylated, and GC retention times of each sugar were compared with those of authentic L-arabinose, D-glucose, and L-rhamnose prepared in the same manner. In this way, the sugar units of **1** were determined to be L-arabinose, D-glucose, and L-rhamnose. Thus, the structure of compound **1** was established as 3β-*O*-(β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-hederagenin-28-*O*-(β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl) ester.

Table **4** *In vitro* anti-proliferative activity of triterpenoid glycosides from *Schefflera rotundifolia*[a]

Compound	Cell Line [IC_{50} µM]		
	J774.A1[b]	HEK-293[c]	WEHI-164[d]
1	1.63	nd[e]	0.64
2	0.51	1.8	1.74
3	0.85	1.1	1.2
4	1.78	2.20	nd
5	0.45	1.85	0.67
6	0.52	1.3	2.1
7	0.32	0.44	0.79
8	0.19	0.26	0.55
6-MP[f]	0.003	0.007	0.015

[a] The IC_{50} value is the concentration of compound that affords 50% reduction in cell growth (after a 3-days incubation).
[b] J774.A1 = murine monocyte/macrophage cell lines.
[c] HEK-293 = human epithelial kidney cell lines.
[d] WEHI-164 = murine fibrosarcoma cell lines.
[e] nd = not detected.
[f] 6-MP = 6-mercaptopurine.

Compound **2** showed the molecular formula $C_{53}H_{86}O_{22}$ by negative-ion FAB-MS (*m/z* = 1073 $[M-H]^-$), ^{13}C-, ^{13}C-DEPT NMR data, and elemental analysis. The ^1H-NMR spectrum of **2** (Table **1**) had signals for a triterpenoid aglycone and sugar units. Comparison of the NMR spectral data of **2** with those of compound **1** revealed that **2** differed from **1** only for the absence of the terminal β-glucopyranosyl moiety linked at C-28 of the aglycone. The D configuration of the glucose units and L configuration of arabinose and rhamnose moieties was obtained as reported for compound **1**. The structure of **2** was thus formulated to be 3β-*O*-(β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-hederagenin-28-*O*-β-D-glucopyranosyl ester.

The FAB-MS of compound **3** ($C_{53}H_{86}O_{22}$) displayed $[M-H]^-$ at *m/z* = 1073, suggesting that **3** was an isomer of **2**. Analysis of NMR of compound **3** and comparison with those of **1** and **2** showed **3** to differ from **1** only in the absence of the terminal glucopyranosyl moiety of the saccharidic chain linked at C-3 of the aglycone (Table **1**). The configuration of the sugar units was determined as described for compound **1**. Therefore, the structure 3β-*O*-(α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-hederagenin-28-*O*-(β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl) ester was assigned to compound **3**.

Compound **4** showed, in the negative ion FAB-MS, a quasi-molecular peak $[M-H]^-$ at *m/z* = 1203 and gave ^{13}C- and ^{13}C-DEPT NMR data consistent with a $C_{59}H_{96}O_{25}$ molecular formula. The aglycone of **4** was identified as oleanolic acid by spectral data [6]. Additionally for **4**, resonances of anomeric protons were observed in the ^1H-NMR spectrum at δ = 4.33 (d, *J* = 6.8 Hz), 4.71 (d, *J* = 7.5 Hz), 4.88 (d, *J* = 1.5 Hz), 4.90 (d, *J* = 1.5 Hz), and 5.36 (d, *J* = 7.5 Hz). 1D-TOCSY, DQF-COSY, and HSQC NMR experiments, together with GC analysis, showed the presence of one α-L-arabinopyranosyl unit, two α-L-rhamnopyranosyl units, and two β-D-glucopyranosyl units (Table **2**). The HSQC spectrum also showed glycosylation shifts for C-2 of the arabinopyranosyl unit (76.8 ppm), C-3 of one rhamnopyranosyl unit (81.0 ppm), and C-4 of the glucopyranosyl moiety (78.4 ppm). An unambiguous determination of the sequence and linkage sites was obtained from the HMBC correlations between $H\text{-}1_{ara}$ (δ = 4.33) and C-3 (87.5 ppm), $H\text{-}1_{rhaI}$ (δ = 4.90) and $C\text{-}2_{ara}$ (76.8), $H\text{-}1_{rhaII}$ (δ = 4.88) and $C\text{-}3_{rhaI}$ (81.0 ppm), $H\text{-}1_{glcI}$ (δ = 5.36) and C-28 (177.0 ppm), and $H\text{-}1_{glcII}$ (δ = 4.71) and $C\text{-}4_{glcI}$ (78.4 ppm). On the basis of this evidence, **4** was established as 3β-*O*-(α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-olean-12-ene-28-*O*-(β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl) ester.

Compound **5** ($C_{53}H_{86}O_{21}$) possessed a FAB-MS showing the $[M-H]^-$ peak at *m/z* = 1057. Analysis of the NMR data of compound **5** and comparison with those of **3** (Table **1**) showed that they both had the same saccharidic chains at C-3 and C-28 while the aglycone was the point of difference. The aglycone of **5** was identified as oleanolic acid, so **5** was determined to be 3β-*O*-(α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-olean-12-ene-28-*O*-(β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl) ester.

The FAB-MS of compound **6** ($C_{53}H_{86}O_{21}$) showed $[M-H]^-$ at *m/z* = 1057. Its ^{13}C- and ^{13}C-DEPT NMR spectra showed 53 signals, of which 30 were assigned to the triterpene moiety and 23 to the saccharide portion. Comparison of the ^1H- and ^{13}C-NMR data of compounds **6** and **5** indicated an identical aglycone moiety and a difference in the sugar chains at C-3 and C-28. The sugar portion of **6** contained, in the ^1H-NMR spectrum (Table **2**), four anomeric proton signals (δ = 4.40, d, *J* = 7.0 Hz; 4.86, d, *J* = 1.8 Hz; 5.35, d, *J* = 7.5 Hz; 5.36, d, *J* = 7.5 Hz). 1D-TOCSY and DQF-COSY experiments allowed analysis of their spin system and assignments of their proton resonances, showing that compound **6** contained one xylopyranosyl, one rhamnopyranosyl, and two glucopyranosyl units. The assignments of their corresponding carbon, made by an HSQC spectrum, indicated that the xylopyranosyl and the rhamnopyranosyl units were the terminal moieties. The HMBC correlation between $H\text{-}1_{glcI}$-C-28 and $H\text{-}1_{glcII}$-$C\text{-}4_{glcI}$ allowed the identification of the sugar sequence. The D configuration of glucose and xylose unit and L configuration of rhamnose were determined by hydrolysis of **6**, trimethylsilylation, and GC analysis. Therefore, **6** was assigned the structure 3β-*O*-β-D-xylopyranosyl-olean-12-ene-28-*O*-(α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl) ester.

Compound **7** had the molecular formula $C_{47}H_{76}O_{16}$ ($[M-H]^-$ *m/z* = 895), as determined by ^{13}C-, ^{13}C-DEPT, and negative-ion FAB-MS. The ^{13}C-NMR spectrum showed 47 signals, of which 30 were assigned to a triterpenoid moiety and 17 to the saccharide portion (Table **2**). The NMR data suggested the structural features of lup-20(29)-en-28-oic acid for the aglycone of compound **7** [1], [15]. The oligosaccharide structure was determined by 2D-NMR and GC analysis. Comparison of sugar portion spectral data of compounds **7** and **5** showed structural similarities: particularly the sugar chain linked at C-3 was the same, while at C-28 was present only one β-glucopyranose unit in **7** instead of two in **5**. Therefore, the structure 3β-*O*-(α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-lup-20(29)-ene-28-*O*-β-D-glucopyranosyl ester was assigned to **7**.

The FAB-MS of compound **8** ($C_{41}H_{66}O_{12}$) showed a prominent fragment at *m/z* = 749. The spectral data of the aglycone moiety of **8** were identical with those of **7**. Comparison of NMR spectral

data of the sugar moiety (Table **2**) of **8** with those of **7** indicated that **8** differed from **7** only by the absence of the terminal rhamnopyranosyl unit linked at C-3. Thus, compound **8** was determined to be 3β-*O*-α-L-arabinopyranosyl-lup-20(29)-ene-28-*O*-β-D-glucopyranosyl ester.

Compound **9** had molecular formula $C_{30}H_{46}O_{20}$ (FAB-MS and NMR spectral data). The ^{1}H-NMR spectrum (Table **3**) exhibited signals ascribable to a benzyl function [7] and four anomeric protons. Analysis of 1D-TOCSY, DQF-COSY, and GC data permitted to recognise the presence of three β-D-glucopyranose and one β-D-apiofuranose moiety. HSQC experiment secured the assignment of all proton and carbon resonances. The absence of any ^{13}C-NMR glycosidation shift for one glucopyranosyl and the apiofuranosyl residue suggested that these sugars were terminal units, while glycosidation shifts at C-4_{glcI}, C-6_{glcI}, and C-4_{glcII} allowed us to define the structure of the saccharide chain of **9**. The interglycosidic linkages were confirmed from the HMBC experiment: correlations were observed between H-1_{glcI} and H_2–7 of the benzyl unit, H-1_{glcII} and C-4_{glcI}, H-1_{apio} and C-6_{glcI}, and H-1_{glcIII} and C-4_{glcII}. Therefore, the structure benzyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→6)]-β-D-glucopyranoside was assigned to **9**.

Finally, to compound **10** was assigned $C_{24}H_{36}O_{15}$ as molecular formula ($[M-H]^-$ at m/z = 563). Analysis of its NMR data and comparison with those of **9** showed **10** differed from **9** only in the absence of the terminal glucopyranosyl unit (Table **3**). Thus, compound **10** was determined to be benzyl β-D-glucopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→6)]-β-D-glucopyranoside.

The antiproliferative activity of all compounds was evaluated against the J774.A1, WEHI-164, and HEK-293 cell lines. Benzyl glycosides **9** and **10** were completely inactive, while data obtained (IC_{50} values) for saponins **1–8** are reported in Table **4**. As can be seen from the results, compounds **1–6** possessed the lowest activity, irrespective of the aglycone structure (hederagenin for compounds **1–3** and oleanolic acid for compounds **4–6**). The absence of the α-hydroxy function at C-16 of oleanane skeleton reduced the antiproliferative activity, as observed for similar derivatives previously tested [2]. Compounds **7** and **8**, having betulinic acid as aglycone, were the most active constituents. Generally, cytotoxic effects of compounds **1–8** were dependent on the number of sugar units: the ones having less sugar moieties were more intense in activity as compared with those having more sugar moieties. A possible explanation is that the number of the sugar moieties determines the hydrophilic properties of a compound; the hydrophilic compounds are less able to pass through the cell membrane of mammalian cells, which is reflected in a lower cytotoxicity.

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