Antiproliferative Triterpene Saponins from *Trevesia palmata*

Nunziatina De Tommasi,*,† Giuseppina Autore,† Aurora Bellino,‡ Aldo Pinto,† Cosimo Pizza,† Raffaella Sorrentino,§ and Pietro Venturella‡

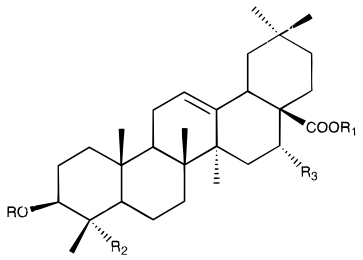
Dipartimento di Scienze Farmaceutiche, Via Ponte Don Melillo, Invariante 11C, Fisciano, Salerno, Italy, Dipartimento di Farmacologia Sperimentale, Via D. Montesano 49, 80131 Napoli, Italy, and Dipartimento di Scienze Botaniche, Sezione Fitochimica, Via Archirafi 20, Palermo, Sicily, Italy

Received May 17, 1999

During the course of a study of plants of the family Araliaceae, antiproliferative activity was demonstrated by the crude saponin fraction of *Trevesia palmata*. After chromatographic purification, six new bisdesmosidic saponins (**1**−**6**), along with two known triterpenoid saponins, (**7** and **8**), were isolated. The structures of **1**−**6** were determined by ^{1}H−^{1}H correlation spectroscopy (COSY−DQF, 1D TOCSY, 2D HOHAHA, 1D ROESY) and ^{1}H−^{13}C (HSQC, HMBC) spectroscopy. The antiproliferative activity of compounds **1**−**8** and of their prosapogenins (**2a**−**7a**) prepared by alkaline hydrolysis, was evaluated using three continuous culture cell lines.

Chemical and pharmacological investigations have indicated that triterpenoid saponins are important bioactive components existing in plants of the family Araliaceae.[1] Saponins isolated and identified from these species are reported to have various pharmacological activities, including increasing mental efficiency, permitting the recovery of physical balance, stimulating metabolic function, and having other general health-promoting effects.[2]

Trevesia palmata (Roxb. ex Lindl.) Vis. (Araliaceae), a plant native to India, is used in folk medicine as a general tonic.[3] The present investigation is part of a series of studies on glycosides from plants in the Araliaceae available at the Botanical Garden of Palermo.[4,5] In this paper we describe the structure determination of six new oleanane saponins (**1**−**6**), using mainly high field (600 MHz) NMR spectroscopy and a combination of 1D and 2D NMR techniques. Also, two glycosides of known structures **7** and **8** were obtained. All of the isolated glycosides are bidesmosides with sugar chains made up of two or three monosaccharide units linked to C-3, as well as sugar chains consisting of three or four monosaccharide units linked to C-28. Because a crude saponin fraction obtained from *T. palmata* showed antiproliferative activity against the J774 cell line, we have determined the antiproliferative activity profile of the purified saponins. Furthermore, to investigate the relationship between antiproliferative activity and the compound structures, we have prepared several prosapogenins by alkaline hydrolysis and also tested them for antiproliferative activity.



	R	R$_1$	R$_2$	R$_3$
1	Glc-(1—>3)-Rha-(1—>2)-Ara	Glc-(1—>3)-Rha-(1—>4)-Glc-(1—>6)-Glc	CH_3	H
2	Glc-(1—>3)-Rha-(1—>2)-Ara	Rha-(1—>4)-Glc-(1—>6)-Glc	CH_3	H
2a	Glc-(1—>3)-Rha-(1—>2)-Ara	H	CH_3	H
3	Qui-(1—>2)-Ara	Rha-(1—>4)-Glc-(1—>6)-Glc	CH_3	H
3a	Qui-(1—>2)-Ara	H	CH_3	H
4	Qui-(1—>2)-Ara	Rha-(1—>4)-Glc-(1—>6)-Glc	CH_2OH	H
4a	Qui-(1—>2)-Ara	H	CH_2OH	H
5	Glc-(1—>3)-Rha-(1—>2)-Ara	Rha-(1—>4)-Glc-(1—>6)-Glc	CH_3	OH
5a	Glc-(1—>3)-Rha-(1—>2)-Ar	H	CH_3	OH
6	Glc-(1—>3)-Rha-(1—>2)-Ara	Rha-(1—>4)-Qui-(1—>6)-Glc (2—>1) Glc	CH_2OH	H
6a	Glc-(1—>3)-Rha-(1—>2)-Ara	H	CH_2OH	H
7	Rha-(1—>2)-Ara	Rha-(1—>4)-Glc-(1—>6)-Glc	CH_3	H
7a	Rha-(1—>2)-Ara	H	CH_3	H
8	Glc-(1—>3)-Rha-(1—>2)-Ara	H	CH_2OH	H

Results and Discussion

The MeOH extract of the aerial parts of *T. palmata* was subjected to Sephadex LH-20 column cromatography, followed by droplet countercurrent chromatography (DCCC) [*n*-BuOH−Me_2CO−H_2O (30:9:11), descending mode] and then reversed-phase HPLC, to give six pure compounds (**1**−**6**). Two known compounds were also isolated. The known compounds were identified as 3*β*-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]olean-12-ene-28-*O*-[α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl] ester (**7**)[6] and hederagenin-3-*O*-*β*-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**8**)[7] by spectral data and direct comparison of their physical properties with those reported previously for these compounds.

Their ^{1}H and ^{13}C NMR spectra indicated that saponins **1**−**3** showed identical aglycon portions but had different saccharide chains. Acid hydrolysis of **1**−**3** afforded oleanolic acid, which was identified by comparing its ^{1}H and ^{13}C NMR data with reported data.[8,9]

In compounds **1**−**6**, C-28 appeared at 178.0 ppm in the ^{13}C NMR spectra and H-18 appeared at δ 2.92 (dd, J = 12.0 and 4.0 Hz) in the ^{1}H NMR spectra, indicating that the carboxyl group was glycosylated.[7] Attachment of an-

* To whom correspondence should be addressed. Tel.: +39-89-962647. Fax: +39-89-962828. E-mail: detommasi@unisa.it.
† Dipartimento di Scienze Farmaceutiche.
‡ Dipartimento di Scienze Botaniche.
§ Dipartimento di Farmacologia Sperimentale.

10.1021/np990231n CCC: $19.00
Published on Web 02/05/2000

Table 1. ^{1}H NMR Data (δ values, J in Hz) for the Oligosaccharide Moieties of Compounds **1**−**3** and **6** in CD_3OD[a]

proton	**1**	**2**	**3**	**6**
Ara 1	4.44 (5.5)	4.45	4.45 d (5.4)	4.44 d (6.2)
2	3.91 dd (5.5, 9.0)	3.91	3.91 dd (5.4, 9.0)	3.94 dd (6.2, 9.0)
3	3.80 t (9.0, 3.0)	3.82	3.80 t (9.0, 3.0)	3.84 t (9., 3.0)
4	4.02 m	4.02	4.03 m	4.03 m
5a	3.96 dd (12.0, 2.0)	3.96	3.99 dd (12.0, 2.0)	3.99 dd (12.0, 2.0)
5b	3.60 dd (12.0, 3.5)	3.61	3.65 dd (12.0, 3.5)	3.64 dd (12.0, 3.5)
Rha I 1	5.17 d (1.5)	5.17		5.18 d (1.5)
2	4.23 dd (1.5, 3.0)	4.23		4.23 dd (1.5, 3.5)
3	3.87 dd (9.0, 3.0)	3.88		3.90 dd (9.0, 3.5)
4	3.59 t (9.0)	3.59		3.60 t (9.0)
5	3.89 dd (9.0, 6.2)	3.87		3.86 dd (9.0, 6.5)
6	1.28 d (6.2)	1.28		1.30 d (6.5)
Glc I 1	4.51 d (7.5)	4.52		4.50 d (7.5)
2	3.35 dd (7.5, 9.0)	3.35		3.34 dd (7.5, 9.0)
3	3.43 t (9.0)	3.37		3.37 t (9.0)
4	3.31 t (9.0)	3.31		3.31 t (9.0)
5	3.40 m	3.39		3.39 m
6a	3.82 dd (12.0, 3.5)	3.85		3.81 dd (12.0, 3.5)
6b	3.67 dd (12.0, 5.0)	3.67		3.67 dd (12.0, 5.0)
Glc C-28 1	5.35 d (7.6)	5.36	5.35 d (7.6)	5.35 d (7.6)
2	3.28 dd (9.5, 7.6)	3.29	3.28 dd (9.5, 7.6)	3.28 dd (9.5, 7.6)
3	3.48 t (9.5)	3.47	3.47 t (9.5)	3.44 t (9.5)
4	3.51 t (9.5)	3.49	3.50 t (9.5)	3.50 t (9.5)
5	3.55 m	3.55	3.55 m	3.54 m
6a	4.11 dd (12.0, 3.0)	4.15	4.12 dd (12.0, 3.0)	4.15 dd (12.0, 3.0)
6b	3.83 dd (12.0, 5.0)	3.82	3.84 dd (12.0, 5.0)	3.88 dd (12.0, 5.0)
Glc II 1	4.48 d (7.6)	4.48	4.48 d (7.6)	4.48 d (7.6)
2	3.42 dd (9.5, 7.6)	3.41	3.45 dd (9.5, 7.6)	3.48 dd (9.5, 7.6)
3	3.60 t (9.5)	3.62	3.63 t (9.5)	3.54 t (9.5)
4	3.25 t (9.5)	3.26	3.26 t (9.5)	3.35 t (9.5)
5	3.28 m	3.24	3.24 m	3.26 m
6a	3.93 dd (12.0, 3.0)	3.93	3.95 dd (12.0, 3.0)	3.97 dd (12.0, 3.0)
6b	3.70 dd (12.0, 5.0)	3.70	3.72 dd (12.0, 5.0)	3.71 dd (12.0, 5.0)
Rha II 1	4.90 d (1.6)	4.87	4. 88 d (1.5)	4.90 d (1.5)
2	4.18 dd (1.6, 3.0)	3.84	3.88 dd (1.5, 3.0)	3.83 dd (1.5, 3.5)
3	3.84 dd (9.0, 3.0)	3.65	3.81 dd (9.0, 3.0)	3.62 dd (9.0, 3.5)
4	3.58 t (9.0)	3.44	3.58 t (9.0)	3.46 t (9.0)
5	3.87 dd (9.0, 6.2)	3.99	3.87 dd (9.0, 6.5)	4.01 dd (9.0, 6.5)
6	1.31 d (6.2)	1.30	1.28 d (6.5)	1.28 d (6.5)
Glc III 1	4.64 d (7.6)			
2	3.21 dd (9.5, 7.6)			
3	3.34 t (9.5)			
4	3.33 t (9.5)			
5	3.44 m			
6	3.78 dd (12.0, 3.0)			
	3.62 dd (12.0, 5.0)			
Qui 1			4.38 d (7.5)	4.38 d (7.5)
2			3.58 dd (9.0, 7.5)	3.58 dd (9.0, 7.5)
3			3.46 t (9.0)	3.42 t (9.0)
4			3.43 t (9.0)	3.45 t (9.0)
5			3.65 dd (9.0, 6.5)	3.61 dd (9.0, 6.5)
6			1.32 d (6.5)	1.32 d (6.5)

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

other glycosidic chain at C-3 was suggested in each case by the significant downfield shift observed for this carbon in **1**−**6** relative to the corresponding signal in oleanolic acid derivatives, and was subsequently confirmed by 2D NMR experiments.[3−10]

Compound **1** had the molecular formula $C_{71}H_{116}O_{35}$, as determined by ^{13}C, ^{13}C−DEPT NMR, and negative-ion FABMS. The FABMS of **1** showed the $[M - H]^-$ ion at m/z 1527 and prominent fragments at m/z 1365 $[(M - H) - 162]^-$ (cleavage of one hexose unit), m/z 1203 $[(M - H) - 162 + 162]^-$ (cleavage of two hexose units, respectively), m/z 1057 (due to the subsequent loss of two hexose units and one deoxyhexose unit), and m/z 587 (due to the sequential loss of a hexose and a deoxyhexose unit) from the m/z 1067 fragment. A peak at m/z 455 was attributed to the aglycon moiety. The ^{13}C NMR spectrum showed 71 signals, of which 30 were assigned to a triterpenoid moiety and 41 to the saccharide portion.

In the ^{1}H NMR spectrum of **1** (Table 1), the sugar portion contained seven anomeric proton signals (δ 4.44, d, J = 5.5 Hz; 4.48, J = 7.6 Hz; 4.51, d, J = 7.5 Hz; 4.64, d, J = 7.6 Hz; 4.90, d, J = 1.6 Hz; 5.17, d, J = 1.5 Hz; 5.35, d, J = 7.6 Hz) and two methyl doublets (δ 1.28, d, J = 6.2 Hz and 1.31, d, J = 6.2 Hz), suggesting the presence of two deoxyhexose units. The other sugar signals were overlapped in the region between δ 3.10 and 4.25. The structures of the oligosaccharide moieties were deduced using 1D TOCSY,[11] 1D ROESY,[12] and 2D NMR experiments. Because of the selectivity of the multistep coherence transfer, the 1D TOCSY method[11] allowed a sub-spectrum of a single monosaccharide unit to be extracted from the crowded overlapped region. The isolated anomeric proton signals resonating in an uncrowded region of the spectrum (between δ 4.44 and 5.35) were the starting point for the 1D TOCSY experiments. Selected 1D TOCSY data were obtained by irradiating each anomeric proton signal to yield

the sub-spectrum of each sugar residue with high digital resolution. Each sub-spectrum contained the scalar-coupled protons within each sugar residue. In some cases, because of the small coupling constants, the distribution of magnetization around the spin system was impeded. For this reason, it was possible to identify only three protons (δ 4.02, 3.91, 3.80) coupled to the anomeric signal at δ 4.44, as in the case of arabinose (Table 1). For the 6-deoxyhexoses, easier identification of all of the proton signals was accomplished by also recording 1D TOCSY experiments and irradiating the methyl doublets. Because, with the TOCSY method, both direct and relayed connectivities occur, we also recorded a DQF−COSY spectrum.[13] The results of 1D TOCSY and DQF−COSY experiments allowed the sequential assignments of all of the proton resonances to the individual monosaccharides, as reported in Table 1. Thus, the shifts of the sugar resonances, as summarized in Table 1, were attributable to the α-L-arabinopyranosyl ($\delta_{H-1_{ara}}$ = 4.44), α-L-rhamnopyranosyl ($\delta_{H-1_{rha\ I}}$ = 5.17), α-L-rhamnopyranosyl ($\delta_{H-1_{rha\ II}}$ = 4.90), β-D-glucopyranosyl ($\delta_{H-1_{glc\ I}}$ = 4.51), β-D-glucopyranosyl ($\delta_{H-1_{glc\ II}}$ = 4.48), β-D-glucopyranosyl ($\delta_{H-1_{glc\ III}}$ = 4.64), and β-D-glucopyranosyl ($\delta_{H-1_{glc}}$ = 5.35) units. In the case of the arabinopyranosyl unit, the J_{H1-H2} coupling constant (5.5 Hz), midway between that observed for methyl-β-L-arabinopyranoside (4 Hz) and methyl-α-L-arabinopyranoside (8 Hz),[14] has been reported not to be diagnostic on its own, owing to the high conformational mobility of arabinopyranosides ($^4C_1 \leftrightarrow {}^1C_4$). As we reported in previous studies,[15,16] evidence of α-L-arabinopyranoside was obtained from the 1D ROESY spectrum. Selected 1D ROESY data obtained by irradiating the anomeric proton signal $\delta_{H-1_{ara}}$ = 4.44 yielded the sub-spectrum of the arabinose residue, which contained the dipolar-coupled protons and showed ROEs from H-1_{ara} to H-$2_{ara,}$ H-3_{ara}, and H-5_{ara}, as expected for an α-L-arabinopyranoside in rapid $^4C_1 \leftrightarrow {}^1C_4$ conformational exchange.

HSQC[17] experiments, which correlated all the proton resonances with those of each corresponding carbon, allowed the assignments of interglycosidic linkages of **1** by comparison of the observed carbon chemical shifts with those of the corresponding methylpyranosides. The absence of any ^{13}C NMR glycosidation shift for two β-D-glucopyranosyl moieties suggested that these sugars were terminal units. Glycosidation shifts were observed for C-2_{ara} (76.3), C-$3_{rha\ I}$ (80.4), C-$3_{rha\ II}$ (80.8), C-$4_{glc\ II}$ (79.0) and C-6_{glc} (68.8) (Table 2). Chemical shifts of H-1_{glc} (δ 5.35) and C-1_{glc} (δ 95.4) indicated that this sugar unit was involved in an ester linkage with the C-28 carboxylic group.[18]

The positions of the sugar residues in **1** were defined unambiguously by the HMBC experiment.[19] A cross-peak due to long-range correlations between C-3 (δ 90.5) of the aglycon and H-1_{ara} (δ 4.44) indicated that arabinose was the residue linked to C-3 of the aglycon; a cross-peak between C-2_{ara} (δ 76.3) and H-1 of the rhamnose I (δ 5.17) indicated that rhamnose was the second unit; and a cross-peak between C-$3_{rha\ I}$ (δ 80.4) and H-1 of terminal glucose (δ 4.51) indicated that glucose was the third unit of the trisaccharide chain at C-3 of the aglycon. Similarly, the sequence of the tetrasaccharide chain at C-28 was indicated by the cross-peaks between C-6_{glc} (δ 68.8) and H-$1_{glc\ II}$ (δ 4.48), C-$4_{glc\ II}$ (δ 79.0), and H-$1_{rha\ II}$ (δ 4.90) and between C-$3_{rha\ II}$ (δ 80.8) and H-1 of the terminal glucose (δ 4.64). A cross-peak between H-1 of glucose (δ 5.35) and the ^{13}C NMR resonance of the aglycon carbonyl group (δ 178.0) provided definitive prove for an ester linkage between the tetrasaccharide chain and the aglycon. On the basis of all of this evidence, compound **1** was identified as 3β-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]olean-12-ene-28-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Table 2. ^{13}C NMR Data for the Oligosaccharide Moieties of Compounds **1**−**3**, and **6** in CD_3OD[a]

carbon	**1**	**2**	**3**	**6**
Ara 1	105.3	104.6	104.8	105.0
2	76.3	76.2	76.6	76.5
3	72.9	73.1	73.0	72.8
4	71.8	72.0	71.8	71.8
5	64.2	64.2	64.8	64.5
Rha I 1	101.4	101.1		101.5
2	70.7	70.5		70.8
3	80.4	80.0		80.8
4	71.9	71.7		71.9
5	69.2	69.5		69.2
6	18.0	18.2		18.0
Glc I 1	105.4	105.6		105.5
2	73.2	73.9		74.1
3	77.6	77.8		77.6
4	70.2	70.3		70.5
5	77.9	77.9		78.1
6	61.0	61.5		61.6
Glc, C-28 1	95.4	95.6	95.4	95.6
2	74.9	74.0	74.5	74.0
3	77.6	77.8	78.0	77.7
4	70.3	71.0	71.4	71.5
5	77.5	77.0	76.9	76.9
6	68.8	69.5	69.0	69.0
Glc II 1	103.9	104.0	104.3	103.4
2	74.7	74.0	74.0	85.3
3	76.8	76.6	77.0	76.1
4	79.0	79.1	79.5	79.1
5	76.7	76.4	76.9	76.8
6	61.9	61.9	62.0	61.7
Rha II 1	102.0	102.6	101.9	102.0
2	70.6	71.8	72.0	70.6
3	80.8	71.6	71.7	71.6
4	71.5	73.0	73.2	73.0
5	69.5	70.0	70.0	69.5
6	18.3	17.8	17.6	18.3
Glc III 1	105.0			
2	74.1			
3	78.0			
4	70.9			
5	78.1			
6	62.0			
Qui 1			106.0	105.8
2			75.5	76.0
3			78.2	78.4
4			72.0	71.7
5			77.4	77.0
6			18.0	18.0

[a] Assignments (δ values) were confirmed by COSY−DQF, 1D TOCSY, HSQC, and HMBC experiments.

Acid methanolysis of **2** gave glucose, rhamnose, and arabinose in a 3:2:1 ratio. The FABMS of compound **2** ($C_{65}H_{106}O_{30}$) showed the $[M - H]^-$ ion at *m/z* 1365 and prominent fragments at *m/z* 1219 $[(M - H) - 146]^-$ and *m/z* 1203 $[(M - H) - 162]^-$ (cleavage of one hexose and one deoxyhexose, respectively) and *m/z* 895 $[(M - H) - (146 + 162 + 162)]^-$ (due to the sequential losses of two hexose units and one deoxyhexose unit). A peak at *m/z* 455 was attributed to the aglycon moiety. The ^{13}C and DEPT ^{13}C NMR spectra showed 65 signals of which 35 were assigned to the saccharide portion and 30 to the triterpene moiety. Analysis of NMR data of compound **2** and comparison with those of **1** showed **2** to differ from **1** only in the absence of the terminal glucopyranosyl unit linked at C-3 of rhamose II (Tables 1 and 2). Therefore, the structure 3β-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-ara-

binopyranosyl]olean-12-ene-28-*O*-[α-L-rhamnopyranosyl (1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl] ester was assigned to **2**.

Compound **3** ($C_{59}H_{96}O_{25}$) was identified as a further oleanolic acid derivative possessing two sugar moieties at C-3 and C-28. The FABMS exhibited a fragmentation pattern consistent with the cleavage of a terminal deoxyhexose unit (146 mass units), followed by sequential losses of another deoxyhexose unit (146 mass units), two hexose units (162 mass units), and a pentose unit (132 mass units) (see Experimental Section). Also, in this case, the proton-coupling network within each sugar residue was traced out, using a combination of DQF−COSY, 1D TOCSY, and HSQC experiments, which indicated that a *β*-D-quinovopyranosyl unit was present instead of the α-L-rhamnopyranosyl unit observed in the disaccharide chain at C-3 of compound **7** (Tables 1 and 2). In fact, the 1D TOCSY subspectrum obtained when irradiating the anomeric proton signal at *δ* 4.38 showed a set of coupled protons at *δ* 3.65, 3.58, 3.46, and 3.43 (all CH), and 1.32 (CH_3), assigned from H-1 to CH_3-6 of a *β*-D-quinovopyranosyl unit. Once again, direct evidence for the sugar sequence and the linkage sites was derived from a HMBC experiment. These results established the same trisaccharide chain as in **2**, linked to the COOH group at C-28, whereas the disaccharide chain was made up by an α-L-arabinopyranosyl unit substituted at position 2 by a *β*-D-quinovopyranosyl unit at C-3 of the aglycon. On the basis of the obtained data the structure of **3** was established as 3*β*-*O*-[*β*-D-quinovopyranosyl-(1→2)-α-L-arabinopyranosyl]olean-12-ene-28-*O*- [α-L-rhamnopyranosyl (1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl] ester.

Comparison of the NMR spectral data of compound **4** ($C_{59}H_{96}O_{26}$) with those of **3** showed these to be identical in the sugar portion but different in the aglycon portion. In particular, hydrogen and carbon NMR signals due to the C, D, and E rings of **4** resonated near the same frequencies as the corresponding signals in oleanolic acid, although the A- and B-ring ^{1}H and ^{13}C signals were shifted somewhat. The NMR spectra of **4** contained one less methyl and more signals [^{1}H NMR *δ* 3.30 (1H, d, J = 11.8 Hz), 3.71 (1H, d, J = 11.8 Hz); ^{13}C NMR *δ* 64.8 (CH_2)] than those of **3**, suggesting that one of the Me groups was replaced by a hydroxymethyl group in **4**. In addition, one of the methyl signals was shifted upfield to *δ* 0.71 in **4** due to the −CH_2-OH group. The most significant features of the ^{13}C NMR spectrum of **4**, which suggested placement of the −CH_2-OH group at C-23, were the downfield shifts exhibited by C-4 and the upfield shifts experienced by C-3, C-5, and Me-24. The aglycon of **4** was identified as hederagenin.[20,21] Thus **4** was determined to be 3*β*-*O*-[*β*-D-quinovopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin 28-*O*-[α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl] ester.

Compound **5** showed, in the negative ion FABMS, a quasi-molecular anion ([M − H]$^-$) at m/z 1381, 16 mass units higher than that of **2** and gave ^{13}C and DEPT ^{13}C NMR data consistent with a $C_{65}H_{106}O_{31}$ molecular formula. The FABMS showed a fragmentation pattern similar to that of **2**. Comparison of ^{1}H and ^{13}C NMR data of both compounds indicated identical saccharide chains at C-3 and C-28 and structural similarity in the aglycon moieties. The main differences were the downfield shifts of C-16 (*δ* 75.0) and C-15 (*δ* 36.0) in the ^{13}C NMR spectrum and the downfield shift of the axial methyl group at C-14 (Me-27, *δ* 1.39) in the ^{1}H NMR spectrum, implying an additional hydroxyl group at C-16 in **5**. This hypothesis was confirmed unambiguously by the HMBC spectrum showing cross-peaks between the proton at *δ* 4.51 (H-16) and the C-14, C-15, C-17, C-18, and C-22 signals. The α configuration of the C-16 hydroxyl group was evident from the chemical shift and the small J value of H-16 (*δ* 4.51, br m), characteristic of an equatorial proton.[15] Thus, the aglycon of **5** was identified as echinocystic acid[22] and compound **5** was defined as 3*β*-*O*-[*β*-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]echinocystic acid 28-*O*-[α-L-rhamnopyranosyl (1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl] ester.

The FABMS of **6** showed a [M − H]$^-$ ion at m/z 1543 with prominent fragments at m/z 1397 [(M − H) − 146]$^-$, 1251 [(M − H) − (146 + 146)]$^-$ (cleavage of two deoxyhexose units), m/z 1089 [(M − H) − (146 + 146 + 162)]$^-$, and 781 [(M − H) − (146 + 146 + 162 + 146 + 162)]$^-$. A peak at m/z 487 was attributed to the aglycon moiety. The ^{13}C and ^{13}C DEPT NMR spectra of **6** showed 71 signals, of which 41 were assigned to the saccharide portion and 30 to the triterpene moiety. The ^{1}H NMR spectrum of the aglycon moiety of **6** showed six singlets assignable to tertiary methyls in the range *δ* 0.72−1.39. An OH-3*β* substitution was evident from the chemical shift and the J value of the proton assigned to C-3 centered at *δ* 3.66. The signal at *δ* 4.46 (br s) indicated the presence of a C-16α hydroxyl group, a conclusion that was further supported by the ^{13}C NMR data (see Experimental Section) and by the C-27 methyl, which resonated at *δ* 1.39, downfield from its usual position.[23] Further features were signals at *δ* 5.27 (1H, t, J = 3.5 Hz), 3.18 (1H, d, J = 11.8 Hz), and 3.61 (1H, d, J = 11.8 Hz), ascribable, respectively, to two olefinic protons, and a hydroxymethyl proton. These data indicated that the aglycon was an oleanolic acid derivative with one of the methyl groups substituted by a −CH_2OH group. Full assignments of the proton and carbon resonances of the aglycon (Experimental Section) were secured from the DQF−COSY and HSQC spectral data. The CH_2OH group was located at C-23 on the basis of the downfield shift exhibited by C-4 and the upfield shift experienced by C-3, C-5, and Me-24. In addition, one of the methyl signals was shifted upfield to *δ* 0.72 in **6** due to the −CH_2OH group. Thus, the aglycon of **6** was 3*β*,16α,23-trihydroxyolean-12-en-28-oic acid.

The oligosaccharide structure of **6** was determined by 2D NMR; 1D and 2D TOCSY spectroscopic[24] experiments allowed resolution of the overlapped spectra of the sugar units into a subset of individual monosaccharide spectra, and, together with the DQF−COSY spectrum, this allowed complete sequential assignments for all proton resonances, starting from the anomeric proton signals of each sugar residue (Tables 1 and 2). HSQC experiments permitted assignments of the interglycosidic linkages by comparing the ^{13}C NMR shifts observed with those of the corresponding methyl pyranosides and taking into account the known effects of glycosidation.[22] The absence of any ^{13}C NMR glycosidation shift for one glucopyranosyl, one rhamnopyranosyl, and one quinovopyranosyl residue suggested that these sugars were terminal units, while glycosidation shifts at C-2 of the arabinopyranosyl unit and at C-3 of rhamnose I, C-6 of the glucose unit linked at C-28, and at C-2 and C-4 of glucose II allowed us to define the structure of the saccharide chains of **6**. The interglycosidic linkages were deduced from an HMBC experiment. Correlations were observed between H-1 of arabinose and C-3 of the aglycon, H-1 of rhamnose I and C-2 of arabinose, H-1 of glucose I and C-3 of rhamnose I, H-1 of glucose II and C-6 of glucose, H-1 of quinovose and C-2 of glucose II, and H-1 of rhamnose

Table 3. In Vitro Anti-proliferative Activity of Saponins and Prosapogenins from *Trevesia Palmata*[a]

	cell line [IC_{50}, μM]		
compound	J774[b]	HEK-293[c]	WEHI-164[d]
1	0.18	0.17	nd
2	nd	0.52	1.8
2a	nd[e]	nd	nd
3	0.1	0.2	nd
3a	nd	nd	nd
4	0.46	nd	1.9
4a	nd	nd	nd
5	0.11	0.15	0.24
5a	0.19	nd	nd
6	0.1	0.32	0.26
6a	0.07	nd	nd
7	0.06	nd	0.6
7a	nd	nd	nd
8	nd	nd	nd
6-MP[f]	0.003	0.007	0.017

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

II and C-4 of glucose II. The ^1H and ^{13}C NMR data indicated a β configuration at the anomeric positions for the glucopyranosyl and quinovopyranosyl units and an α configuration at the anomeric positions for the rhamnopyranosyl and arabinopyranosyl units (Tables 1 and 2). Therefore, the structure 3β-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]16α, 23 dihydroxyolean-12-ene-28-*O*-{-α-L-rhamnopyranosyl (1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]}ester was assigned to **6**.

Basic hydrolysis of compounds **2**−**7** gave prosapogenins **2a**−**7a** that showed the free carboxyl group of the aglycon at ca. 181.6 ppm in the ^{13}C NMR spectrum, whereas it resonated at ca. 178.0 ppm when esterified, as in **2**−**7**. Analysis of NMR data of compounds **2a**−**7a** and comparison with those of **2**−**7** showed that **2a**−**7a** differed from **2**−**7** only in the absence of the saccharide chain esterified at C-28 (see Experimental Section). The antiproliferative activity of saponins **1**−**8** and their prosapogenins **2a**−**7a** was evaluated against the J774, WEHI-164, and HEK-293 cell lines, with the IC_{50} values obtained shown in Table 3. Comparison of the data obtained for compounds **1**−**8** and their prosapogenins **2a**−**7a** clearly showed that a saccharide chain esterified at C-28 is crucial for the antiproliferative activity. However, the saccharide chain at C-3 seems not to be necessary for cytotoxicity owing to the inactivity of prosapogenins **2a**−**4a** and compounds **7a**, **8**. The hydroxyl group at C-16 of the aglycon may play an important role in mediating antiproliferative activity among these compounds because compounds **5** and **6** showed the strongest cytotoxicity, and their prosapogenins **5a** and **6a** still showed activity against the J774 cell line, while prosapogenins **2a**−**4a** and **7a** were completely inactive.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm on 1% w/v solution in MeOH. A Bruker DRX-600 spectrometer operating at 599.19 MHz for ^1H and 150.858 MHz for ^{13}C, and the UX-NMR software package was used for NMR measurements in CD_3OD solutions. The 2D experiments, ^1H−^1H DQF−COSY,[12] 2D HOHAHA,[23] inverse detected ^1H−^{13}C HSQC,[16] HMBC,[18] and ROESY,[11] were obtained using UX-NMR software. 1D TOCSY[10] spectra were acquired using waveform generator-based GAUSS shaped pulses, mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5-ms trim pulse. FABMS were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (Xe atoms of energy of 2−6 kV). DCCC was performed on an apparatus manufactured by Büchi, equipped with 300 tubes. HPLC separations were performed on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector and with a Waters μ-Bondapak C_{18} column and a U6K injector. GC were run using a Hewlett-Packard 5890 gas chromatograph equipped with mass-selective detector MSD 5970 MS, a split/splitness injector, and a Hewlett−Packard HP-5 fused-silica column (25 m × 0.2 mm; i.d. 0.33 mm film; Wilmington, DE).

Plant Material. The plant *Trevesia palmata* was collected in Palermo, Italy, in September 1995; a sample (1224/30BP) has been deposited in the Herbarium of the Botanical Garden of Palermo.

Extraction and Isolation. The dried leaves (800 g) were defatted with petroleum ether (6 g) then extracted with EtOH to give 11 g of residue. The ethanolic extract was dissolved in H_2O. The H_2O extract was partitioned between EtOAc and *n*-BuOH to afford an *n*-BuOH-soluble portion (8.2 g) and an EtOAc portion (2.5 g). The *n*-BuOH extract (4 g) was chromatographed on a Sephadex LH-20 column (100 × 5 cm) with MeOH as the eluent. Fractions (8 mL) were collected and checked by TLC [Si gel plates, *n*-BuOH−AcOH−H_2O (12:3:5)]. Fractions 15−26 containing the crude glycosidic mixture were further purified by DCCC using *n*-BuOH−Me_2CO−H_2O (30:9:11), in which the stationary phase consisted of the lower phase (descending mode, flow 14 mL/h). DCCC fractions 80−220 (435 mg) were chromatographed on a C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d., flow rate 3.5 mL/min), eluting with MeOH−H_2O (2:3), to yield pure compounds **1** (19 mg, t_R 6 min), **2** (50 mg, t_R 12.5 min), **5** (62 mg, t_R 11 min), and **6** (45 mg, t_R 4 min). DCCC fractions 240−270 (110 mg) afforded **7** (20 mg, t_R 19 min) and **4** (39 mg, t_R 18.5 min) using MeOH−H_2O (1:1) as the eluent (flow rate 3.5 mL/min). DCCC fractions 280−400 (320 mg) afforded **2** (21 mg, t_R 5.5 min), **7** (35 mg, t_R 11 min), **3** (56 mg, t_R 10.5 min), and **8** (23 mg, t_R 22 min) using MeOH−H_2O (55:45) as the eluent (flow rate 3 mL/min).

Methanolysis of Compounds 1−6. A solution of each compound (2 mg) in anhydrous 2 N HCl−MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial for 12 h. On cooling, the solution was neutralized with Ag_2CO_3 and centrifuged. The supernatant was evaporated to dryness under N_2. The residue was reacted with Trisil-Z (Pierce; Rockford, IL) and the carbohydrates analyzed by GLC.

Basic Hydrolysis of Compounds 2−7. Saponins **2** (30 mg), **3** (30 mg), **4** (30 mg), **5** (30 mg), **6** (30 mg), and **7** (30 mg), in 0.5 M KOH (1 mL), were each heated at 110 °C in a stoppered reaction vial for 2 h. Each reaction mixture was adjusted to pH 7 and then extracted with *n*-BuOH. The organic phase was evaporated to dryness, dissolved in CD_3OD, and analyzed by ^1H and ^{13}C NMR spectroscopy. Hydrolysis of saponins **2**−**7** gave sapogenins **2a** (15 mg), **3a** (13 mg), **4a** (11 mg), **5a** (16 mg), **6a** (18 mg), and **7a** (12 mg).

Compound 1: white amorphous powder; $[\alpha]^{25}_D$ +28° (*c* 0.9, MeOH); for NMR data of aglycon moiety, see Mahvidadze et al.[7] and Miyase et al.;[8] ^1H and ^{13}C NMR of sugar moieties, see Tables 1 and 2; FABMS *m*/*z* 1527 [M − H]$^-$, 1203 [(M − H) − (162 + 162)]$^-$, 1057 [(M − H) − (162 + 162 + 146)]$^-$, 911 [(M − H) − (162 + 162 + 146 + 146)]$^-$, 587 [(M − H) − (162 + 162 + 162 + 162 + 146 + 146)]$^-$, 455 [(M − H) − (162 + 162 + 162 + 162 + 146 + 146 + 132)]$^-$.

Compound 2: white amorphous powder: $[\alpha]^{25}_D$ +38° (*c* 1, MeOH); for NMR data of aglycon moiety, see Mahvidadze et al.[7] and Miyase et al.;[8] ^1H and ^{13}C NMR of sugar moieties, see Tables 1 and 2; FABMS *m*/*z* 1365 [M − H]$^-$, 1219 [(M − H) − 146]$^-$, 1203 [(M − H) − 162]$^-$, 1057 [(M − H) − (146 + 162)]$^-$, 749 [(M − H) − (162 + 162 + 146 + 146)]$^-$, 455 [(M − H) − (162 + 162 + 162 + 132 + 146 + 146)]$^-$.

Compound 3: white amorphous powder: $[\alpha]^{25}_D$ +30° (*c* 1, MeOH); for NMR data of aglycon moiety, see Mahvidadze et

al.[7] and Miyase et al.;[8] ^{1}H and ^{13}C NMR of sugar moieties, see Tables 1 and 2; FABMS *m/z* 1203 $[M - H]^-$, 1057 $[(M - H) - 146]^-$, 911 $[(M - H) - (146 + 146)]^-$, 749 $[(M - H) - (146 + 146 + 162)]^-$, 455 $[(M - H) - (162 + 162 + 132 + 146 + 146)]^-$.

Compound 4: white amorphous powder: $[\alpha]^{25}_D$ +25° (*c* 1, MeOH); for NMR data of aglycon moiety, see refs 19 and 20; ^{1}H and ^{13}C NMR of sugar moieties, see Tables 1 and 2; FABMS *m/z* 1219 $[M - H]^-$, 1073 $[(M - H) - 146]^-$, 927 $[(M - H) - (146 + 146)]^-$, 765 $[(M - H) - (146 + 146 + 162)]^-$, 603 $[(M - H) - (162 + 162 + 146 + 146)]^-$, 471 $[(M - H) - (162 + 162 + 132 + 146 + 146)]^-$.

Compound 5: white amorphous powder; $[\alpha]^{25}_D$ +22° (*c* 1, MeOH); for NMR data of aglycon moiety, see ref 21; ^{1}H and ^{13}C NMR of sugar moieties, see Tables 1 and 2; FABMS *m/z* 1381 $[M - H]^-$, 1219 $[(M - H) - 162]^-$, 1073 $[(M - H) - (162 + 146)]^-$, 765 $[(M - H) - (146 + 146 + 162 + 162)]^-$, 603 $[(M - H) - (162 + 162 + 162 + 146 + 146)]^-$, 471 $[(M - H) - (162 + 162 + 132 + 146 + 146)]^-$.

Compound 6: white amorphous powder; $[\alpha]^{25}_D$ +11° (*c* 1, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 5.27 (1H, t, J = 3.4 Hz, H-12), 4.46 (1H, dd, br m, H-16), 3.66 (1H, dd, J = 11.8, 4.0, Hz H_2-3), 3.61 (1H, d, J = 11.8 Hz, H-23a), 3.18 (1H, d, J = 11.8 Hz, H-23b), 2.82 (1H, dd, J = 13.0, 3.5 Hz, H-18), 1. 39 (1H, s, Me-27), 1. 00 (1H, s, Me-25), 0.96 (1H, s, Me-30), 0.91 (1H, s, Me-29), 0.81 (1H, s, Me-26), 0.72 (1H, s, Me-24); ^{13}C NMR data of the aglycon (CD_3OD, 600 MHz) δ 178.3 (C-28), 145.0 (C-13), 123.9 (C-12), 82.2 (C-3), 74.3 (C-16), 64.00 (C-23), 49.6 (C-17), 48.7 (C-9), 47.9 (C-5), 47.0 (C-19), 43.0 (C-4), 42.4 (C-14), 41.9 (C-18), 40.8 (C-8), 39.3 (C-1), 36.7 (C-10), 36.0 (C-15), 34.5 (C-22), 33.0 (C-29), 32.9 (C-7), 32.3 (C-21), 31.0 (C-20), 26.1 (C-2), 25.8 (C-27), 23.7 (C-11), 23.6 (C-30), 17.3 (C-26), 17.3 (C-6), 15.9 (C-25), 13.8 (C-24); ^{1}H and ^{13}C NMR of sugar moieties, see Tables 1 and 2; FABMS *m/z* 1543 $[M - H]^-$, 1397 $[(M - H) - 146]^-$, 1251 $[(M - H) - (146 + 146)]^-$, 1089 $[(M - H) - (146 + 146 + 162)]^-$, 781$[(M - H) - (162 + 162 + 146 + 146 + 146)]^-$, 619 $[(M - H) - (162 + 162 + 162 + 146 + 146 + 146)]^-$, 487 $[(M - H) - (162 + 162 + 162 + 146 + 146 + 146 + 132)]^-$.

Compound 7: identified as 3β-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]olean-12-ene-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, previously isolated from *Hedera colchica* by spectral data comparison.[6]

Compound 8: identified as hederagenin-3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, previously isolated from *Patrinia scabiosaefolia*, by spectral data comparison.[7]

Compound 2a: identified as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosil olean-12-en-28-oic acid, by spectral data comparison.[25]

Compound 3a: [3-*O*-β-D-quinovopyranosyl-(1→2)-α-L-arabinopyranosyl olean-12-en-28-oic acid]: obtained as a yellowish amorphous powder; $[\alpha]^{25}_D$ −12° (*c* 1, MeOH); for NMR data of aglycon moiety, see refs 7 and 8; ^{1}H NMR data (CD_3OD, 600 MHz) of sugar moiety δ 4.46 (1H, J = 7.5 Hz, H-1_{Ara}), 4.38 (1H, d, J = 7.5 Hz, H-1_{Qui}), 1.32 (3H, d, J = 6.5 Hz, H-6_{Qui}); ^{13}C NMR data (CD_3OD, 600 MHz) of sugar moiety δ 106.0 (C-1_{Qui}), 104.6 (C-1_{Ara}), 78.2 (C-3_{Qui}), 77.4 (C-5_{Qui}), 76.6 (C-2_{Ara}), 75.6 (C-5_{Qui}), 73.1 (C-3_{Ara}), 72.0 (C-4_{Ara}), 72.4 (C-4_{Qui}), 65.0 (C-5_{Ara}), 18. 0 (C-6_{Qui}).

Compound 4a [hederagenin-3-*O*-β-D-quinovopyranosyl-(1→2)-α-L-arabinopyranosyl: obtained as whitish amorphous powder]; $[\alpha]^{25}_D$ −7° (*c* 1, MeOH); for NMR data of aglycon moiety, see refs 19 and 20; ^{1}H NMR data (CD_3OD, 600 MHz) of sugar moiety δ 4.45 (1H, d, J = 7.5 Hz, H-1_{Ara}), 4.38 (1H, d, J = 7.5 Hz, H-1_{Qui}), 1.32 (3H, d, J = 6.5 Hz, H-6_{Qui}); ^{13}C NMR data (CD_3OD, 600 MHz) of sugar moiety δ 106.2 (C-1_{Qui}), 104.6 (C-1_{Ara}), 78.5 (C-3_{Qui}), 77.7 (C-5_{Qui}), 76.5 (C-2_{Ara}), 75.2 (C-5_{Qui}), 73.1 (C-3_{Ara}), 72.4 (C-4_{Ara}), 72.2 (C-4_{Qui}), 64.5 (C-5_{Ara}), 17.8 (C-6_{Qui}).

Compound 5a [3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl echinocistyc acid]: obtained as whitish amorphous powder; $[\alpha]^{25}_D$ +19° (*c* 1, MeOH); for NMR data of aglycon moiety, see ref 21; ^{1}H NMR data (CD_3OD, 600 MHz) of sugar moiety δ 4.45 (1H, d, J = 7.5 Hz, H-1_{Ara}), 5.18 (1H, d, J = 1.5 Hz, H-1_{Rha}), 4.52 (1H, d, J = 7.5 Hz, H-1_{Glc}), 1.28 (3H, d, J = 6.2 Hz, H-6_{Rha}); ^{13}C NMR data (CD_3OD, 600 MHz) of sugar moiety δ 105.3 (C-$1_{Glc\ I}$), 104.2 (C-1_{Ara}), 101.0 (C-$1_{Rha\ I}$), 80.2 (C-$3_{Rha\ I}$), 78.2 (C-$5_{Glc\ I}$), 77.9 (C-$3_{Glc\ I}$), 76.4 (C-2_{Ara}), 73.9 (C-$2_{Glc\ I}$), 73.3 (C-3_{Ara}), 72.4 (C-4_{Ara}), 71.9 (C-$4_{Rha\ I}$), 70.8 (C-$2_{Rha\ I}$), 70.4 (C-$4_{Glc\ I}$), 69.8 (C-$5_{Rha\ I}$), 64.2 (C-5_{Ara}), 61.8 (C-$6_{Glc\ I}$), 18. 0 (C-$6_{Rha\ I}$).

Compound 6a: identified as caulophyllogenin3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosidic, by spectral data comparison.[25]

Compound 7a: identified as 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid, by spectral comparison.[26]

Cells. J774 (murine monocyte/macrophage) cells were grown in adhesion on Petri dishes and maintained with Dulbecco's modified Eagle's medium (DMEM) at 37 °C in DMEM supplemented with 10% fetal calf serum (FCS), 25 mM HEPES, 2 mM glutamine, 100 u/mL penicillin, and 100 μg/mL streptomycin. WEHI-164 (murine fibrosarcoma) cells were maintained in adhesion on Petri dishes with DMEM supplemented with 10% heat-inactivated FCS, 25 mM HEPES, 100 u/mL penicillin, and 100 μg/mL streptomycin. HEK-293 (human epithelial kidney) cells were maintained and grown in adhesion on Petri dishes supplemented with DMEM, and 10% FCS, 25 mM HEPES, 100 u/mL penicillin, and 100 μg/mL streptomycin. All reagents for cell culture were from Hy-clone (Euroclone, Paignton Devon, U.K.); MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide] and 6-mercaptopurine (6-MP) were from Sigma Chemicals (Milan, Italy).

Antiproliferative Assays. J774, 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.[27,28] Briefly, 25 μL of MTT (5 mg/mL) were added, and the cells were incubated for an additional 3 h. Thereafter, cells were lysed and the dark blue crystals solubilized with 100 μL of a solution containing 50% (v:v) *N*,*N*-dimethylformamide, 20% (w:v) SDS with an adjusted pH of 4.5.[29] 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 was calculated as: % dead cells = 100 − (OD treated/OD control) × 100. Table 3 shows the results obtained expressed as an IC_{50} value (μM), the concentration that inhibited cell growth by 50% as compared to the control.

References and Notes

(1) Hu, M.; Ogawa, K.; Sashida, Y.; Xiao, P. *Phytochemistry* **1995**, *39*, 179−184.
(2) Quetin-Leclercq, J.; Elias, R.; Balansard, G.; Bassleer, R.; Angenot, L. *Planta Med.* **1992**, *58*, 279−281.
(3) Hegnauer, R. *Chemotaxonomie der Pflanzen*; Birkhäuser: Basel, 1964; Vol. 3, p 173.
(4) De Tommasi, N.; Pizza, C.; Bellino, A.; Venturella, P. *J. Nat. Prod.* **1997**, *60*, 663−668.
(5) De Tommasi, N.; Pizza, C.; Bellino, A.; Venturella, P. *J. Nat. Prod.* **1997**, *60*, 1070−1074.
(6) Hu, M.; Ogawa, K.; Sashida, Y.; Xiao, P. *Phytochemistry* **1995**, *39*, 179−184.
(7) Quetin-Leclercq, J.; Elias, R.; Balansard, G.; Bassleer, R.; Angenot, L. *Planta Med.* **1992**, *58*, 279−281.
(8) Jung, K. Y.; Do, J. C.; Son, K. H. *J. Nat. Prod.* **1993**, *56*, 1912−1916.
(9) Mahvidadze, V. D.; Dekanosidze, G. E.; Shashkov, A. S.; Kemertelidze, E. P. *Biorg. Khim.* **1993**, *19*, 1001−1007.
(10) Miyase, T.; Shiokawa, K.; Zhang, D. M.; Ueno, A. *Phytochemistry* **1996**, *41*, 1411−1418.
(11) Heinzmann, B. M.; Schenkel, E. P. *J. Nat. Prod.* **1995**, *58*, 1419−1422.
(12) Srivastava, S. K.; Jain, D. C. *Phytochemistry* **1989**, *28*, 644−647.
(13) Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 7198−7199.
(14) Homans, S. W. *Prog. Nucl. Magn. Reson. Spectrosc.* **1990**, *22*, 55−59.
(15) Bodenhausen, G.; Freeman, R.; Morris, G. A.; Neidermeyer, R.; Turner, J. *J. Magn. Reson.* **1977**, *25* , 559−564.
(16) Ishiu, H.; Kitagawa, I.; Matsushita, K.; Shirakawa, K.; Tori, K.; Tozyo, T.; Yoshikawa, M.; Yoshimura, Y. *Tetrahedron Lett.* **1981**, 1529−1535.

(17) De Tommasi, N.; Piacente, S.; De Simone, F.; Pizza, C. *J. Nat. Prod.* **1993**, *56*, 1669−1675.
(18) Piacente, S.; Pizza, C.; De Tommasi, N.; De Simone, F. *J. Nat. Prod.* **1995**, *58*, 512−519.
(19) Bodenhausen, G.; Ruben, D. J. *Chem. Phys. Lett.* **1980**, *69*, 185−186.
(20) Elgamal, M. H. A.; Soliman, H. S. M.; Karawya, M. S.; Mikhova, B.; Duddeck, H. *Phytochemistry* **1995**, *38*, 1481−1485.
(21) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565−569.
(22) Caliskan, O. A.; Anil, H.; Stephenson, G. R. *J. Nat. Prod.* **1994**, *57*, 1001−1003.
(23) Tschesche, R.; Rehkamper, H.; Wulff, G. *Liebigs Ann. Chem.* **1969**, *726*, 125−129.
(24) Nagao, T.; Tanaka, R.; Iwase, Y.; Okabe, H. *Chem. Pharm. Bull* **1993**, *41*, 659−665.
(25) Asada, Y.; Ikeno, M.; Furuya, T. *Phytochemistry* **1994**, *93*, 93−141.
(26) Geen, H.; Freeman, R. *J. Magn. Reson.* **1991**, *67*, 565−569.
(27) Setsuo, S.; Shgeya, S.; Norihiro, T.; Yoichi, N.; Keishi, N.; Madoka, I.; Isao, I. *Chem. Pharm. Bull.* **1990**, *38*, 411−414.
(28) Kinjo, J.; Uemura, H.; Nakamura, M.; Nohara, T. *Chem. Pharm. Bull.* **1994**, *42*, 1339−1341.
(29) Mosman, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(30) Green, L. M.; Reade, J. L.; Ware, C. F. *J. Immunol. Methods* **1984**, *70*, 257−268.
(31) Opipari, A. W. J.; Hu, H. M.; Kabkowitz R.; Dixit, Y. M. *J. Biol. Chem.* **1992**, *267*, 12424−12427.

NP990231N