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Porrigenins A and B, Novel Cytotoxic and Antiproliferative Sapogenins Isolated from *Allium porrum*[1]

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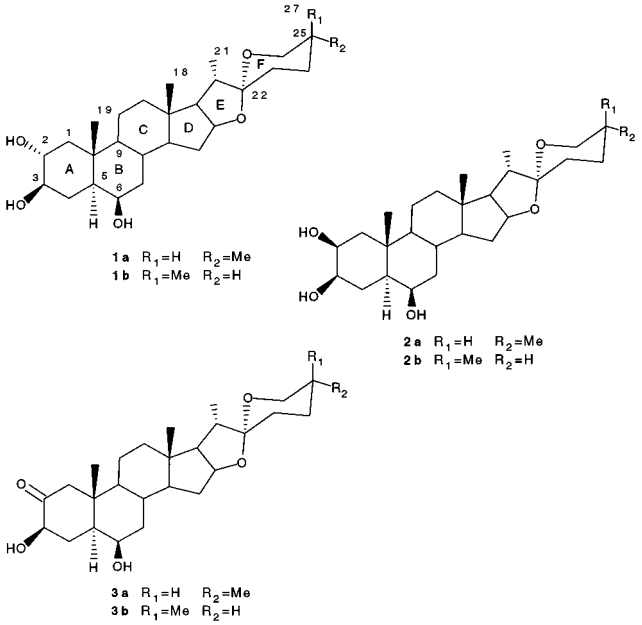
Four new sapogenins, porrigenins A (**2a**) and B (**3a**), identified as (25*R*)-5α-spirostan-2*β*,3*β*,6*β*-triol and (25*R*)-2-oxo-5α-spirostan-3*β*,6*β*-diol, respectively, and neoporrigenins A (**2b**) and B (**3b**) were also isolated from *Allium porrum*. In addition, the known agigenin (**1a**) and its 25*S* epimer, neoagigenin (**1b**), were also identified. Their structure elucidation was provided by comprehensive spectroscopic analyses. Compounds **1a**, **2a**, and **3a** exhibited cytotoxicity and high antiproliferative acivity on four different tumor cell lines in vitro.

As part of our efforts to isolate novel bioactive agents from *Allium* species,[2,3] we have investigated *Allium porrum* L. (Liliaceae), a bulbous perennial plant commonly called leek and widely cultivated as a vegetable throughout the world.[4,5] Fresh juice of the plant is claimed to be a bactericide. Diuretic, hypotensive, and digestive properties are also attributed to this plant.[6] Examinations of the $CHCl_3$−MeOH (9:1) extracts of the bulbs revealed the presence of two novel cytotoxic and antiproliferative sapogenins, porrigenins A (**2a**) and B (**3a**). Small quantities of their 25*S* isomers, the new neoporrigenin A (**2b**) and neoporrigenin B (**3b**), have also been isolated. Compounds **3a** and **3b** possess a novel ketone function at C-2. Agigenin (**1a**) and neoagigenin (**1b**),[7] have also been found in the extract. In this paper, we report the structural elucidation and biological activities of the isolated compounds.

Results and Discussion

Samples of collected *Allium porrum* were briefly air dried and exhaustively extracted with *n*-hexane, $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH. The $CHCl_3$−MeOH (9:1) extracts were separated by sequential chromatography to afford the new porrigenin A (**2a**), neoporrigenin A (**2b**), porrigenin B (**3a**), and neoporrigenin B (**3b**), in addition to the known agigenin (**1a**) and neoagigenin (**1b**).

Compound **1a** was identified as agigenin, a sapogenin previously isolated from *Allium giganteum* L. (Liliaceae) by Kel'ginbaev *et al.*[7] Because the spectroscopic characterization of **1a** was incomplete in the literature, its identification was achieved from a detailed NMR analysis, including the use of 2D NMR COSY, HOHAHA, and HMQC, which allowed the assignment of all the ^{1}H resonances, reported in Table 1. Neoagigenin (**1b**),[7] the 25*S* epimer of **1a** was identified by analysis of HREIMS, ^{1}H-NMR and ^{13}C-NMR data.



Porrigenin A (**2a**), isolated as a colorless powder, showed in the HREIMS a molecular ion peak at *m/z* 448.3181 in accordance with the empirical formula $C_{27}H_{44}O_5$, also deduced on the basis of ^{13}C-NMR data. A comparison of HREIMS, ^{1}H-NMR and ^{13}C-NMR data of porrigenin A (Tables 1 and 2) with those of agigenin suggested an isomeric relationship between them. In particular, the ^{1}H and ^{13}C chemical shifts of the two compounds were almost identical for the C−F rings' atoms. Analysis of the COSY, HOHAHA, and HMQC spectra of **2a** immediately allowed definition of chemical shifts, multiplicities, and coupling constants for the C−F rings (Table 1 and 2).

The structure of the remaining part of the molecule was then derived by extensive analysis of 2D NMR. A cross peak in the 2D HMBC spectrum between the singlet at δ 1.24, assigned to H_3-19, and the methylene carbon signal at δ 46.1 allowed us to ascribe this resonance to C-1. HMQC correlations observed between this carbon signal and proton resonances at δ 1.15 and δ 1.95 identified H_2-1 signals, which in a COSY spectrum was correlated to the H-2 signal (δ 3.97). The

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Table 1. ^{1}H-NMR Data (CD_3OD) for Compounds **1a**−**3a**

proton	δ_H (mult, *J* [Hz]) **1a**	**2a**	**3a**
1α	0.95[a]	1.15[a]	2.19 (bd, 12.6)
β	1.89[a]	1.95[a]	2.31 (d,12.6)
2	3.54 (ddd, 4.7, 8.8, 11.6)	3.97 (td, 2.5, 2.5, 4.2)	
3	3.37[a]	3.61 (td, 4.2, 4.2, 11.3)	4.27 (dd, 7.7, 11.6)
4α	1.65[a]	1.38[a]	2.06[a]
β	1.83[a]	2.13 (q, 11.3, 11.3, 11.3)	2.06[a]
5	1.25 (td, 2.8, 2.8, 12.0)	1.19[a]	1.83 (td, 2.8, 2.8, 5.3)
6	3.82 (td, 2.5, 2.5, 5.3)	3.82 (td, 3.2, 3.2, 4.5)	3.95 (td, 2.8, 2.8, 5.3)
7α	1.24[a]	1.21[a]	1.29[a]
β	1.85 (td, 1.4, 1.4, 11.6)	1.85 (td, 3.2, 3.2, 13.7)	1.92 (td, 3.5, 3.5, 11.9)
8	1.98[a]	2.00[a]	1.94[a]
9	0.81 (dt, 4.2,11.9,11.9)	0.71 (dt, 4.6,12.3,12.3)	1.07[a]
11α	1.61[a]	1.59[a]	1.47[a]
β	1.47[a]	1.45[a]	1.47[a]
12α	1.21[a]	1.17[a]	1.20[a]
β	1.78[a]	1.77[a]	1.79[a]
14	1.21[a]	1.19[a]	1.23[a]
15α	2.03[a]	2.02[a]	2.05[a]
β	1.36[a]	1.34[a]	1.34[a]
16	4.42 (td, 6.3, 6.3, 7.8)	4.41 (td, 6.3, 6.3, 7.8)	4.44 (td, 6.3, 6.3, 7.8)
17	1.78[a]	1.77[a]	1.79[a]
18	0.86 (s)	0.85 (s)	0.86 (s)
19	1.10 (s)	1.24 (s)	1.10 (s)
20	1.94[a]	1.95[a]	1.94[a]
21	0.99 (d, 7.0)	0.98 (d, 7.0)	0.99 (d, 7.0)
23α	1.59[a]	1.60[a]	1.59[a]
β	1.74 (dt, 4.6,13.3,13.3)	1.74 (dt, 4.5,13.2,13.2)	1.74 (dt, 4.6,13.3,13.3)
24α	1.46[a]	1.42[a]	1.45[a]
β	1.67[a]	1.66[a]	1.66[a]
25	1.62[a]	1.62[a]	1.63[a]
26α	3.33	3.31	3.33
β	3.48 (ddd, 1.8, 4.2, 10.9)[a]	3.47 (ddd, 1.8, 4.2, 10.6)	3.48 (ddd, 1.8, 4.2, 10.5)
27	0.82 (d, 6.3)	0.82 (d, 6.3)	0.83 (d, 6.3)

[a] Submerged by other signals.

Table 2. ^{13}C-NMR Data (CD_3OD) of **2a** and **3a** in Comparison with That of Agigenin (**1a**)[7]

carbon	δ_C (mult) **1a**	**2a**	**3a**
1	48.1 (CH_2)	46.1 (CH_2)	53.9 (CH_2)
2	73.4 (CH)	71.2 (CH)	212.5 (C)
3	77.4 (CH)	74.1 (CH)	76.5 (CH)
4	34.5 (CH_2)	31.0 (CH_2)	37.3 (CH_2)
5	48.8 (CH)	49.8 (CH)	47.8 (CH)
6	71.6 (CH)	72.5 (CH)	70.8 (CH)
7	40.8 (CH_2)	40.7 (CH_2)	40.8 (CH_2)
8	30.7 (CH)	30.8 (CH)	30.7 (CH)
9	55.6 (CH)	56.5 (CH)	54.8 (CH)
10	38.2 (C)	36.8 (C)	42.3 (C)
11	22.1 (CH_2)	22.0 (CH_2)	22.1 (CH_2)
12	41.1 (CH_2)	41.1 (CH_2)	40.7 (CH_2)
13	41.8 (C)	41.8 (C)	41.7 (C)
14	57.2 (CH)	57.2 (CH)	57.0 (CH)
15	32.7 (CH_2)	32.7 (CH_2)	32.4 (CH_2)
16	82.2 (CH)	82.3 (CH)	82.1 (CH)
17	63.9 (CH)	63.8 (CH)	63.8 (CH)
18	17.0 (CH_3)	16.9 (CH_3)	16.9 (CH_3)
19	17.3 (CH_3)	18.6 (CH_3)	16.3 (CH_3)
20	42.9 (CH)	43.0 (CH)	42.9 (CH)
21	14.9 (CH_3)	14.8 (CH_3)	14.8 (CH_3)
22	110.5 (C)	110.5 (C)	110.5 (C)
23	32.4 (CH_2)	32.3 (CH_2)	32.7 (CH_2)
24	29.9 (CH_2)	29.9 (CH_2)	29.9 (CH_2)
25	31.4 (CH)	31.4 (CH)	31.4 (CH)
26	67.9 (CH_2)	67.9 (CH_2)	67.9 (CH_2)
27	17.5 (CH_3)	17.5 (CH_3)	17.5 (CH_3)

chemical shift of the last resonance (δ 3.97, ddd, J = 2.5, 2.5, 4.2 Hz) was in agreement with a hydroxymethine nature for C-2. Further analysis of the COSY spectrum allowed determination of all proton resonances of A and B rings (Table 1). The midfield chemical shifts of H-3 (δ 3.61, ddd, J = 4.2, 4.2, 11.3 Hz) and of H-6 (δ 3.82, ddd, J = 3.2, 3.2, 4.5 Hz) located two additional hydroxyl groups at positions 3 and 6. Moreover, the H-2 and H-6 signals' multiplicities, all characterized by small coupling constants (J = 2.5−4.5), defined the equatorial orientation of both protons. Conversely, the J values observed for H-3 signal (J_{2-3} = 4.2, $J_{3-4\beta}$ = 11.3, $J_{3-4\alpha}$ = 4.2 Hz) established its axial orientation and confirmed the equatorial nature of H-2. In addition, H_β-4 signal (δ 2.13) possessed three large couplings (J = 11.3 Hz, each), thus defining the axial orientation of H-5. An intense NOE effect observed between H-5 (δ 1.19, overlapped) and H-9 (δ 0.71, ddd, J = 4.6, 12.3, 12.3 Hz) indicated the α-orientation of the former and a *trans* junction between the A and B rings. The *β*-configuration of the hydroxyl at C-2 was further supported by the chemical shift of H_β-4 (2.13) and H_3-19 (1.24), which are shifted downfield when compared to the corresponding protons in **1a** (δ 1.83 and 1.10, respectively). Their deshielded resonances could be ascribed to the influence of the 1,3-diaxial interaction with the hydroxyl group at C-2. Finally, the observed ^{13}C chemical shift values of C-2 (δ 71.2) and C-3 (δ 74.1) fully agreed with the calculated values, according to the additional rules on model compounds.[8]

Neoporrigenin A (**2b**), isolated as a colorless powder, displayed in the HREIMS a molecular ion peak at *m/z* 448.3185 in accordance with the empirical formula $C_{27}H_{44}O_5$. Comparison of the ^{1}H- and ^{13}C-NMR resonances of **2b** (Experimental Section) with those of **2a** (Tables 1 and 2) suggested an isomeric relationship between them, and that the most significant differences were found in the F ring atoms. Comparison of the ^{13}C

chemical shifts of neoporrigenin A (**2b**) with porrigenin A (**2a**) showed upfield shifts of all the F ring carbon atoms in **2b** [$\delta_{2a} - \delta_{2b} = 5.4$ (C-23), 3.1 (C-24), 0.2 (C-25), 1.8 (C-26), 1.1 (C-27)], characteristic of an inversion at the chiral center C-25.[9] In particular, the C-23 resonance exhibited a dramatic shielding of 5.4 ppm (δ 26.9 in **2b** vs. δ 32.3 in **2a**) due to the existence of γ-gauche interactions. Thus, the new neoporrigenin A (**2b**) was identified as the 25*S* epimer of porrigenin A (**2a**).

Porrigenin B (**3a**), isolated as a colorless powder, showed in the HREIMS a molecular ion peak at *m/z* 446.3018 in accordance with the empirical formula $C_{27}H_{42}O_5$. The ^{13}C-NMR and DEPT ^{13}C-NMR spectra confirmed the presence of 27 carbon atoms (Table 2), including one unsaturated functionality (a carbonyl at δ 212.5), thus indicating the hexacyclic nature of the molecule, on the basis of the formal unsaturations implied by the molecular formula.

The most striking feature of the proton NMR spectrum of **3a** (Table 1) was the presence of four methyl signals [(two singlets at δ 0.86 and δ 1.10, and two doublets at δ 0.83 ($J = 6.3$ Hz) and δ 0.99 ($J = 7.0$ Hz)], three methine signals (δ 3.95, 4.27, 4.44) and one methylene (signals at δ 3.33 and 3.48) all in the region characteristic of protons on sp^3 carbons linked to heteroatoms. These data together with the presence in the ^{13}C-NMR spectrum of a ketal carbon (singlet at δ 110.5 ppm) suggested a hydroxylated spirostane skeleton for porrigenin B (**3a**). A comparative analysis of ^{1}H- and ^{13}C-NMR spectra of **3a** with those of **1a** and **2a** suggested a similar structure for the C−F rings and indicated the presence of a carbonyl function in **3a** instead of a hydroxyl group in **1a** and **2a**. Further analysis of ^{1}H- and ^{13}C-NMR data evidenced the presence of an isolated methylene group [^{1}H NMR, δ 2.31 (1H, d, $J = 12.6$ Hz, H$_\beta$-1) and δ 2.19 (1H, br d, $J = 12.6$ Hz, H$_\alpha$-1); ^{13}C-NMR, δ 53.9]. A proton signal at δ 2.19 (H$_\alpha$−1) exhibited in the ^{1}H−^{1}H COSY a four-bond coupling with H$_3$-19 (br s, δ 1.10), thus suggesting its linkage at C-1. The downfield chemical shift of the C-1 resonance in the ^{13}C-NMR spectrum of **3a** (δ 53.9), assigned by 2D HMQC experiments, when compared to the relevant carbon atom in **1a** and **2a** (δ 48.1 and 46.1, respectively), as well as the splitting pattern of the H$_2$-1 proton signals (isolated AB system), was in agreement with the carbonyl function located on the adjacent C-2. The observed long-range correlation between H$_2$-1 (δ 2.19 and 2,31) and C-2 (δ 212.5) in the 2D HMBC experiments confirmed this structural feature. Additional cross peaks in the same spectrum correlated C-1 (δ 53.9) and C-2 (δ 212.5) with a proton signal at δ 4.27 (dd, $J = 7.7$, 11.6 Hz), which was consequently identified as H-3.

Using as a starting point the signal at δ 4.27 (H-3), analysis of ^{1}H−^{1}H COSY and HOHAHA allowed us to complete the assignment of all proton resonances of the A and B rings. In particular, the signal at δ 3.95 (td, $J = 2.8$, 2.8, 5.3 Hz) was attributed to H-6, thus determining the position of the remaining hydroxyl group, while its β orientation was deduced by the small coupling constants of H-6. Intense NOE contacts in the ROESY spectrum of H$_\alpha$-1(δ 2.19)/H-3 (δ 4.27), H$_\alpha$-1(δ 2.19)/H-5 (δ 1.83), and H-9 (δ 1.07)/H-5 (δ 1.83) established the β-orientation of 3-OH and the α-orientation of H-5, defining the *trans* junction of the A and B rings.

Table 3. *In Vitro* Cytotoxicity (24 h) and Antiproliferative (48 and 72 h) Activity of Compounds **1a**, **2a**, **3a**, and 6-MP on IGR-1, J774, WEHI 164 and P-388 cell lines[a]

cell line	h	**1a**	**2a**	**3a**	6-MP
IGR-1	24	n.d.	942.0 ± 63.0	823.0 ± 41.0	51.7 ± 21.0
	48	627.0 ± 42.0	471.0 ± 27.0	330.0 ± 12.0	12.3 ± 1.1
	72	264.0 ± 50.0	184.0 ± 50.0	45.0 ± 13.0	1.1 ± 0.3
J-774	24	896.0 ± 5.7	754.0 ± 63.0	552.0 ± 33.0	61.0 ± 3.5
	48	655.0 ± 81.0	515.0 ± 35.0	111.0 ± 27.0	3.6 ±1.2
	72	321.0 ± 25.0	270.0 ± 41.0	51.0 ± 21.0	0.5 ± 0.1
WEHI 164	24	836.0 ± 4.1	n.d.	655.0 ± 11.0	83.0 ± 5.0
	48	548.0 ± 62.0	277.0 ± 23.0	344.0 ± 22.0	42.0 ± 7.0
	72	210.0 ± 4.1	110.0 ± 37.0	92.0 ± 19.0	2.7 ± 0.3
P-388	24	609.0 ± 41.0	617.0 ± 31.0	837.0 ± 44.0	37.0 ± 4.0
	48	471.0 ± 14.0	270.0 ± 16.0	185.0 ± 17.0	2.1 ± 0.5
	72	354.0 ± 18.0	115.0 ± 9.0	74.0 ± 22.0	0.3 ± 0.08

[a] The results (IC_{50} in μg/mL) are expressed as mean ±SEM of three separate experiments in triplicate. IC_{50} is a concentration that reduces by 50% (after 24, 48, and 72 h of incubation) cell growth as compared to control cultures.

Neoporrigenin B (**3b**), isolated as a colorless powder, gave in the HREIMS a molecular ion peak at *m/z* 446.3015 in accordance with the empirical formula $C_{27}H_{42}O_5$. ^{1}H- and ^{13}C-NMR data of **3b** (Experimental Section) were almost identical to those of **3a** (Tables 1 and 2), apart from the F ring atoms. The foregoing observation suggested an isomeric relationship between **3b** and **3a**. Comparison of the ^{13}C chemical shifts of neoporrigenin B (**3b**) with porrigenin B (**3a**) showed upfield shifts of all the F ring carbon atoms in **3b** [$\delta_{3a} - \delta_{3b} = 5.5$ (C-23), 3.1 (C-24), 0.2 (C-25), 1.8 (C-26), 1.1 (C-27)], characteristic of an inversion at the chiral center C-25.[9] In particular, the C-23 resonance exhibited a shielding of 5.5 ppm (δ 27.2 in **3b** vs. δ 32.7 in **3a**) ascribed to γ-gauche interactions. Thus, the new neoporrigenin B (**2b**) was identified as the 25*S* epimer of porrigenin B (**2a**).

Table 3 summarizes the data about the growth-inhibitory activity (IC_{50}) of compounds **1a**, **2a**, and **3a** on four different cell lines in vitro. Cytotoxicity and antiproliferative activity were evaluated by the inhibition of cell growth at different times. All the tested compounds exhibited higher antiproliferative than cytotoxic activity. In addition, porrigenin B (**3a**) showed higher antiproliferative activity than porrigenin A (**2a**) and agigenin (**1a**) on all cell lines studied, as shown in Table 3.

Experimental Section

General Experimental Procedures. HREIMS were obtained by electron impact at 55 eV on a VG Prospec Fisons mass spectrometer. Optical rotations were determined on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and 10-cm microcell. ^{1}H- and ^{13}C-NMR spectra were recorded at 500.13 and 125.795 MHz, respectively, on a Bruker AMX-500 spectrometer in CD_3OD solution. Proton chemical shifts were referenced to the residual solvent signal (δ 3.34). ^{13}C-NMR spectra were referenced to the center peak of the quintet at 49.0 ppm. The ^{13}C resonances' multiplicities were determined by DEPT experiments. ^{1}H connectivities were determined by using COSY and HOHAHA experiments; the 2D HOHAHA experiments[10] were performed in the phase-sensitive mode (TPPI) using the MLEV-17 (mixing time 125 ms)

sequence for mixing. $^{1}H-^{13}C$ connectivities were determined with 2D HMQC experiments,[11] with a BIRD pulse 0.5 s before each scan to suppress the signal from protons not directly bonded to ^{13}C. The interpulse delays were adjusted for an average $^{1}J_{CH}$ of 135 Hz. Two- and three-bond heteronuclear $^{1}H-^{13}C$ connectivities were determined with 2D HMBC experiments,[12] optimized for $^{2-3}J_{CH}$ of 8 Hz. NOE measurements were performed by 2D ROESY experiments. Medium-pressure liquid chromatography (MPLC) was performed on a Buchi 861 apparatus using a SiO_2 (230−400 mesh) and RP-8 columns. HPLC in isocratic mode was performed on a Varian apparatus equipped with an RI-3 refractive index detector. Hibar LiChrospher RP-18 columns and Hibar LiChrospher SiO_2 columns were used.

Extraction and Isolation. Samples of *Allium porrum* L. were collected in May 1995, near Salerno (Campania, Italy). A reference specimen has been deposited at the Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II, Italy. The plants were air dried immediately after collection (820 g, dry) and extracted at room temperature with the following solvents: *n*-hexane, $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH. The $CHCl_3$−MeOH (9:1) extract (81g) was concentrated *in vacuo* to afford 8.54 g of a crude organic extract that was chromatographed by MPLC on a RP-8 column using a gradient solvent system from H_2O to MeOH. The fraction eluted with MeOH−H_2O 8:2 was rechromatographed by MPLC on a SiO_2 column using sequential mixtures of increasing polarity from $CHCl_3$ 100% to $CHCl_3$−MeOH 8:2. Fractions of 30 mL were collected and analyzed by TLC on SiO_2 with $CHCl_3$−MeOH 9:1. Fractions eluted with $CHCl_3$−MeOH 95:5 (65 mg), were purified by HPLC on a Hibar LiChrospher Si 60 column with mobile phase $CHCl_3$−MeOH 9:1, to give a mixture of **1a** and **1b** (54 mg) and a mixture of **2a** and **2b** (18 mg). Fractions eluted with $CHCl_3$−MeOH 98:2 (53 mg) were rechromatographed by HPLC on a Hibar LiChrospher RP-18 column with a mobile phase MeOH−H_2O 8:2 to give a mixture of **3a** and **3b** (13 mg). Mixture of **1a,b** subjected to HPLC (eluent: *n*-hexane−EtOAc 1:9) afforded compounds **1a** (45 mg) and **1b** (2.5 mg). Mixture of **2a,b**, subjected to the same treatment, gave compounds **2a** (16 mg) and **2b** (0.8 mg). Finally, mixture of **3a,b**, purified on HPLC (eluent: *n*-hexane−EtOAc, 2:8), afforded compounds **3a** (12 mg) and **3b** (0.6 mg).

Porrigenin A (2a): $[\alpha]^{25}_{D}$ −20° (c 0.03, $CHCl_3$); ^{1}H and ^{13}C-NMR spectra see Table 1 and 2; HREIMS (70 eV) obsd *m/z* 448.3181, $C_{27}H_{44}O_5$, calcd *m/z* 448.3177.

Neoporrigenin A (2b): $[\alpha]^{25}_{D}$ −37° (*c* 0.003, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 3.97 (1H, td, J = 2.5, 2.5, 4.2 Hz, H-2), 3.61 (1H, td, J = 4.2, 4.2, 11.3 Hz, H-3), 3.82 (1H, td, J = 3.2, 3.2, 4.5 Hz, H-6), 4.41 (1H, td, J = 7.8, 6.3, 6.3 Hz, H-16), 0.85 (3H, s, H_3-18), 1.24 (3H, s, H_3-19), 1.12 (3H, d, J = 7.0 Hz, H_3-21), 3.30 (1H, overlapped, H-26a), 3.95 (1H, dd, J = 11.3, 3.0 Hz, H-26b), 1.02 (3H, d, J = 7.0 Hz, H_3-27); ^{13}C-NMR (CD_3OD) δ 46.1 (t, C-1), 71.2 (d, C-2), 74.1 (d, C-3), 31.0 (t, C-4), 49.8 (d, C-5), 72.5 (d, C-6), 40.7 (t, C-7), 30.8 (d, C-8), 56.5 (d, C-9), 36.8 (s, C-10), 22.0 (t, C-11), 41.1 (t, C-12), 41.8 (s, C-13), 57.2 (d, C-14), 32.7 (t, C-15), 82.4 (d, C-16), 63.7 (d, C-17), 16.9 (q, C-18), 18.6 (q, C-19), 43.5 (d, C-20), 14.4 (q, C-21), 111.0 (s, C-22), 26.9 (t, C-23), 26.8 (t, C-24), 31.2 (d, C-25), 66.1 (t, C-26), 16.4 (q, C-27); HREIMS (70 eV) obsd *m/z* 448.3185, $C_{27}H_{44}O_5$, calcd *m/z* 448.3177.

Porrigenin B (3a): $[\alpha]^{25}_{D}$ −28° (*c* 0.03, $CHCl_3$−CH_3-OH); ^{1}H and ^{13}C-NMR spectra see Tables 1 and 2; HREIMS (70 eV) obsd *m/z* 446.3018, $C_{27}H_{42}O_5$ calcd *m/z* 446.3023.

Neoporrigenin B (3b): $[\alpha]^{25}_{D}$ −42° (*c* 0.003, $CHCl_3$−CH_3OH); ^{1}H NMR (CD_3OD) δ 4.27 (1H, dd, J = 7.7, 11.6 Hz, H-3), 3.95 (1H, td, J = 2.8, 2.8, 5.3 Hz, H-6), 4.44 (1H, td, J = 7.8, 6.3. 6.3 Hz, H-16), 0.86 (3H, s, H_3-18), 1.10 (3H, s, H_3-19), 1.12 (3H, d, J = 7.0 Hz, H_3-21), 3.30 (1H, overlapped, H-26a), 3.97 (1H, dd, J = 11.5, 3.0 Hz, H-26b), 1.02 (3H, d, J = 7.0 Hz, H_3-27); ^{13}C-NMR (CD_3-OD) δ 53.9 (t, C-1), 212.5 (s, C-2), 76.5 (d, C-3), 37.3 (t, C-4), 47.8 (d, C-5), 70.8 (d, C-6), 40.8 (t, C-7), 30.7 (d, C-8), 54.8 (d, C-9), 42.3 (s, C-10), 22.1 (t, C-11), 40.7 (t, C-12), 41.7 (s, C-13), 57.0 (d, C-14), 32.4 (t, C-15), 82.2 (d, C-16), 63.6 (d, C-17), 16.9 (q, C-18), 16.3 (q, C-19), 43.4 (d, C-20), 14.3 (q, C-21), 111.0 (s, C-22), 27.2 (t, C-23), 26.8 (t, C-24), 31.2 (d, C-25), 66.1 (t, C-26), 16.4 (q, C-27); HREIMS (70 eV) obsd *m/z* 446.3015, $C_{27}H_{42}O_5$, calcd *m/z* 446.3023.

Cells. WEHI 164 cells (murine fibrosarcoma cell line) were maintained in adhesion on Petri dishes with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 25 mM HEPES, penicillin (100 U/mL), and streptomycin (100 μg/mL). J-774 cells (murine monocyte/macrophage cell line) were grown in suspension culture, in Techne stirrer bottles, spun at 25 rpm and incubated at 37 °C in DMEM supplemented with 10% FBS, 25 mM HEPES, glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 μg/m). IGR-1 cells (human melanoma cell line) were grown in adhesion on Petri dishes with Minimum Essential Medium Eagle (MEM) supplemented with 10% FBS, 25 mM HEPES, penicillin (100 U/mL), and streptomycin (100 μg/mL). P-388 cells (murine leukemia cell line) was grown in adhesion on Petri dishes with L-15 (Leibovitz) medium supplemented with 10% FBS, 25 mM HEPES, penicillin (100 U/mL), and streptomycin (100 μg/mL). All reagents for cell culture were from Cellbio; MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2*H*-tetrazolium bromide] and 6-mercaptopurine from Sigma.

Antiproliferation and cytotoxicity assay. WEHI 164, J-774, IGR-1, P-388 (1×10^4 cells) were plated on 96-well microtiter plates and allowed to adhere at 37 °C in 5% CO_2/95% air for 2 h. Thereafter, the medium was replaced with 50 μL of fresh medium, and a 75 μL aliquot of 1.2 v/v serial dilution of each test compound **1a**, **2a**, **3a** was added and then the cells incubated for 24 h (cytotoxicity assay) and 48 and 72 h (proliferation assay). In some experiments 6-mercaptopurine (6-MP) was added. The cells viability was assessed through an MTT conversion assay.[13,14] Briefly, 25 μL of MTT (5 mg/mL) was added, and the cells were incubated for additional 3 h. Following this time, the 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.[15] 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 compounds **1a**, **2a**, **3a,** and 6-MP was calculated as: % dead cells = 100 −

(OD treated/OD control) × 100. Table 3 shows the results expressed as IC_{50} (the concentration that inhibited the cell growth by 50% as compared to the control cultures).

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References and Notes

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