Cytotoxic and Anti-oxidant Activities of Lanostane-Type Triterpenes Isolated from *Poria cocos*

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A new lanostane-type triterpene, 29-hydroxypolyporenic acid C (8), was isolated from the dried sclerotia of *Poria cocos* together with eight other known compounds pachymic acid (1), dehydropachymic acid (2), 3-acetyloxy-16α-hydroxytrametenolic acid (3), polyporenic acid C (4), 3-*epi*-dehydropachymic acid (5), 3-*epi*-dehydrotumulosic acid (6), tumulosic acid (7), and dehydrotumulosic acid (9). The compounds were identified by spectral analysis and comparison with spectroscopic data reported in the literatures. Although none of the nine (1 to 9) compounds showed promising antioxidant activity, 1 through 6 and 8 showed good cytotoxicity against human lung cancer cell line A549 and human prostate cancer cell line DU145. Interestingly, all these compounds exhibited better cytotoxicity towards A549 than DU145 cells.

Key words *Poria cocos*; lanostane; cytotoxicity; antioxidant; phytochemistry

Poria cocos (*P. cocos*), which is also known as Fu Ling, is often used in Chinese traditional medicine for its diuretic and sedative effects.[1] Compounds with anti-cancer properties have been isolated from both the aqueous and alcoholic extracts of *P. cocos* in various studies.[2—7] Polysaccharides from aqueous extracts have also been shown to exhibit various anti-tumor and cytotoxic effects against certain cancer cell lines.[8—10] Likewise, lanostane-type triterpenes from alcoholic extracts were shown to possess certain anti-cancer properties, including inhibition of DNA polymerases,[4,11] inhibition of tumor cell growth induced by TPA (12-*O*-tetradecanoylphorbol-13-acetate),[2,12,13] and inhibition of DNA topoisomerase II.[4] Cytotoxicity of lanostane-type triterpenes against certain human cancer cell lines have also been reported.[2,3,14] Recently, we have shown that pachymic acid (**1**), a lanostane-type triterpene found in *P. cocos* alcoholic extract, is effective in preventing the growth of both androgen-responsive and -insensitive prostate cancer cells by inducing apoptosis in a dose- and time-dependent fashion.[5] This finding, together with the observation that various lanostane-type triterpenes have been isolated from this plant, have motivated us to conduct further research on *P. cocos* to identify other pachymic acid-like compounds with equal or greater cytotoxicity. We report here the isolation and characterization of one novel and eight known lanostane-type triterpenes from the sclerotia of *P. cocos*, and the cytotoxic and antioxidant activity of these triterpenes.

Results and Discussion

To isolate compounds from *P. cocos*, dry sclerotia of *P. cocos* were crushed and extracted using 95% ethanol at 60 °C under reflux. Subsequently, the alcoholic extracts were dried and fractionated using flash column chromatography. In all, four fractions, PC A—D, were obtained and evaluated for their cytotoxic activity against DU145 and A549 cells. Of these fractions, PCB gave the best response, which, at 37 μg/ml, caused cell viability of DU145 and A549 to decrease to 67.7% and 44%, respectively, after 72 h treatment (data not shown to conserve space). This was followed by PCC, which had slightly lower cytotoxicity against DU145 growth (84.7% *vs.* 67.7% growth inhibition at 37 μg/ml over a 72 h treatment period) relative to PCB but similar inhibiting activity as PCB on A549 cells (data not shown). PCB and PCC were then subjected to repeated column chromatography to isolate pure compounds. In all, nine lanostane-type triterpenes, including pachymic acid (**1**),[15] dehydropachymic acid (**2**),[15] 3-acetyloxy-16α-hydroxytrametenolic acid (**3**),[16] polyporenic acid C (**4**),[4] 3-*epi*-dehydropachymic acid (**5**),[16] 3-*epi*-dehydrotumulosic acid (**6**),[3,17] tumulosic acid (**7**), 29-hydroxypolyporenic acid C (**8**), and dehydrotumulosic acid (**9**)[17] were isolated and identified. Identification of known compounds was conducted by comparison of their physical and spectroscopic data (^{1}H-, ^{13}C-NMR and MS) with the corresponding compounds reported in the literatures. The chemical structures of compounds **1**—**9** are shown in Fig. 1.

Compound **8**, which exhibited a positive Libermann–Burchard reaction, was obtained as white amorphous powder with $[\alpha]_D^{20}$ +27 (c=0.0762, MeOH). Its molecular formula was deduced as $C_{31}H_{46}O_5$ on the basis of its HR-ESI-MS ([M−H$^-$], m/z 497.3265) in tandem with the ^{1}H- and ^{13}C-

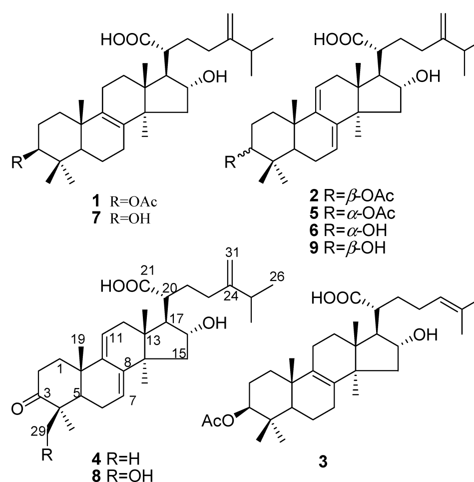

Fig. 1. Chemical Structure of Compounds **1**—**9**

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NMR spectra. IR spectrum of compound **8** reveals the presence of a carboxyl group with characteristic absorption at 1710 cm^{-1}. The ^{1}H-NMR spectrum of **8** indicated the presence of six methyl protons (δ_H 0.99, 1.01, 1.07, 1.07, 1.14, 1.42), an exomethylene group at δ 4.86, 4.99 (each 1H, br s), which were characteristic of 24-methylenelanostane, and one oxymethine proton at δ 4.53 (t, J=6.5 Hz). The UV spectrum showed an absorption maxima at 236, 244 and 252 nm, suggesting the presence of a $\Delta^{7,9,11)}$ conjugated diene system.[18] This was fully supported by ^{13}C-NMR data of **8** (Table 1), which showed the signals of C-7 (δ 120.9), C-8 (δ 142.8) and C-9 (δ 144.4), C-11 (δ 118.0) revealing the presence[18] of Δ.[7,9,11] In addition, there was one carboxyl resonance at δ 178.6 (C-21), one ketone carbon at δ 215.5 (C-3), and an oxymethine resonance at δ 75.6 (C-16) in the ^{13}C-NMR of **8**. On the basis of comparison of the NMR spectral data with those of polyporenic acid C (**4**),[10] **8** could be assigned as a close derivative of polyporenic acid C. A significant difference in the ^{1}H-NMR spectrum showed that H-29 appeared at δ 3.59, 4.17 (each 1H, d, J=10.5 Hz), rather than at δ 1.14, which correlated with a hydroxymethyl carbon at δ 66.9 in the HMQC spectrum. These shifts can be explained by hydroxymethylation of one methyl linked to C-4, which also can be supported by the cross peak between methyl and C-3 at HMBC spectrum (Fig. 2). Consequently, NOESY experiment revealed it was the methyl-29 group that had been hydroxymethylated since a cross peak was observed between methyl-19 and hydroxymethyl-29 in the spectrum (Fig. 2). The configuration assignment at C-20 was based upon a comparison in the chemical shift with known facts. When there is α-COOH group at the C-20 position in naturally-occurring lanostanes, chemical shift of C-20 should be above δ 48.3[3,16,17]; otherwise, for β-COOH, the chemical shift is less than δ 47.6.[19] Based on the above evidences, the structure of **8** was elucidated to be 29-hydroxypolyporenic acid C.

The growth inhibitory effects of compounds **1** through **6** and **8** on DU145 and A549 cells were examined as a preliminary evaluation of their potential to inhibit cancer cell growth. The result (Table 2) indicated that all compounds except compound **2** showed good inhibitory activity towards the growth of DU145 and A549 cells. Most interestingly, all compounds showed better cytotoxicity towards A549 cells than DU145 cells. This effect is most obvious for compound **6**, which exhibits an IC_{50} value of 25.3 and 418.6 μM, respec-

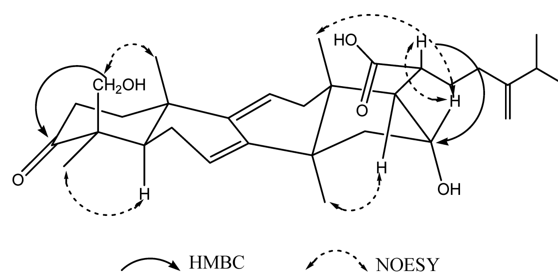

Fig. 2. Characteristic Correlations Observed by HMBC and NOESY NMR for Compound **8**

Table 1. ^{13}C-NMR Spectral Data for Compounds **1**—**9** (75 MHz in C_5D_5N; δ in ppm)

Position	1	2	3	4	5	6	7	8	9
1	35.3	35.6	35.4	36.8	31.1	30.7	36.1	36.4	36.2
2	24.5	24.5	24.5	34.9	23.5	26.7	28.7	35.3	28.6
3	80.7	80.6	80.7	215.2	78.0	75.2	78.1	215.5	78.0
4	38.0	37.8	38.0	47.5	36.9	38.0[b)]	39.6	43.1	39.1
5	50.7	49.6	50.7	51.0	45.0	43.8	51.0	52.7	49.7
6	18.4	23.1	18.4	23.8	23.4	23.5	18.8	23.8	23.5
7	26.7	120.7	26.7	120.7	121.2	121.3	27.0	120.9	121.2
8	134.4[b)]	142.7	134.4[b)]	142.8	142.7	142.8	134.7[b)]	142.8	142.6
9	*pyr*[b)]	145.7	*pyr*[b)]	144.7	146.4	146.7	*pyr*[b)]	144.4	146.4
10	37.1	37.6	37.1	37.5	37.7	37.9[b)]	37.4	37.2	37.6
11	20.9	117.0	20.9	117.6	116.5	116.2	21.0	118.0	116.4
12	29.7	36.2	29.7	36.2	36.2	36.3	29.8	36.2	36.2
13	46.2	45.0	46.3	45.0	45.2	45.2	46.3	45.1	45.1
14	48.8	49.4	48.8	49.3	49.6	49.5	48.8	49.4	49.2
15	43.6	44.4	43.6	44.3	44.6	44.5	43.8	44.4	44.3
16	76.6	76.4	76.6	76.4	76.7	76.5	76.7	76.4	76.3
17	57.3	57.6	57.5	57.6	57.6	57.6	57.4	57.6	57.5
18	17.8	17.6	17.8	17.6	17.6	17.7	17.9	17.7	17.6
19	19.2	20.8	19.2	22.3	22.9	23.0	19.4	22.5	23.0
20	48.7	48.5	48.6	48.5	48.6	48.6	48.8	48.5	48.4
21	178.8	178.7	178.8	178.6	178.8	178.7	178.7	178.6	178.6
22	31.6	31.4	33.2	31.4	31.6	31.5	31.6	31.5	31.5
23	33.2	33.2	27.1	33.2	33.5	33.2	33.3	33.2	33.1
24	156.1	156.0	135.2	156.0	156.1	156.1	156.2	156.1	156.1
25	34.1	34.1	131.5	34.1	34.2	34.2	34.2	34.2	34.2
26	22.0[a)]	22.0[a)]	25.8	22.0[a)]	22.1[a)]	22.1[a)]	22.1[a)]	22.1[a)]	22.0[a)]
27	21.9[a)]	21.8[a)]	17.7	21.8[a)]	22.0[a)]	21.9[a)]	21.9[a)]	21.9[a)]	21.8[a)]
28	28.0	28.1	28.0	22.0	27.9	29.2	28.7	18.7	28.4
29	16.8	17.1	16.8	26.3	22.5	23.2	16.3	66.9	16.7
30	25.4	26.5	25.4	25.6	26.8	26.7	25.5	26.1	26.6
31	107.0	107.0		107.0	107.0	107.1	107.0	107.0	107.0
<u>CH₃</u>CO	21.1	21.1	21.1		21.2				
CH₃<u>CO</u>	170.7	170.5	170.6		170.6				

a), *b*) Assignments in the same column may be interchangeable; *pyr*: peak hidden in pyridine.

Table 2. Inhibitory Effects of Compounds **1**—**9** on Growth of A549 and DU145 Cells

	Compound	IC_{50} (μM)±S.D.	
		A549	DU145
1	Pachymic acid	30.5±9.7	52.4±20.9
2	Dehydropachymic acid	194.3±247.3	368.2±266.8
3	3-Acetyloxy-16α-hydroxy-trametenolic acid	44.3±39.1	140±50.7
4	Polyporenic acid C	19.8±5.2	73.4±20.3
5	3-*epi*-Dehydropachymic acid	24.0±12.8	56.8±12.1
6	3-*epi*-Dehydrotumulosic acid	25.3±9.2	418.6±204.1
7	Tumulosic acid	—	—
8	29-Hydroxypolyporenic acid C	38.1±19.9	49.7±15.2
9	Dehydrotumulosic acid	—	—

tively, for the lung and prostate carcinoma cell lines.

Reactive oxygen species such as superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radical have been implicated in the etiology of a wide array of human diseases, including cancer and heart diseases.[20] Hence, compounds that have antioxidant activity may play an important role in reducing cancer risk. Lanostane-type triterpenes were previously implicated to possess antioxidant and free radical scavenging activities.[21] To determine if our compounds possess any antioxidant and free radical scavenging activity, we studied their ability to scavenge DPPH free radicals. None of the compounds was powerful scavenger of DPPH free radicals (data not shown). These results contradict those reported by Sekiya *et al.*,[21] who found that lanostane-type triterpenes from *P. cocos* had inhibitory activities against AAPH [2,2′-azobis(2-amidinopropane)dihydrochloride]-induced lysis of red blood cells. Sekiya *et al.* found that incubation of red blood cells with triterpenes and APPH, a peroxyl radical generator, inhibited cell lysis and reduced residual APPH. In our study, we analyzed whether triterpenoids alone could interact with and scavenge DPPH. AAPH and DPPH share similar chemical properties, therefore, we speculate whether the presence of cellular components provided by red blood cells contributed to or augmented the antioxidant activity of triterpenoids found by Sekiya *et al.* as a plausible explanation for the existing discrepancy between the two studies.

In conclusion, we have reported the isolation of one novel and eight known lanostane-type triterpenes from the scleortia of *P. cocos*. Compounds, **1** through **6** and **8**, show good cytotoxicity against two human cancer cell lines. Most interestingly, these compounds exhibit better cytotoxicity towards A549 lung cancer cells than DU145 prostate cancer cells. The results point to *P. cocos* as a potentially rich source for identification of novel lanostane-type triterpenes that could be further modified to give better cytotoxicity and selectivity as anti-cancer agents.

Experimental

General Experimental Procedures All chemicals were purchased from Tedia Company, Inc. (Fairfield, OH, U.S.A.) unless otherwise noted. The deuteriated solvent, pyridine-d_5, for NMR measurement was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, U.S.A.). All sorbents for chromatographic isolation, such as Silica gel 60 (40—63 μm), LiChroprep® RP-18 (40—63 μm), and pre-coated silica gel 60 TLC plates, were purchased from Merck KGaA (Darmstadt, Germany). Melting points were determined on apparatus of Gallenkamp product (Cat. No. MPD035.BM2.5), which was uncorrected. IR spectra were recorded on Perkin-Elmer 983G spectrometer. All NMR spectra were obtained on a Bruker ACF300 spectrometer (Karlsruhe, Germany) operating at 300 MHz for proton and 75 MHz for carbon. Optical rotations were measured on a Perkin-Elmer 243B polarimeter (Waltham, MA, U.S.A.) with MeOH as solvent. Mass spectra and HR-ESI-MS were obtained on QTrap® 2000 (Applied Biosystems Inc., U.S.A.) ESI mass spectrometer and a Finnigan MAT95XL (Finnigan, Bremen, Germany) mass spectrometer, respectively. Data were acquired in continuum mode until acceptable averaged data were obtained. DU145 and A549 cell lines were purchased from the American Type Culture Collection (Rockville, MD, U.S.A.). DU145 was propagated at 37 °C in a 5% CO_2 humidified environment in RPMI-1640 culture medium (Gibco, Invitrogen; Eugene, OR, U.S.A.) containing 4.5 g/l D-glucose, 1.5 g/l sodium bicarbonate, 2.38 g/l HEPES, 1 mM sodium pyruvate, 10% fetal bovine serum (FBS; Hyclone Laboratories; Logan, UT, U.S.A.) and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin G). A549 was maintained at 37 °C in a 5% CO_2 humidified incubator in Ham's F12K culture medium (Sigma-Aldrich) containing 4.5 g/l D-glucose, 1.5 g/l sodium bicarbonate, 2.38 g/l HEPES, 1 mM sodium pyruvate, 10% FBS and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin G).

Plant Material The sclerotia of *P. cocos* Wolf (Polyporaceae) harvested in Anhui, China was purchased from a local reputable Chinese herb store.

Extraction and Isolation of Compounds 1—9 Pulverized sclerotia of *P. cocos* (4 kg) were extracted three times with 95% ethanol (12 l) under reflux for 3 h. The ethanol solution was combined and evaporated in vacuum to give a crude extract (18 g). The crude extract was mixed with silica gel (Merck, Darmstadt, Hesse, Germany, size: 63—200 μm), and fractionated on silica (Merck, size: 40—63 μm) column chromatography (600×80 mm i.d.) by gradient elution using $CHCl_3$ and MeOH (100 : 0→95 : 5→80 : 20, with methanol added at 1% gradient). Fractions were collected, combined and subjected to further chromatography on a silica gel 60 column (600×80 mm i.d.) by step gradients of $CHCl_3$–CH_3OH (100 : 0→85 : 15). The collected fractions were combined on the basis of their TLC characteristics to give four pooled fractions: PCA (1 g), PCB (3.6 g), PCC (4.12 g), and PCD (1.85 g), listed in increasing order of polarity. PCB (3.6 g) was subjected to chromatography on an ODS column (500 g) with step gradient elution conducted with CH_3OH–H_2O (60 : 40→85 : 15), which gave rise to two sub-fractions: PCB-a and PCB-b. Both sub-fractions had similar cytotoxicity against A549 and DU145 cells, and as such they were taken into the next phase for further separation and isolation of active pure compounds. PCB-b was subjected to chromatography on a silica gel 60 column (500×40 mm i.d.), from which two pure compounds, **4** (230 mg) and **5** (24 mg), were isolated with step gradient elution of $CHCl_3$–CH_3OH (98 : 2→93 : 7). PCB-a was subjected to an ODS column chromatography with step gradient elution conducted with CH_3OH–H_2O (70 : 30→85 : 15) to give compound **1** (1.5 g) and a mixture of compounds. The mixture was further fractionated on silica gel 60 column chromatograph (500×40 mm i.d.) to give compounds **2** (43 mg) and **3** (7 mg), using isocratic elution of hexane–ethyl acetate–CH_3OH (70 : 30 : 5). PCC (4.12 g) was subjected to chromatography on a silica gel 60 column (600×80 mm i.d.). Step gradient elution was conducted with $CHCl_3$–CH_3OH (97 : 3→85 : 15) to give 2 sub-fractions, PCC-a and PCC-b. PCC-a was subjected to an ODS column chromatography with step gradient elution conducted with CH_3OH–H_2O (55 : 45→70 : 30) to give compound **7** (80 mg) and **9** (17 mg). PCC-b was fractionated on silica gel 60 column chromatograph (500×40 mm i.d.) to give compounds **6** (110 mg) and **8** (28 mg), with isocratic elution of $CHCl_3$–CH_3OH (93 : 7).

Pachymic Acid (**1**): White powder, mp 293—294 °C. ESI-MS *m/z*: 529 $[M+H]^+$; ^{1}H-NMR (300 MHz, Pyr-d_5) δ: 0.92 (3H, s, H-28), 0.94 (3H, s, H-29), 0.97 (3H, s, H-19), 0.99, 1.01 (ea. 3H, d, J=6.5 Hz, H-26, H-27), 1.13 (3H, s, H-18), 1.49 (3H, s, H-30), 2.07 (3H, s, CH_3CO), 2.27 (1H, m, H-25), 2.84 (1H, dd, J=11, 5.5 Hz, H-17), 2.94 (1H, m, H-20), 4.54 (1H, t, J=6 Hz, H-16), 4.68 (1H, dd, J=11, 4 Hz, H-3), 4.86, 4.99 (ea. 1H, s, H-31); ^{13}C-NMR (75 MHz, Pyr-d_5); Table 1.

Dehydropachymic Acid (**2**): White powder, mp 265—267 °C. ESI-MS *m/z*: 527 $[M+H]^+$; ^{1}H-NMR (300 MHz, Pyr-d_5) δ: 0.90 (3H, s, H-28), 0.99 (3H, s, H-29), 1.01 (3H, s, H-19), 0.99, 1.01 (ea. 3H, d, J=6.5 Hz, H-26, H-27), 1.05 (3H, s, H-18), 1.50 (3H, s, H-30), 2.05 (3H, s, CH_3CO), 2.30 (1H, m, H-25), 2.88 (1H, dd, J=11, 5.5 Hz, H-17), 2.94 (1H, m, H-20), 4.54 (1H, t, J=6 Hz, H-16), 4.70 (1H, dd, J=11, 4 Hz, H-3), 4.86, 4.99 (ea. 1H, s, H-31), 5.34 (1H, d, J=6 Hz, H-11), 5.59 (1H, br s, H-7); ^{13}C-NMR (75 MHz, Pyr-d_5); Table 1.

3-Acetyloxy-16α-hydroxytrametenolic Acid (**3**): White powder, mp >300 °C. ESI-MS *m/z*: 515 $[M+H]^+$; ^{1}H-NMR (300 MHz, Pyr-d_5) δ: 0.90 (3H, s, H-28), 0.93 (3H, s, H-29), 0.96 (3H, s, H-19), 1.13 (3H, s, H-18), 1.48 (3H, s, H-30), 1.60, 1.62 (ea. 3H, s, H-26, H-27), 2.05 (3H, s, CH_3CO),

2.80 (1H, dd, J=11, 6 Hz, H-17), 2.94 (1H, m, H-20), 4.53 (1H, t, J=6 Hz, H-16), 4.70 (1H, dd, J=11, 4 Hz, H-3), 5.35 (1H, br s, H-24); ^{13}C-NMR (75 MHz, Pyr-d_5); Table 1.

Polyporenic Acid C (**4**): White needles, mp 261—263 °C. ESI-MS m/z: 483 $[M+H]^+$; ^{1}H-NMR (300 MHz, Pyr-d_5) δ: 1.06 (3H, s, H-28), 1.14 (3H, s, H-29), 1.14 (3H, s, H-19), 0.99, 1.01 (ea. 3H, d, J=6.5 Hz, H-26, H-27), 1.07 (3H, s, H-18), 1.46 (3H, s, H-30), 1.64 (1H, m, H-5), 2.88 (1H, m, H-17), 2.94 (1H, m, H-20), 4.55 (1H, t, J=6 Hz, H-16), 4.86, 4.99 (ea. 1H, s, H-31), 5.37 (1H, d, J=6 Hz, H-11), 5.60 (1H, d, J=6 Hz, H-7); ^{13}C-NMR (75 MHz, Pyr-d_5); Table 1.

3-*epi*-Dehydropachymic Acid (**5**): White powder, mp 276—278 °C. ESI-MS m/z: 527 $[M+H]^+$; ^{1}H-NMR (300 MHz, Pyr-d_5) δ: 0.90 (3H, s, H-28), 0.93 (3H, s, H-29), 1.03 (3H, s, H-19), 0.99, 1.01 (ea. 3H, d, J=6.5 Hz, H-26, H-27), 1.07 (3H, s, H-18), 1.44 (3H, s, H-30), 1.75 (1H, m, H-5), 2.86 (1H, dd, J=11, 6 Hz, H-17), 2.94 (1H, m, H-20), 4.54 (1H, t, J=6 Hz, H-16), 4.85 (1H, br s, H-3), 4.86, 4.99 (ea. 1H, s, H-31), 5.37 (1H, d, J=6 Hz, H-11), 5.60 (1H, br s, H-7); ^{13}C-NMR (75 MHz, Pyr-d_5); Table 1.

3-*epi*-Dehydrotumulosic Acid (**6**): White powder, mp 236—239 °C. ESI-MS m/z: 485 $[M+H]^+$; ^{1}H-NMR (300 MHz, Pyr-d_5) δ: 1.19 (3H, s, H-28), 0.98 (3H, s, H-29), 1.11 (3H, s, H-19), 0.99, 1.01 (ea. 3H, d, J=6.5 Hz, H-26, H-27), 1.08 (3H, s, H-18), 1.43 (3H, s, H-30), 2.30 (1H, m, H-25), 2.88 (1H, m, H-17), 2.94 (1H, m, H-20), 4.52 (1H, t, J=6 Hz, H-16), 3.63 (1H, br s, H-3), 4.85, 4.98 (ea. 1H, s, H-31), 5.47 (1H, d, J=6 Hz, H-11), 5.64 (1H, br s, H-7); ^{13}C-NMR (75 MHz, Pyr-d_5); Table 1.

Tumulosic Acid (**7**): White powder, mp 253—256 °C. ESI-MS m/z: 487 $[M+H]^+$; ^{1}H-NMR (300 MHz, Pyr-d_5) δ: 1.01 (3H, s, H-28), 1.06 (3H, s, H-29), 1.14 (3H, s, H-19), 0.96, 0.98 (ea. 3H, d, J=6.5 Hz, H-26, H-27), 1.23 (3H, s, H-18), 1.47 (3H, s, H-30), 2.27 (1H, m, H-25), 2.84 (1H, dd, J=11, 5.5 Hz, H-17), 2.94 (1H, m, H-20), 4.52 (1H, t, J=6 Hz, H-16), 3.43 (1H, t, J=7 Hz, H-3), 4.86, 4.99 (ea. 1H, s, H-31); ^{13}C-NMR (75 MHz, Pyr-d_5); Table 1.

29-Hydroxypolyporenic Acid C (**8**): White powder, mp 255—258 °C. UV (MeOH) λ_{max} nm: 236, 244, 252; IR (KBr) ν_{max} cm^{-1}: 3400, 2960, 1710, 1675, 1642, 1380, 1100, 900; HR-ESI-MS m/z: 497.3265 $[M-H]^-$; ^{1}H-NMR (300 MHz, Pyr-d_5) δ: 0.99, 1.01 (ea. 3H, d, J=6.5 Hz, H-26, H-27), 1.07 (6H, s, H-18, H-28), 1.14 (3H, s, H-19), 1.42 (3H, s, H-30), 1.64 (1H, m, H-5), 2.25 (1H, m, H-25), 2.86 (1H, m, H-17), 2.92 (1H, m, H-20), 3.59, 4.17 (each 1H, d, J=10.5 Hz, H-29), 4.53 (1H, t, J=6 Hz, H-16), 4.86, 4.99 (ea. 1H, s, H-31), 5.44 (1H, d, J=6 Hz, H-11), 5.59 (1H, br s, H-7); ^{13}C-NMR (75 MHz, Pyr-d_5); Table 1.

Dehydrotumulosic acid (**9**): White powder, mp 272—275 °C. ESI-MS m/z: 485 $[M+H]^+$; ^{1}H-NMR (300 MHz, Pyr-d_5) δ: 1.07 (3H, s, H-28), 1.08 (3H, s, H-29), 1.14 (3H, s, H-19), 0.99, 1.01 (ea. 3H, d, J=6.5 Hz, H-26, H-27), 1.22 (3H, s, H-18), 1.52 (3H, s, H-30), 2.28 (1H, m, H-25), 2.74 (1H, dd, J=11, 5.5 Hz, H-17), 2.91 (1H, m, H-20), 4.57 (1H, t, J=6 Hz, H-16), 3.48 (1H, dd, J=11, 4 Hz, H-3), 4.85, 4.98 (ea. 1H, s, H-31), 5.38 (1H, br s, J=6 Hz, H-11), 5.64 (1H, br s, H-7); ^{13}C-NMR (75 MHz, Pyr-d_5); Table 1.

Cell Growth Assay Cell growth was quantified using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich). DU145 or A549 cells were seeded in 96-well (7500 or 5000 cells per well, respectively) tissue culture plates and cultured for 72 h. Cells were treated with media containing pure tested compounds or DMSO vehicle in triplicate for each concentration and time point. After treatment, cell growth was analyzed by addition of MTT following the manufacturers' protocol. The absorbance of MTT was determined at 590 nm using a Tecan Spectra Fluor spectrophotometer (MTX Lab Systems Inc., Vienna, VA, U.S.A.). Absorbance values were normalized to relevant media and vehicle controls. Data from three independent experiments were analyzed using GraphPad Prism (San Diego, CA, U.S.A.) to determine IC_{50} values after nonlinear regression (curve fit). IC_{50} values were expressed as concentration±standard deviation (S.D.). The IC_{50} and S.D. reported are within the 95% confidence interval.

Evaluation of Antioxidant Activity To measure the ability of the compounds to scavenge free oxygen radicals and inhibit oxidation, the method of scavenging stable DPPH free radical by the compounds of interest was used. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was purchased from Sigma-Aldrich. For measurement of free radical scavenging activity, the compounds (200 μM, 100 μl) were added to an ethanol solution of DPPH (200 μM, 100 μl), and the reaction mixtures were shaken vigorously in the dark. DDPH plus vitamin E was used as a positive assay control. After 2 h of incubation, the absorbance of the remaining DPPH was read at 516 nm and compared to negative control solutions containing only DPPH. Data were expressed as mean absorbance±S.E.M., and Student's t-test was used to evaluate the significant difference between groups. Compounds **1**—**9** did not show significant antioxidant activity with $p<0.05$ considered statistically significant (data not shown).

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