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QUINONE-METHIDE TRITERPENES AND SALASPERMIC ACID FROM *KOKOONA OCHRACEA*[1]

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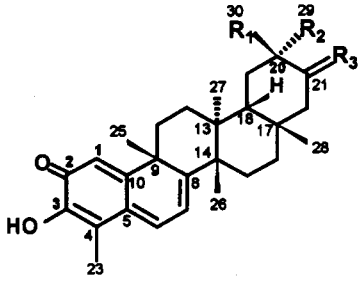
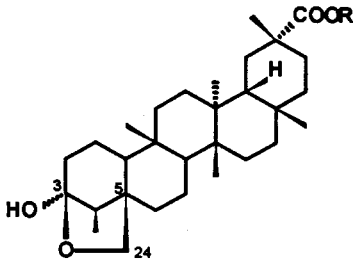
ABSTRACT.—Tingenone [**1**], 20-hydroxy-20-*epi*-tingenone [**2**], celastrol [**3**], and salaspermic acid [**4**] have been isolated from *Kokoona ochracea* stem bark. The quinone-methide triterpenes **1**–**3** exhibited strong but non-specific in vitro cytotoxicity against P-388 murine lymphocytic leukemia cells and a panel of human cancer cell lines. Salaspermic acid [**4**] was not active in all the cancer cell lines used in this investigation. ^{13}C-nmr spectra assignments for salaspermic acid have been accomplished through the application of 1D and 2D nmr spectral techniques, and ^{13}C-nmr assignments for celastrol [**3**] have been revised.

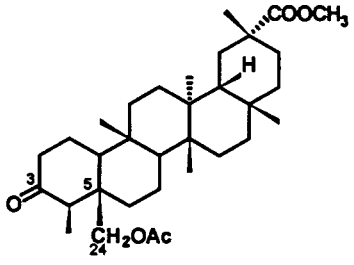
There is scarce biological and phytochemical information on *Kokoona ochracea* (Elm.) Merrill (Celastraceae). Thus, the Philippine plant was collected as a potential source of anticancer agents. In preliminary screening, the stem bark of *K. ochracea* was found to possess significant in vitro cytotoxicity against P-388 murine lymphocytic leukemia cells and a number of human cancer cell lines (ED_{50} values < 20 μg/ml). Bioassay-guided fractionation of the active extracts using BC-1 (breast cancer) cells afforded four major cytotoxic fractions, and three new cytotoxic compounds, ochraceolides A, B, and C, had been previously isolated (1). We currently report the isolation of tingenone [**1**], 20-



1 R_1=Me, R_2=H, R_3=O
2 R_1=OH, R_2=Me, R_3=O
3 R_1=Me, R_2=COOH, R_3=H_2



4 R=H
5 R=Me



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[1]Parts of this study were derived from the Ph.D. thesis of Olipa Ngassapa, University of Illinois at Chicago (1992).

hydroxy-20-*epi*-tingenone [**2**], celastrol [**3**], and salaspermic acid [**4**] from *K. ochracea*, and the in vitro cytotoxic activity of these isolates. This is the first report on the cytotoxic potential of 20-hydroxy-20-*epi*-tingenone [**2**]. We report also ^{13}C-nmr assignments for salaspermic acid [**4**] and a revision of the assignments made previously for celastrol [**3**] (2).

Compounds **1–4** were identified by analysis of their spectral data (uv, ir, ms, and nmr), and by comparison with those reported previously (2–7). Compound **2** was first isolated from *Euonymus tingens* Wall. (Celastraceae), and was named 20-hydroxytingenone (3). The C-20 hydroxyl group was originally thought to be α-oriented (3), but recent nmr studies have shown it to be β-oriented (8); hence the name 20-hydroxy-20-*epi*-tingenone. Tingenone [**1**] and celastrol [**3**] have been shown to have antitumor (9,10) and cytotoxic (11–13) activity in previous studies, but in those studies only one or two cancer cell systems were used. In the present study compounds **1–4** have been evaluated for in vitro cytotoxic activity against P-388 cells and twelve human cancer cell lines, to determine their cytotoxic potential and to determine whether they could demonstrate selective activity. As summarized in Table 1, the quinone-methide triterpenes **1–3** exhibited strong cytotoxic activity. However, none of these compounds was selective for a particular cancer cell line; they were broadly cytotoxic against all the cell systems used in this investigation. Consequently, these compounds may be of high priority for development as cancer chemotherapeutic agents. Salaspermic acid [**4**] and its methyl ester **5** were inactive with all cancer cell systems. However, methyl 24-acetoxypolpunonate [**6**], an acetylation product of salaspermic acid methyl ester, was marginally cytotoxic against A431 (human epidermoid carcinoma) and ZR-75-1 (human hormone-dependent breast cancer) cells. This compound also exhibited weak activity with other cell lines, including a multidrug-resistant cell line, KB-V1 (Table 1).

^{13}C-Nmr assignments for celastrol [**3**] were made previously (2). However, several of the assignments need to be revised. With the current availability of 1D and 2D nmr techniques, unambiguous ^{13}C-nmr assignments have been made for quinone-methide triterpenes related to celastrol (6). In this study, using ^{1}H-^{1}H DQCOSY, APT, ^{1}H-^{13}C HETCOR, and selective INEPT techniques, unambiguous ^{13}C-nmr assignments for celastrol have been accomplished and are in good agreement with those made for related compounds (6). Protonated carbons were assigned based on correlations observed in the ^{1}H-^{13}C HETCOR spectrum of **3**. Carbon signals at δ 135.62, 120.59, and 118.26 were assigned to C-6, C-1 and C-7,

TABLE 1. In Vitro Cytotoxic Activity of Compounds **1–6**.

Cell line	Compound tested (ED_{50}, μg/ml)					
	1	**2**	**3**	**4**	**5**	**6**
BC-1	0.3	0.9	0.3	>20	>20	>20
HT-1080	0.7	2.1	0.5	>20	>20	10.0
LU-1	0.6	2.2	1.3	>20	>20	11.9
MEL-2	0.2	1.5	0.6	>20	>20	>20
COL-2	0.9	5.8	2.3	>20	>20	>20
KB	0.5	1.7	1.2	>20	>20	10.1
KB-V1 (+VLB)	0.4	3.3	1.7	>20	>20	12.0
KB-V1-(−VLB)	0.4	3.0	2.4	>20	>20	9.8
P-388	0.1	2.0	3.5	>5	>5	>5
A431	0.7	1.1	0.6	>20	>20	5.2
LNCaP	0.2	1.0	0.3	>20	>20	11.8
ZR-75-1	0.6	1.0	0.4	>20	>20	6.2
U373	0.2	0.8	0.4	>20	>20	>20

respectively, based on their correlations with the olefinic protons at δ 7.04 (H-6), 6.47 (H-1), and 6.30 (H-7). The signals of methyl carbons at δ 38.32, 32.33, 31.40, 21.37, 18.61, and 10.45 were assignable to C-25, C-30, C-28, C-26, C-27, and C-23, respectively. The most downfield carbon signal at δ 182.65 was assigned to the carboxyl carbon, C-29, and that at δ 178.20 was assignable to C-2. Other carbon signals were assigned through the use of selective INEPT experiments (14). Irradiation of a proton at δ 7.04 (H-6; $^3J_{CH}$=8 Hz) enhanced the carbon signals at δ 120.64, 164.99, and 172.95, which were assigned to C-4, C-10, and C-8, respectively. When H-1, at δ 6.47, was irradiated ($^3J_{CH}$=8 Hz), carbon signals at δ 146.99, 127.40, and δ 43.04 were enhanced and were subsequently assigned to C-3, C-5, and C-9, respectively. Finally, irradiation of H-7 (δ 6.30; $^3J_{CH}$=8 Hz) led to enhancement of carbon signals at δ 43.04 (C-9), 45.27, and 127.40 (C-5), permitting assignment of the signal at δ 45.27 to C-14. The ^{13}C-nmr assignments for celastrol are presented in Table 2.

Salaspermic acid [**4**] was first isolated from *Salacia macrosperma* Wight (Celastraceae) and its structure elucidated by chemical, ^{1}H-nmr, and X-ray crystallographic studies

TABLE 2. ^{13}C-Nmr Spectral Data (δ, $CDCl_3$) for Compounds **3–6**.

Carbon	Compound			
	3	**4**[a]	**5**	**6**
C-1	120.59 d	20.35 t	19.88 t	21.87 t
C-2	178.20 s	38.90 t	37.40 t	40.79 t
C-3	146.99 s	105.86 s	105.82 s	211.00 s
C-4	120.64 s	53.74 d	52.86 d	55.96 d
C-5	127.40 s	46.96 s	46.88 s	44.11 s
C-6	135.62 d	33.88 t	33.76 t	38.53 t
C-7	118.26 d	19.41 t	19.22 t	18.61 t
C-8	172.95 s	50.03 d	49.82 d	50.47 d
C-9	43.04 s	37.35 s	37.40 s	37.50 s
C-10	164.99 s	57.14 d	56.98 d	58.35 d
C-11	33.72 t	34.60 t	34.35 t	35.30 t
C-12	29.21 t	29.41 t	29.12 t	29.45 t
C-13	39.22 s	39.37 s	39.04 s	39.07 s
C-14	45.27 s	39.13 s	39.04 s	39.31 s
C-15	28.64 t	29.33 t	29.12 t	28.95 t
C-16	36.27 t	36.54 t	36.15 t	36.06 t
C-17	30.59 s	30.29 s	30.06 s	30.06 s
C-18	44.16 d	44.65 d	44.43 d	44.47 d
C-19	30.99 t	30.72 t	30.37 t	30.33 t
C-20	39.86 s	40.53 s	40.50 s	40.51 s
C-21	29.39 t	30.34 t	29.92 t	29.87 t
C-22	34.40 t	37.21 t	36.51 t	36.46 t
C-23	10.45 q	8.45 q	7.29 q	6.91 q
C-24		72.88 t	73.03 t	66.10 t
C-25	38.32 q	16.72 q	16.73 q	18.13 q
C-26	21.37 q	17.91 q	17.33 q	17.38 q
C-27	18.61 q	16.48 q	16.18 q	15.93 q
C-28	31.40 q	31.94 q	31.78 q	31.76 q
C-29	182.65 s	181.25 s	179.16 s	179.16 s
C-30	32.33 q	32.25 q	31.92 q	31.90 q
Me			51.47 q	51.47 q
Me				170.28 s
Me				20.54 q

[a]Spectra were recorded in pyridine-d_5.

(5,7,15). Later it was isolated from several other genera of the Celastraceae (16–18). Despite the wide occurrence of salaspermic acid in the Celastraceae, no nmr assignments have been made for this compound. Using APT, ^{1}H-^{13}C HETCOR, and selective INEPT nmr spectral techniques, ^{13}C-nmr assignments for salaspermic acid [**4**] and its derivatives **5** and **6** have been accomplished in this study. Protonated carbons were assigned through analysis of the ^{1}H-^{13}C HETCOR spectrum of **4**, and by comparison with ^{13}C-nmr assignments made for a related compound, maytenonic acid (19). Thus, methyl carbon signals at δ 32.25, 31.94, 17.91, 16.75, 16.48, and 8.45 were assignable to C-30, C-28, C-26, C-25, C-27, and C-23, respectively. The carbinolic methylene signal at δ 72.88 was assigned to C-24, and the quaternary carbon signal at δ 105.86 was assigned to C-3. In a selective INEPT experiment (Table 3), irradiation of the downfield H_a-24 (δ 4.21; $^{3}J_{CH}$=5 Hz) led to the enhancement of carbon signals at δ 105.86 (C-3), 57.14, 53.74, and 46.96. This confirmed the assignment of C-3 and permitted assignment of the signals at δ 57.14, 53.74, and 46.96 to C-10, C-4, and C-5, respectively.

When a methyl group at δ 0.87 (Me-25) was irradiated ($^{3}J_{CH}$=4 Hz), signals of a methylene at δ 34.60, a quarternary carbon at δ 37.45, and methines at δ 50.03 and 57.14 (C-10) were enhanced. Thus, the signals at δ 34.60, 37.45, and 50.03 were assigned to C-11, C-9 and C-8, respectively. Magnetization transfer from Me-27 (δ 0.80; $^{3}J_{CH}$=4 Hz) led to the enhancement of a methylene signal at δ 29.41 (C-12) and quaternary carbon signals at δ 39.13 and 39.37. The signal at δ 39.37 was also enhanced when H-19 (δ 2.64) was irradiated ($^{3}J_{CH}$=5 Hz). Thus, the signal at δ 39.37 was assigned to C-13, and that at δ 39.13 to C-14. Signals of C-17 (δ 30.29), C-20 (δ 40.53), C-18 (δ 44.65), and C-29 (δ 181.25) were also enhanced following the irradiation of H-19. Irradiation involving H-21 at δ 2.48 ($^{3}J_{CH}$=5 Hz) also enhanced the signals of C-17, C-20, and C-29, further confirming their assignments. These ^{13}C-nmr assignments for salaspermic acid [**4**] are summarized in Table 2. Based on similar nmr experiments, ^{13}C-nmr assignments were also made for the derivatives of salaspermic acid, **5** and **6** (Table 2).

TABLE 3. Selective INEPT Nmr Data for Celastrol [**3**] and Salaspermic acid [**4**].

Proton irradiated (δ, $^{3}J_{CH}$)	Carbon enhanced δ (C #)	
	Celastrol [**3**]	Salaspermic acid [**4**]
H-1 (δ 6.47, 8 Hz)	146.99 (C-3), 127.40 (C-5), 43.04 (C-9)	
H-2 (δ 2.16, 5 Hz)		105.86 (C-3), 57.14 (C-10), 53.74 (C-4)
H-6 (δ 7.04, 8 Hz)	172.95 (C-8), 164.99 (C-10), 120.64 (C-4)	
H-7 (δ 6.30, 8 Hz)	127.40 (C-5), 45.27 (C-14), 43.04 (C-9)	
H-19 (δ 2.64, 5 Hz)		181.25 ((C-29, 44.65 (C-18), 40.53 (C-20), 39.37 (C-13), 30.29 (C-17)
H-21 (δ 2.48, 5 Hz)		181.25 (C-29), 40.53 (C-20), 30.29 (C-17)
H_a-24 (δ 4.21, 5 Hz)		105.86 (C-3), 57.14 (C-10), 53.74 (C-4), 46.96 (C-5)
Me-25 (δ 0.87, 4 Hz)		57.14 (C-10), 50.03 (C-8), 37.35 (C-9), 34.60 (C-11)
Me-27 (δ 0.80, 4 Hz)		39.37 (C-13), 39.13 (C-14), 29.41 (C-12)

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Nmr spectra were obtained in $CDCl_3$ or pyridine-d_5 with TMS as an internal standard. ^{1}H-nmr spectra were recorded on a Varian XL-300 spectrometer operating at 300 MHz. ^{13}C-nmr spectra were obtained on Varian XL-300 (75.4 MHz) and Nicolet NMC-360 (90.8 MHz) spectrometers. The eims was obtained on a Varian MAT-112S spectrometer. Ir and uv spectra were recorded with Nicolet MX-1 FT-IR and Beckman DU-7 spectrophotometers, respectively. Cc was performed on Si gel 60 (70–230 mesh, E. Merck, Darmstadt, Germany). All solvents were spectral grade or redistilled before use.

COLLECTION AND EXTRACTION OF PLANT MATERIAL.—The stem bark of *K. ochracea* was collected in July 1988 on Palawan Island, Philippines, and identified by one of us (D.D.S.) and Dr. D.A. Madulid (Philippine National Herbarium, Manila, Philippines). Voucher specimens (Soejarto and Madulid 6098) are deposited in the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, and the Philippine National Herbarium, Manila.

Air-dried powdered stem bark (5 kg) was extracted and fractionated as previously described (1). Briefly, the plant material was extracted with petroleum ether, then with MeOH to afford a petroleum ether extract (35.8 g) and an MeOH extract (320 g). Partitioning of the latter (310 g) between H_2O and $CHCl_3$, followed by drying, afforded a $CHCl_3$-soluble (155 g), an interphase (93.2 g), and an H_2O-soluble (59.1 g) fraction. The petroleum ether extract and the H_2O-soluble material were found to possess *in vitro* cytotoxic activity against the P-388 (murine lymphocytic leukemia) and various human cancer cell lines. These extracts had similar tlc profiles; they were therefore combined (188 g) and fractionated further by cc (petroleum ether/EtOAc; EtOAc/ MeOH) to afford eight major fractions (F1–F8). The breast cancer (BC-1) cell line was used to monitor the fractionation process. BC-1 cytotoxicity was retained in fractions F3–F6. The major compound, ochraceolide A (15 g) was obtained from fraction F3 (1).

Tingenone [**1**].—Further purification of fraction F4 by cc (petroleum ether/$CHCl_3$; $CHCl_3$/MeOH) afforded six major fractions (F4-I–F4-VI). Cytotoxicity with BC-1 cells was retained in fractions F4-II to F4-VI, with F4-II being the most active (ED_{50} 1.0 μg/ml), followed by F4-IV (ED_{50} 3.4 μg/ml). Fraction F4-II (6.2 g) was purified further by Si gel chromatography (180 g, 4.3×30 cm, petroleum ether/EtOAc), to afford tingenone [**1**] (1.42 mg), in addition to ochraceolide B (282 mg) and ochraceolide C (190 mg) (1). Tingenone [**1**] was obtained as orange-red crystals ($CHCl_3$/petroleum ether): mp 147–149°; $[\alpha]^{25}D$ −341° (c=0.1, MeOH); uv (MeOH) λ max (log ε) 210 (3.33), 242 (sh, 3.02), 288 (sh, 2.21), 421 (3.16) nm; ir (dry film) ν max 3324 (OH), 2973–2872 (CH, aliphatic), 1706 (C=O, ketone), 1595, 1514, 1437, 1289, 1217, 1184, 1084, 870, 756, 677 cm^{-1}; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 7.13 (1H, br s, OH), 7.05 (1H, br d, J=6.0 Hz, H-6), 6.55 (1H, br s, H-1), 6.39 (1H, d, J=7.2 Hz, H-7), 2.92 (1H, d, J=14.3 Hz, H-22), 2.51 (1H, m, H-20), 2.23 (3H, s, Me-23), 1.51 (3H, s, Me-25), 1.35 (3H, s, Me-26), 1.01 (3H, s, Me-28), 1.00 (3H, d, J=6.1 Hz, Me-30), 0.98 (3H, s, Me-27); ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 213.50 (s, C-21), 178.27 (s, C-2), 168.69 (s, C-8), 164.61 (s, C-10), 145.96 (s, C-3), 133.69 (d, C-6), 127.57 (s, C-5), 119.67 (d, C-1), 118.06 (d, C-7), 117.17 (s, C-4), 52.45 (t, C-22), 44.53 (s, C-14), 43.35 (d, C-18), 42.63 (s, C-9), 41.82 (d, C-20), 40.51 (s, C-13), 38.95 (q, C-25), 38.11 (s, C-17), 35.38 (t, C-16), 33.65 (t, C-11), 32.48 (q, C-28), 31.97 (t, C-19), 29.81 (t, C-12), 28.38 (t, C-15), 21.45 (q, C-26), 19.64 (q, C-27), 15.03 (q, C-30), 10.23 (q, C-23); eims (70 eV) *m/z* (% rel. int.) $[M]^+$ 420 (15), 406 (4), 241 (15), 202 (100), 201) (59), 187 (6), 163 (13), 135 (14), 121 (14), 95 (29), 69 (23), 41 (45).

*20-Hydroxy-20-*epi*-tingenone* [**2**].—Further purification of fraction F4-IV (10.1 g) by Si gel flash chromatography (EtOAc/toluene), gravity cc ($CHCl_3$/MeOH), and gel filtration (Sephadex^R LH-20, MeOH) led to the isolation of 20-hydroxy-20-*epi*-tingenone [**2**] (43 mg). Compound **2** was obtained as orange-red crystals (Me_2CO/$CHCl_3$): mp 194–196°; $[\alpha]^{25}D$ −23°; uv (MeOH) λ max (log ε) 209 (3.30), 242 (sh, 2.98), 288 (sh, 2.21), 421 (3.10) nm; ir (dry film) ν max 3351 (OH), 2977-2874 (CH, aliphatic), 1715 (C=O, ketone), 1593, 1514, 1439, 1287, 1186, 1084, 868, 750 cm^{-1}; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 7.02 (1H, br d, J=5.3 Hz, H-6), 6.53 (1H, br s, H-1), 6.37 (1H, d, J=6.4 Hz, H-7), 2.99 (1H, d, J=14.2 Hz, H-22), 2.22 (3H, s, Me-23), 1.46 (3H, s, Me-25), 1.35 (3H, s, Me-26), 1.35 (3H, s, Me-30), 1.12 (3H, s, Me-28), 0.88 (3H, s, Me-27); ^{13}C-nmr ($CDCl_3$ 90.8 MHz) δ 215.06 (s, C-21), 178.30 (s, C-2), 168.92 (s, C-8), 164.22 (s, C-10), 146.00 (s, C-3), 133.64 (d, C-6), 127.56 (s, C-5), 119.59 (d, C-1), 118.26 (d, C-7), 117.17 (s, C-4), 73.62 (s, C-20), 50.31 (t, C-22), 44.04 (s, C-14), 43.15 (d, C-18), 42.84 (s, C-9), 39.82 (s, C-13), 38.40 (q, C-25), 36.72 (t, C-19), 35.91 (s, C-17), 35.50 (t, C-16), 33.02 (t, C-11), 33.02 (q, C-28), 29.68 (t, C-12), 29.15 (t, C-15), 28.86 (q, C-30), 23.04 (q, C-26), 19.33 (q, C-27), 10.25 (q, C-23); eims (70 eV) *m/z* (% rel. int.) $[M]^+$ 436 (6), 422 (2), 241 (15), 202 (65), 189 (5), 175 (6), 149 (12), 83 (34), 55 (19), 42 (100).

Celastrol [**3**].—Fraction F5 (34 g) was purified by vacuum liquid cc (Si gel for tlc; 200 g; 10×6 cm; petroleum ether/$CHCl_3$ and $CHCl_3$/MeOH) to afford four major fractions, F5-I to F5-IV. Further purification F5-I by Si gel cc (petroleum ether/$CHCl_3$, then $CHCL_3$/MeOH) afforded 20-hydroxy-20-*epi*-

tingenone [**2**] (270 mg). Purification of fraction F5-II (5 g) by Si gel cc (petroleum ether/$CHCl_3$ and $CHCl_3$/MeOH), and reversed phase Si gel (RP8) cc [MeOH-H_2O(8:2)] afforded celastrol [**3**] (70 mg). Compound **3** was obtained as an amorphous solid: uv (MeOH) λ max (log ε) 207 (3.24), 244 (sh, 2.92), 288 (sh, 2.24), 424 (3.01); ir (dry film) ν max 3252 (br, OH), 2990–2880 (CH, aliphatic), 1703 (C=O), 1582, 1510, 1445, 1225, 870, 756 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 7.04 (1H, br d, J=6.8 Hz, H-6), 6.47 (1H, br s, H-1), 6.30 (1H, d, J=7.1 Hz, H-7), 2.48 (1H, d, J=16.1 Hz, H-19), 2.18 (3H, s, Me-23), 1.40 (3H, s, Me-25), 1.25 (3H, s, Me-26), 1.21 (3H, s, Me-30), 1.06 (3H, s, Me-28), 0.90 (1H, br d, J=13.7 Hz, H-22), 0.54 (3H, s, Me-27); ^{13}C nmr ($CDCl_3$, 75.4 MHz) see Table 2; eims (70 eV) *m/z* (% rel. int.) $[M]^+$ 450 (14), 436 (2), 281 (1), 269 (2), 267 (94), 265 (3), 253 (12), 241 (20), 239 (9), 227 (12), 215 (18), 202 (23), 201 (100), 200 (15), 135 (10), 121 (16), 109 (20).

Salaspermic acid [**4**].—Fraction F5-IV was suspended in $CHCl_3$. After filtration the residue material was crystallized from $CHCl_3$/MeOH to afford colorless crystals of salaspermic acid [**4**] (302 mg): mp 277–280°; $[\alpha]^{25}D$ +2° (*c*=0.1, MeOH); uv (MeOH) λ max 204 (end absorption); ir (KBr) ν max 3308 (OH), 2975 - 2872 (CH, aliphatic), 1696 (C=O, carboxyl), 1457, 1384, 1225, 1146, 1036 cm^{-1}; ^1H-nmr (pyridine-d_5; 300 MHz), δ 4.21 (H, d, J=7.8 Hz, H_a-24), 3.67 (1H, d, J=8.2 Hz, H_b-24), 2.64 (1H, br d, J=15.0 Hz, H-19), 2.48 (1H, br d, J=13.5 Hz, H-21), 2.32 (1H, td, J=13.5, 3.4 Hz, H-22), 2.16 (1H, dd, J=11.6, 4.9 Hz, H-2), 1.39 (3H, s, Me-30), 1.22 (3H, s, Me-26, 1.16 (3H, d, J=7.1 Hz, Me-23), 1.10 (3H, s, Me-28), 0.87 (3H, s, Me-25), 0.80 (3H, s, Me-27); ^{13}C nmr (pyridine-d_5, 90.8 MHz) see Table 2; eims (70 ev) *m/z* (% rel. int.) $[M]^+$ 472 (27), 442 (2), 395 (1), 289 (5), 235 (11), 191 (9), 155 (21), 125 (100), 55 (79).

Salaspermic acid methyl ester [**5**].—Salaspermic acid [**4**] (100 mg) was methylated with excess ethereal CH_2N_2 at room temperature. A portion of the dried crude methyl ester (56 mg) was purified by crystallization (MeOH) to afford colorless crystals of salaspermic acid methyl ester [**5**] (46 mg): ir (dry film) ν max (3493–3327 (OH), 2967–2865 (CH, aliphatic), 1721 (C=O), 1460, 1385, 1215, 1140, 999, 754, 667 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 4.14 (1H, d, J=8.1 Hz, H_a-24), 3.63 (3H, s, OMe), 3.57 (1H, d, J=8.5 Hz, H_b-24), 2.90 (1H, s, OH), 2.33 (1H, br d, J=13.7 Hz, H-19), 2.15 (1H, br d, J=13.7 Hz, H-21), 2.01 (1H, td, J=13.9, 3.9 Hz, H-22), 1.17 (3H, s, Me-30), 1.06 (3H, s, Me-28), 0.96 (3H, d, J=7.1 Hz, Me-23), 0.95 (3H, s, Me-25), 0.82 (3H, s, Me-26), 0.81 (3H, s, Me-27); ^{13}C nmr ($CDCl_3$, 75.4 MHz) see Table 2; eims (70 eV) *m/z* (% rel. int.) $[M]^+$ 486 (6), 375 (1), 319 (1), 289 (1), 249 (3), 223 (3), 191, (4), 169 (14), 125 (100), 121 (18), 109 (35), 95 (27), 81 (26), 55 (38).

Methyl 24-acetoxypolpunonate [**6**].—The remaining portion of crude salaspermic acid methyl ester (46 mg) was acetylated [Ac_2O-pyridine (1:1)] at 40° for 48 h. The product was purified by Si gel cc to afford a white amorphous solid of methyl 24-acetoxypolpunonate [**6**] (36 mg): uv (MeOH) λ max 209 (log ε 2.93); ir (dry film) ν max 2951–2872 (CH, aliphatic), 1732 (C=O, esters), 1715 (C=O, ketone), 1454, 1388, 1233, 1152, 1038, 754 (cm^{-1}). ^1H-nmr ($CDCl_3$ 300 MHz), δ 4.30 (1H, d, J=12.3 Hz, H_a-24), 4.07 (1H, d, J=12.3 Hz, H_b-24), 3.65 (2H, s, OCH_3), 2.53 (1H, m, H-2), 2.34 (1H, br d, J=13.4 Hz, H-19), 1.93 (3H, s, CH_3CO), 1.18 (3H, s, Me-30), 1.08 (3H, s, Me-28), 0.98 (3H, s, Me-25), 0.95 (3H, d, J=6.8 Hz, Me-23), 0.86 (3H, s, Me-26), 0.86 (3H, s, Me-27); ^{13}C nmr ($CDCl_3$, 75.4 MHz) see Table 2; eims (70 ev) *m/z* (% rel. int.) $[M]^+$ 528 (1), 496 (1), 468 (2), 395 (1), 332 (2), 271 (6), 223 (9), 191 (10), 189 (9), 169 (51), 163 (23), 149 (12), 137 (22), 109 (77), $[Ac]^+$ 43 (100).

CYTOTOXICITY ASSAYS.—P-388, human fibrosarcoma (HT-1080), human oral epidermoid carcinoma (KB), human epidermoid carcinoma (A431), and the human glioblastoma (U373) cell lines were purchased from the American Type Culture Collection, Rockville, Maryland. Other human cancer cell types, including breast (BC-1), colon (COL-2), lung (LU-1) cancers, and melanoma (MEL-2) were established from primary human tumors in the Specialized Cancer Center, University of Illinois College of Medicine at Chicago. The multidrug-resistant cell line, KB-V1, was supplied by Dr. Igor B. Roninson, Department of Genetics, University of Illinois College of Medicine at Chicago. It was developed from KB cells by treatment of the latter with sublethal doses of vinblastine over an extended period of time (20). The human hormone-dependent breast (ZR-75-1) and prostate (LNCaP) cancer cell lines were obtained from Glaxo Group Research, Greenford, United Kingdom.

The human breast (BC-1) and colon (COL-2) cancer cell lines were cultured and maintained in Eagle's Minimum Essential Medium with Earle's salts (MEME) supplemented with 1% non-essential amino acids (NAA) and heat-inactivated (56° for 30 min) fetal bovine serum (FBS). The fibrosarcoma (HT-1080) and lung (LU-1) cancer cell lines were maintained in MEME supplemented with 1% NAA and 10% heat-inactivated FBS. The oral epidermoid carcinoma (KB), KB-V1, and A431 cells were maintained in Dulbecco's modified Minimum Essential Medium (D-MEM) containing 10% heat-inactivated FBS. The medium for KB-V1 cells was also supplemented with 1 μg/ml of vinblastine. Melanoma (MEL-2) cells were cultured in Minimum Essential Medium with Hank's salts (MEMH), supplemented with 10% heat-inactivated FBS. Murine P-388 cells were maintained in Fishers's Medium containing 10% heat-inactivated FBS. The human hormone-dependent breast (ZR-75-1) and prostate (LNCaP) cell lines were cultured in

Rosewell Park Memorial Institute (RPMI) 1640 medium (phenol red-free) supplemented with 10% heat-inactivated hormone-free FBS, and 0.1 nM estradiol (ZR-75-1) or 0.1 nM testosterone (LNCaP). Human glioblastoma cells (U373) were maintained in the RPMI 1640 medium supplemented with10% heat-inactivated FBS. All media were supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), 0.25 μg/ml of fungizone (except media for P-388, and U373) and 1.5–3% of 7.5% $NaHCO_3$ solution, and the pHs were adjusted to 7.1–7.2. All cell lines except MEL-2 were maintained at 37° in a humidified atmosphere containing 5% CO_2 in air. Melanoma (MEL-2) cells were maintained in a closed tissue culture container.

Cytotoxicity assays were performed in 96-well microtiter plates, basically by a procedure described previously (21). Briefly, compounds were dissolved initially in DMSO; then serial dilutions were made, so that the final concentration of DMSO in each well was 0.5% v/v. Cultured cells were treated, in triplicate, with four concentrations (0.16–20 μg/ml) of test compounds. Treated cell cultures were incubated at 37° in a humidified atmosphere of 5% CO_2 in air (except MEL-2) for 72 h, or 48 h in the case of P-388 and HT-1080, and 96 h for U373 cells. Melanoma (MEL-2) cells were incubated at 37° for 72 h in the absence of CO_2. After the incubation period, the cells were fixed with cold 50% trichloroacetic acid (TCA) or 20% TCA for P-388 cells. Then the cells were stained with 0.4% w/v sulforhodamine B dye in 1% HOAc. Unbound dye was rinsed off with 1% HOAc, and protein-bound dye was solubilized with 10 mM unbuffered Tris base [tris (hydroxymethyl) aminomethane]. Protein (cell quantity) was measured by the determination of optical density at 515 nm with a BT 2000 MicroKinetics Reader (Bio-Tek Instruments, Inc.). The averaged data were expressed as percent relative to controls treated only with DMSO. The dose that inhibited cell growth by 50% (ED_{50}) was then determined. The results are presented in Table 1.

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