Cytotoxic Constituents of the Roots of *Ekmanianthe longiflora*

Sergio R. Peraza-Sánchez,[†] Daniel Chávez,[†] Hee-Byung Chai,[†] Young Geun Shin,[†] Ricardo García,[‡] Miliciades Mejía,[‡] Craig R. Fairchild,[§] Kate E. Lane,[§] Ana T. Menendez,[§] Norman R. Farnsworth,[†] Geoffrey A. Cordell,[†] John M. Pezzuto,[†] and A. Douglas Kinghorn*[,†]

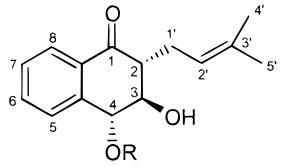
Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, Jardín Botánico Nacional "Dr. Rafael Moscoso", Santo Domingo, Dominican Republic, and Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543

Received October 20, 1999

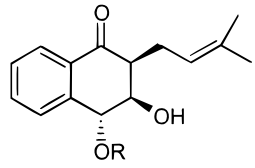
Bioactivity-directed fractionation of the $CHCl_3$ extract of the roots of *Ekmanianthe longiflora* resulted in the isolation of three new natural products, (2*R*,3*R*,4*R*)-3,4-dihydro-3,4-dihydroxy-2-(3-methyl-2-butenyl)-1(2*H*)-naphthalenone (**1**), (2*S*,3*R*,4*R*)-3,4-dihydro-3,4-dihydroxy-2-(3-methyl-2-butenyl)-1(2*H*)-naphthalenone (**2**), and (2*R**,3a*R**,9*R**,9a*R**)-9-hydroxy-2-(1-hydroxy-1-methylethyl)-2,3,3a,4,9,9a-hexahydronaphtho[2,3-*b*]furan-4-one (**3**), together with the known compounds 2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-quinone (**4**), 2-acetylnaphtho[2,3-*b*]furan-4,9-quinone (**5**), dehydro-*iso*-α-lapachone (**6**), α-lapachone (**7**), catalponol, and *epi*-catalponol. The structures of **1**−**3** were determined using a combination of NMR spectroscopic techniques, and the absolute configurations of compounds **1** and **2** were obtained using Mosher ester methodology. Compounds **4**−**6** showed significant cytotoxicity in a panel of human cancer cells. α-Lapachone (**7**) exhibited only marginal activity, and catalponol and *epi*-catalponol were inactive in this regard. When tested at 72 mg/kg/injection in an in vivo mouse P-388 leukemia system, compound **4** was inactive (110% T/C).

Ekmanianthe longiflora (Griseb.) Urb. (Bignoniaceae) is distributed in the Dominican Republic and Cuba. Known as "roble de Puerto Rico" or "roble real", this tree is abundant in dry-type forests that grow on flat, well-developed soils. Originally, *E. longiflora* was described as *Tecoma longiflora* Grisebach.[1] No previous reports of chemical constituents of this species have been published. However, plants of the genus *Tecoma* have been studied in some detail, with most of the studies concentrated on *T. stans*, for its content of flavonoids,[2] monoterpene alkaloids,[3] and indole derivatives.[4] In addition, *T. ipe*,[5] *T. capensis*,[6,7] and *T. grandiflora*[8] have proved to be rich sources of furanonaphthoquinones, iridoids and phenylpropanoid glucosides, and flavanone disaccharides, respectively. Preparations made from *T. stans* have been used in traditional medicine to treat diabetes, high blood pressure, and gastric pain.[9,10] Antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria and fungi has been reported for methanolic extracts of the leaves and stem bark of *T. stans*.[11]

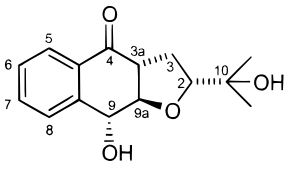
In a continuing search for novel, plant-derived anticancer agents, we have investigated a chloroform-soluble extract of the roots of *E. longiflora*, which showed significant cytotoxic activity against human breast cancer (BC1) and lung cancer (Lu1) cell lines. Chromatographic separation of this extract guided by these two cell lines yielded nine naphthoquinone analogues, with three being dihydronaphthoquinones of novel structure (**1**−**3**) and six of known composition, 2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-quinone (**4**), 2-acetylnaphtho[2,3-*b*]furan-4,9-quinone (**5**), dehydro-*iso*-α-lapachone (**6**), α-lapachone (**7**), catalponol, and *epi*-catalponol. All compounds were evaluated for their in vitro cytotoxicity in a small human-tumor cell-line panel, with compound **4** then being selected for more detailed in vitro evaluation and in vivo testing. The isolation and structural characterization of **1**−**3** and the biological evaluation of **4**−**7** are described in this paper.



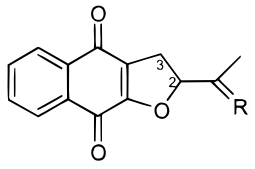
1 R = H
1s R = (*S*)-MTPA
1r R = (*R*)-MTPA



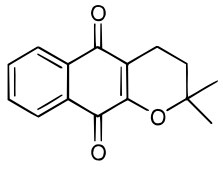
2 R = H
2s R = (*S*)-MTPA
2r R = (*R*)-MTPA



3



4 R = OH, H; $\Delta^{2,3}$
5 R = O; $\Delta^{2,3}$
6 R = CH_2



7

Results and Discussion

The initial bioactive chloroform-soluble extract of the roots of *E. longiflora* was fractionated using the BC1 and Lu1 human cancer cell lines to monitor cytotoxicity, result-

* To whom correspondence should be addressed. Tel.: (312) 996-0914. Fax: (312) 996-7107. E-mail: kinghorn@uic.edu.
[†] University of Illinois at Chicago.
[‡] Jardín Botánico Nacional "Dr. Rafael Moscoso".
[§] Bristol-Myers Squibb Pharmaceutical Research Institute.

10.1021/np990528l CCC: $19.00
Published on Web 03/10/2000

Table 1. ^{1}H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) Spectral Data of Compounds **1** and **2**[a]

position	δ_H (mult., *J* Hz)		δ_C (mult.)[b]	
	1	**2**	**1**	**2**
1			197.1 s	198.6 s
2	2.63 (td, 11.3, 4.6)	3.05 (td, 7.1, 3.3)	53.5 d	50.1 d
3	3.85 (dd, 11.3, 9.2)	4.24 (dd, 5.7, 3.3)	75.3 d	74.0 d
4	4.83 (d, 9.2)	4.83 (d, 5.7)	73.9 d	70.9 d
4a			142.5 s	141.4 s
5	7.77 (d, 7.8)	7.52 (dd, 7.0, 1.3)	126.1 d	129.4 d
6	7.63 (td, 7.5, 1.3)	7.58 (ddd, 7.5, 7.0, 1.3)	134.8 d	134.6 d
7	7.41 (t, 7.5)	7.40 (ddd, 7.5, 7.0, 1.5)	128.5 d	129.0 d
8	8.00 (dd, 7.8, 1.3)	7.97 (dd, 7.8, 1.1)	127.4 d	127.4 d
8a			131.4 s	131.5 s
1′	2.76 (m)	2.52 (t, 7.1)	24.7 t	24.1 t
2′	5.13 (t-sept, 7.2, 1.3)	5.20 (tt, 7.3, 1.3)	120.9 d	121.6 d
3′			134.9 s	134.6 s
4′	1.68 (s)	1.69 (s)	26.2 q	26.2 q
5′	1.64 (br)	1.60 (s)	18.3 q	18.2 q

[a] All assignments are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY). [b] Multiplicities determined by APT (s = C, d = CH, t = CH_2, q = CH_3).

ing in the isolation of three new metabolites, **1**−**3**. Additionally, six known compounds were isolated, and these were identified by comparison of their published ^{1}H and ^{13}C NMR spectral data as 2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-quinone (**4**),[12,13] 2-acetylnaphtho[2,3-*b*]furan-4,9-quinone (**5**),[12,13] dehydro-*iso*-α-lapachone (**6**),[14,15] α-lapachone (**7**),[16,17] catalponol,[18,19] and *epi*-catalponol.[19,20]

Compound **1** was isolated as a colorless oil. Its IR spectrum showed absorptions for hydroxyl (3467 cm^{-1}), carbonyl (1692 cm^{-1}), and aromatic (1603 cm^{-1}) functionalities. Compound **1** exhibited characteristic UV bands for a dihydronaphthoquinone nucleus (λ_{max} at 247 and 287 nm).[18] The HREIMS of **1** revealed a $[M]^+$ peak at *m*/*z* 246.1258 that corresponded to a molecular formula of $C_{15}H_{18}O_3$. The base peak at *m*/*z* 105 and a fragment at *m*/*z* 131 in the EIMS of **1** indicated the presence of an unsubstituted aromatic ring.[21] This finding was further confirmed in the ^{1}H NMR spectrum of **1** (Table 1) in which four signals were seen in the aromatic region at δ 7.77, 7.63, 7.41, and 8.00. In addition, the ^{1}H NMR spectrum showed two carbinol protons at δ 3.85 (H-3) and 4.83 (H-4), which were attributed to a vicinal diol according to their coupling constants (J = 9.2 Hz). A third aliphatic proton at δ 2.63 was coupled to H-3 and assigned to H-2. Inspection of the COSY spectrum confirmed this proposal and showed that H-2 was substituted with an isopropenyl moiety. Signals for an isopropenyl group were apparent in the ^{1}H NMR spectrum of **1** at δ 2.76, 5.13 (C=C*H*), 1.68 (Me), and 1.64 (Me), and a fragment peak observed at *m*/*z* 178 ($[M]^+$ − 68) in the EIMS was consistent with the presence of this functionality. Also, the loss of one hydroxyl group as water was indicated in the EIMS by a peak at *m*/*z* 228. The ^{13}C NMR spectrum showed only one carbonyl group signal at δ 197.1 and two signals for carbons bearing a hydroxyl group (δ 75.3 and 73.9), thereby confirming **1** to be a dihydronaphthoquinone derivative.[22]

The ^{13}C NMR data (Table 1) of compound **1** were very similar to those of (2*S*,3*S*,4*R*)-3,4-dihydro-3,4-dihydroxy-2-(3-methyl-2-butenyl)-1(2*H*)-naphthalenone, a known compound from *Oroxylum indicum* Vent. (Bignoniaceae),[23] but these compounds were slightly different in their ^{1}H NMR spectra. The *O. indicum* constituent exhibited the coupling pairs H-2/H-3 and H-3/H-4 oriented trans ($J_{2,3}$ = 8.1 Hz) and cis ($J_{3,4} \approx$ 3.0 Hz), respectively,[23] whereas the analogous coupling constants in compound **1** were observed as $J_{2,3}$ = 11.3 Hz and $J_{3,4}$ = 9.2 Hz, suggesting that both coupling pairs were arranged in a *trans*-diaxial configuration. In addition, in the NOESY spectrum of **1**, a strong cross-peak between H-2 and H-4 confirmed that these protons are on the same side of the molecule. The absolute configuration of one of the stereogenic centers in **1** was established using Mosher ester methodology.[24] Although there are two secondary carbinols in **1**, the hydroxyl group affixed to C-3 is the more sterically hindered. Thus, on treatment of **1** with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride, the mono-(*S*)-ester (**1s**) and the mono-(*R*)-ester (**1r**) derivatives at C-4 were obtained as the major products (see Experimental Section). Analysis of the $\Delta\delta_{H(S-R)}$ data (Table 2) showed a negative chemical shift difference for H-2, H-3, and the isopropenyl moiety. Consequently, the absolute configuration at C-4 was established as *R*, and because of the trans arrangements of the pairs H-2/H-3 and H-3/H-4, the absolute configurations at C-2 and C-3 could also be determined as *R*. In this way, compound **1** was established as (2*R*,3*R*,4*R*)-3,4-dihydro-3,4-dihydroxy-2-(3-methyl-2-butenyl)-1(2*H*)-naphthalenone.

Table 2. Partial ^{1}H NMR Spectral Data of the Mono-(*S*)- and -(*R*)-Mosher Esters of **1** and **2**[a]

proton	δ_H **1s**	δ_H **1r**	$\Delta\delta_{S-R}$	δ_H **2s**	δ_H **2r**	$\Delta\delta_{S-R}$
H-5				7.61	7.56	+0.05
H-4	6.39	6.42	4 *R*[b]	6.29	6.30	4 *R*[b]
H-3	4.03	4.09	−0.06	4.39	4.47	−0.08
H-2	2.73	2.76	−0.03	2.79	2.94	−0.15
H_a-1′	2.73	2.76	−0.03	2.67	2.70	−0.03
H_b-1′	2.73	2.76	−0.03	2.42	2.50	−0.08
H-2′	5.10	5.16	−0.06	5.04	5.16	−0.12
H-4′	1.65	1.68	−0.03	1.72	1.73	−0.01
H-5′	1.65	1.70	−0.05	1.65	1.78	−0.03

[a] Obtained in $CDCl_3$ at 300 MHz. [b] Absolute configuration.

The molecular formula of **2** was established as $C_{15}H_{18}O_3$ by HREIMS. Analysis of the EIMS, IR, and ^{1}H and ^{13}C NMR spectra of **2** suggested that it was a stereoisomer of **1** (Table 1). The coupling constants between H-2/H-3 (3.3 Hz) and H-3/H-4 (5.7 Hz) were smaller compared to those of **1**, indicating that H-2/H-3 were cis oriented and H-3/H-4 were trans oriented. The relative stereochemistry of **2** was corroborated by the NOESY spectrum. H-2 showed an NOE correlation with H-3, but not with H-4, and an NOE cross-peak was observed between H-3 and H-4. The absolute configurations of the stereogenic centers of **2** were determined by analysis of the ^{1}H NMR data of the (*S*)- and (*R*)-mono-Mosher ester derivatives **2s** and **2r** (Table 2), in a manner similar to the procedure described for **1**. The negative values ($\Delta\delta_{S-R}$) obtained for H-2, H-3, and the isopropenyl moiety, and the positive difference for H-5 showed that the absolute stereochemistry of the chiral center at C-4 was *R*. Thus, the absolute stereochemistry for C-2 and C-3 was deduced as *S* and *R*, respectively. Therefore, compound **2** was assigned as (2*S*,3*R*,4*R*)-3,4-dihydro-3,4-dihydroxy-2-(3-methyl-2-butenyl)-1(2*H*)-naphthalenone.

Compound **3** was obtained as an optically inactive colorless oil. The ^{13}C NMR and APT spectra displayed 15 carbons as two methyls, one methylene, eight methines, and four quaternary carbons. The HREIMS of **3** showed the molecular ion at *m*/*z* 262.1192, consistent with the elemental formula $C_{15}H_{18}O_4$ and seven unsaturated sites. The presence of hydroxyl and carbonyl groups in **3** was immediately evident from its IR spectrum (3472, 1690 cm^{-1}). Considering that **3** was obtained as a colorless oil and did not show a UV spectrum characteristic of a naphthoquinone skeleton, it was apparent that **3** was a

Table 3. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Spectral Data of Compound **3**[a]

proton	δ_H (mult., *J* Hz)	carbon	δ_C (mult.)[b]
H-2	3.62 (dd, 4.4, 12.0)	C-2	74.2 d
H_a-3	2.47 (dt, 4.4, 12.8)	C-3	28.5 t
H_b-3	1.69 (dd, 12.0, 12.8)	C-3a	49.2 d
H-3a	2.51 (td, 4.1, 11.6)	C-4	196.2 s
H-5	8.00 (dd, 1.3, 7.8)	C-4a	130.9 s
H-6	7.42 (tt, 1.0, 7.4, 7.8)	C-5	127.1 d
H-7	7.66 (td, 1.3, 7.4, 7.8)	C-6	128.4 d
H-8	7.83 (d, 7.8)	C-7	135.0 d
H-9	4.88 (d, 9.0)	C-8	126.8 d
H-9a	3.66 (dd, 9.0, 11.6)	C-8a	142.7 s
Me-11	1.19 (s)	C-9	72.3 d
Me-12	1.38 (s)	C-9a	75.1 d
OH-9	3.23 (br)	C-10	76.2 s
OH-10	3.82 (br)	C-11	28.2 q
		C-12	16.9 q

[a] All assignments are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY). [b] Multiplicities determined by APT (s = C, d = CH, t = CH_2, q = CH_3).

dihydronaphthoquinone derivative with the conjugated ketone system absent.[22] This inference was supported by the ^{13}C NMR spectrum, in which only one carbonyl carbon was observed at δ 196.2 (C-4). An aromatic unsubstituted moiety in the molecule was indicated from the ^{1}H and ^{13}C NMR spectra of **3** (Table 3). The EIMS showed a peak at *m/z* 204 ($[M]^+ - 58$), compatible with the presence of a 1-hydroxy-1-methylethyl moiety attached to the naphthoquinone skeleton. This was also consistent with the ^{13}C NMR spectrum, in which the tetrasubstituted C-10 carbon (δ 76.2) was judged to be attached to one hydroxyl (δ 3.82) and two methyl groups (δ 28.2 and δ 16.9). The HMBC spectrum of **3** displayed a long-range correlation between the aromatic carbon C-8a (δ 142.7) and H-9 (δ 4.88), revealing the site of bonding of the second hydroxyl group (δ 3.23) to be at C-9. A fourth oxygen was positioned as part of a tetrahydrofuran ring. Signals in the ^{1}H NMR spectrum of **3** for two oxymethine resonances at δ 3.62 (H-2) and 3.66 (H-9a) and for a methylene group [δ 2.47 (H_a-3) and 1.69 (H_b-3)] were consistent with a tetrahydrofuran moiety. Placement of the 1-hydroxy-1-methylethyl unit at C-2 of the tetrahydrofuran ring was determined based on the HMBC associations between H-2 (δ 3.62) and C-10 (δ 76.2). Signals for the protonated aromatic carbons were assigned on the basis of the HMQC spectrum, while connectivities obtained from HMBC data enabled assignment of all the quaternary carbon shifts. The relative stereochemistry of **3** was elucidated from the relevant coupling constants and NOE correlations. Thus, the values of $J_{3a,9a}$ = 11.6 Hz and $J_{9,9a}$ = 9.0 Hz suggested that each pair of protons (H-3a/H-9a and H-9/H-9a) occurs in a *trans*-diaxial orientation. In the NOESY spectrum, cross-peaks were observed between H-3a (δ 2.51) and H-9 (δ 4.88) and between H-3a and H-2 (δ 3.62). Due to the fact that **3** was isolated as a racemate ($[\alpha]^{20}_D$ 0°), the absolute stereochemistry is not reported. Thus, **3** is the new compound (2*R**,3a*R**,9*R**,9a*R**)-9-hydroxy-2-(1-hydroxy-1-methylethyl)-2,3,3a,4,9,9a-hexahydronaphtho[2,3-*b*]furan-4-one.

Compounds **4**−**6** exhibited broad cytotoxic activity in the range 0.2−4.0 μg/mL against a panel of nine human cancer cell lines and one murine cell line, while α-lapachone (**7**) showed marginal activity against the SW626 (ovarian adenocarcinoma) cell line (3.0 μg/mL) (Table 4). Compounds **1**−**3** and the other two known compounds, catalponol and *epi*-catalponol, were noncytotoxic, probably due to the absence of an α,*β*-unsaturated quinone system in their molecules.

Compound **4** was further evaluated for cytotoxicity in a 25 cell-line Oncology Diverse Cell Assay, representing a diverse group of mouse and human tumors, fibroblasts, and normal bovine endothelial cells.[25] It was found to be significantly active, exhibiting a mean IC_{50} value of 2.9 μM. Compound **4** was also evaluated in an in vivo mouse P-388 leukemia system (injected intraperitoneally).[26] When tested at 72 mg/kg/injection, administered intraperitoneally, compound **4** was inactive (T/C value of 110%).

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. IR spectra were obtained with an ATI Mattson Genesis Series FT-IR spectrophotometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded at room temperature on a Bruker DPX-300 instrument with tetramethylsilane (TMS) as internal standard. COSY, APT, HMBC, HMQC, and NOESY NMR experiments were run using the manufacturer's software. EIMS and HREIMS were obtained on a Finnigan MAT 90 instrument. Column chromatography was carried out with Si gel G (Merck, 70−230 and 230−400 mesh). Thin-layer chromatography (TLC) was performed on precoated 0.25-mm thick Merck Si gel 60 F_{254} aluminum plates. TLC plates were dipped into a 5% (v/v) aqueous solution of H_2SO_4, containing 4% (w/v) phosphomolybdic acid and 0.5% (w/v) ceric ammonium sulfate, and heated at 120 °C on a hot plate to visualize the spots. All solvents were double-distilled prior to use.

Plant Material. Roots of *E. longiflora* were collected in December 1995, in a dry-type forest, 4 km east of Cabral, Barahona Province, Dominican Republic, and identified by one of us (R. G.). Voucher specimens representing this collection are deposited in the Field Museum of Natural History, Chicago, IL, under the reference numbers 2169789 and 2169790.

Extraction and Isolation. The roots (423 g) of *E. longiflora* were air-dried, ground, and extracted with MeOH (3 × 1.8 L) in a percolator. The extracts were concentrated in vacuo and combined to give a residue (61.4 g), which was submitted to solvent partitioning. Three fractions were obtained after removal of solvent: petroleum ether (3.65 g), $CHCl_3$ (10.06 g), and EtOAc (3.76 g). Only the $CHCl_3$ extract showed significant

Table 4. Cytotoxicity of Compounds **4**−**7** Isolated from *E. longiflora*

	cell line[a,b]									
compound	BC1	Lu1	Col2	KB	KB−V (+VLB)	KB−V (−VLB)	LNCaP	SW626	SKNSH	M109
4	3.2	0.2	1.8	0.5	1.2	2.0	3.5	0.3	4.0	1.8
5	0.4	0.3	0.4	0.2	0.9	0.9	0.2	0.2	0.8	0.9
6	1.0	0.2	0.2	0.2	0.3	0.5	0.2	0.3	0.2	0.4
7	6.7	5.3	9.9	13.0	8.3	12.9	7.1	3.0	5.1	9.0

[a] Key to cell lines (human unless stated): BC1 = breast cancer, Lu1 = lung cancer, Col2 = colon cancer, KB = oral epidermoid carcinoma, KB−V (+VLB) = multidrug-resistant KB assessed in the presence of vinblastine (1 μg/mL), KB−V (−VLB) = multidrug-resistant KB assessed in the absence of vinblastine, LNCaP = prostate cancer, SW626 = ovarian cancer, SKNSH = neuroblastoma, M109 = mouse lung carcinoma. [b] Results are expressed as ED_{50} values (μg/mL) and were obtained using standard protocols.[27] Compounds showing values ≤ 4.0 μg/mL are considered active.

cytotoxic activity against the BC1 (ED_{50}, 2.5 μg/mL) and Lu1 (ED_{50}, 3.4 μg/mL) cell lines.

The $CHCl_3$ extract (9.98 g) was subjected to fractionation using a Si gel column (436 g of Si gel, 70−230 mesh) and eluted with a gradient of increasing polarity with hexane−$CHCl_3$−EtOAc, resulting in the collection of 20 fractions (F1−F20). Active fraction F14 (BC1, 4.1 μg/mL) was subjected to Si gel column chromatography [70−230 mesh, eluent: $CHCl_3$−EtOAc (8:2)] to afford three new compounds: **1** (16.0 mg), **2** (36.8 mg), and **3** (9.5 mg). By crystallization from a solution in MeOH of fractions F10 (BC1, 1.8 μg/mL) and F11 (BC1, 1.3 μg/mL), compound **4** was obtained (138.0 mg). Purification of fraction F8 (BC1, 3.2 μg/mL), using $CHCl_3$−acetone (99:1) to elute the column, yielded **5** (15.6 mg), catalponol (64.0 mg), and *epi*-catalponol (23.9 mg). Further fractionation over a Si gel column of F4 (BC1, 4.6 μg/mL), eluting with hexane−$CHCl_3$ (7:3), yielded **6** (48.8 mg). Finally, purification of F6 (BC1, 4.9 μg/mL), using hexane−$CHCl_3$ (2:8) as the solvent system, afforded **7** (172.0 mg).

(2*R*,3*R*,4*R*)-3,4-Dihydro-3,4-dihydroxy-2-(3-methyl-2-butenyl)-1(2*H*)-naphthalenone (1): colorless oil; $[\alpha]^{20}_D$ −5.4° (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (4.21), 247 (4.03), 287 (3.24) nm; IR (film) ν_{max} 3467, 3070, 2970, 2912, 2855, 1692, 1603 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* $[M]^+$ 246 (32), 228 (21), 199 (33), 178 (43), 160 (61), 144 (42), 134 (40), 131 (64), 105 (100); HREIMS *m*/*z* 246.1258 (calcd for $C_{15}H_{18}O_3$ 246.1256).

(2*S*,3*R*,4*R*)-3,4-Dihydro-3,4-dihydroxy-2-(3-methyl-2-butenyl)-1(2*H*)-naphthalenone (2): colorless oil; $[\alpha]^{20}_D$ +9.1° (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 252 (3.99), 284 (3.47); IR (film) ν_{max} 3436, 2969, 2912, 1673, 1601, cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* $[M]^+$ 246 (65), 228 (9), 213 (17), 199 (21), 195 (22), 185 (27), 178 (22), 173 (40), 172 (51), 160 (41), 159 (85), 144 (25), 134 (30), 131 (64), 115 (22), 105 (100); HREIMS *m*/*z* 246.1253 (calcd for $C_{15}H_{18}O_3$ 246.1256).

(2*R,3a*R**,9*R**,9a*R**)-9-Hydroxy-2-(1-hydroxy-1-methylethyl)-2,3,3a,4,9,9a-hexahydronaphtho[2,3-*b*]furan-4-one (3):** colorless oil; $[\alpha]^{20}_{\lambda}$ 0° (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ϵ) 252 (3.95), 282 (3.50), 294 (sh) (3.44), 335 (3.10); IR (film) ν_{max} 3472, 2974, 2930, 2870, 1690, 1600 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; EIMS (70 eV) *m*/*z* $[M]^+$ 262 (1), 258 (3), 222 (5), 204 (48), 202 (17), 186 (96), 173 (32), 161 (60), 160 (65), 158 (54), 157 (100), 147 (42), 134 (33), 131 (56), 115 (49), 105 (64); HREIMS *m*/*z* 262.1192 (calcd for $C_{15}H_{18}O_4$ 262.1205).

Preparation of Mono-(*S*)- and Mono-(*R*)-MTPA Ester Derivatives of 1 and 2. To a solution of **1** or **2** (1.9 mg in 0.5 mL of $CHCl_3$) were added sequentially pyridine (100 μL), 4-(dimethylamino)-pyridine (0.5 mg), and *R*-(−)α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (8.0 μL). Each mixture was heated at 50 °C for 4 h under N_2 and was then passed through a disposable Pasteur pipet (0.6 × 5 cm) packed with Si gel and eluted with 5 mL of $CHCl_3$. Solvent was removed in vacuo, and each residue was subjected to preparative TLC to give the purified mono-(*S*)-Mosher ester **1s** (1.6 mg) or **2s** (1.7 mg). Treatment of **1** or **2** (1.9 mg) with (*S*)-(+)α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride as described above yielded the mono-(*R*)-Mosher ester **1r** (1.7 mg) or **2r** (1.5 mg) (^1H NMR data, Table 2).

Bioassay Evaluation Procedures. All nine compounds obtained in this investigation were evaluated for cytotoxic activity against a panel of human cancer cell lines according to established protocols,[27] and the results for the four compounds (**4**−**7**) that exhibited significant cytotoxicity in one or more cell lines are reported in Table 4.

Oncology Diverse Cell Assays. Compound **4** was assessed in more detail for cytotoxicity at Brystol-Myers Squibb in a panel of 25 tumor cell lines, using an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium, inner salt] assay,[28] according to a previously described procedure.[25]

In Vivo Evaluation of Compound 4. To evaluate the therapeutic effectiveness of compound **4**, the P-388 leukemia model was used.[26] Briefly, each of six female CDF1 mice was injected intraperitoneally with 10^6 P-388 tumor cells in 0.5 mL of RMPI-1X medium (GIBCO Life Technologies, Grand Island, NY). One day later, compound **4** was injected at a dose of 72 mg/kg i.p. every day for 5 days, and the therapeutic effectiveness was determined based on survival time of the mice. The therapeutic effectiveness of compound **4** was also tested at two lower doses (8 and 24 mg/kg/injection), but the highest dose used (72 mg/kg/injection) was determined as the probable maximum tolerated dose, based on an approximately 20% body weight loss observed after dosing. Survival time is expressed as % T/C value (median survival time of test mice/median survival time of control mice × 100%). Threshold activity is deemed significant for T/C ≥ 125%.

Acknowledgment. This investigation was supported by grant U19-CA52956 from the National Cancer Institute, NIH, Bethesda, MD. We wish to acknowledge Mr. Richard B. Dvorak, College of Pharmacy, University of Illinois at Chicago (UIC), for the mass spectral data, and the Research Resources Center, UIC, for the provision of spectroscopic equipment used in this investigation. We also thank Ms. Laurie Cornell, Ms. Kathy Johnson, Ms. Judy Macbeth, and Mr. Russ Peterson, for conducting the 25 cell-panel cytotoxicity assays, and Ms. Michele Arico and Ms. Christine Burke, for their technical expertise in the in vivo experiments, conducted at Bristol-Myers Squibb. S.R.P.-S. thanks the Consejo Nacional de Ciencia y Tecnología (CONACYT) from Mexico for a graduate student scholarship.

References and Notes

(1) Gentry, A. H. In *Flora Neotropica*; The New York Botanical Garden: Bronx, New York, 1992; Vol. 25 (II), pp 42−47.
(2) Shrivastava, B. K.; Reddy, M. V. R. K. *Asian J. Chem*. **1995**, *7*, 679−680.
(3) Lins, A. P.; Felicio, J. D. *Phytochemistry* **1993**, *34*, 876−878.
(4) Kunapuli, S. P.; Vaidyanathan, C. S. *Phytochemistry* **1984**, *23*, 1826−1827.
(5) Nagata, K.; Hirai, K.-I.; Koyama, J.; Wada, Y.; Tamura, T. *Antimicrob. Agents Chemother*. **1998**, *42*, 700−702.
(6) Guiso, M.; Marra, C.; Piccioni, F.; Nicoletti, M. *Phytochemistry* **1997**, *45*, 193−194.
(7) Bianco, A.; Passacantilli, P.; Righi, G. *J. Nat. Prod*. **1983**, *46*, 314−319.
(8) Ahmad, M.; Jain, N.; Kamil, M.; Ilyas, M. *J. Chem. Res., Synop*. **1991**, 109.
(9) Dimayuga, R. E.; Agundez, J. *J. Ethnopharmacol*. **1986**, *17*, 183−193.
(10) Winkelman, M. *Med. Anthropol*. **1989**, *11*, 255−268.
(11) Binutu, O. A.; Lajubutu, B. A. *Afr. J. Med. Sci*. **1994**, *23*, 269−273.
(12) Rao, M. M.; Kingston, D. G. I. *J. Nat. Prod*. **1982**, *45*, 600−604.
(13) Zani, C. L.; De Oliveira, A. B.; De Oliveira, G. G. *Phytochemistry* **1991**, *30*, 2379−2381.
(14) Joshi, K. C.; Prakash, L.; Singh, P. *Phytochemistry* **1973**, *12*, 942−943.
(15) Inoue, K.; Chen, C.-C.; Inouye, H. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2764−2770.
(16) Inouye, H.; Okuda, T.; Hayashi, T. *Chem. Pharm. Bull*. **1975**, *23*, 384−391.
(17) Duarte-Weinberg, M. de L.; Gottlieb, O. R.; De Oliveira, G. G. *Phytochemistry* **1976**, *15*, 570.
(18) Inouye, H.; Okuda, T.; Hayashi, T. *Tetrahedron Lett*. **1971**, 3615−3618.
(19) Inouye, H.; Hayashi, T.; Shingu, T. *Chem. Pharm. Bull*. **1975**, *23*, 392−399.
(20) Inoue, K.; Inouye, H.; Taga, T.; Fujita, R.; Osaki, K.; Kuriyama, K. *Chem. Pharm. Bull*. **1980**, *28*, 1224−1229.
(21) Bowie, J. H.; Cameron, D. W.; Williams, D. H. *J. Am. Chem. Soc*. **1965**, *87*, 5094−5099.
(22) Fujiwara, A.; Mori, T.; Iida, A.; Ueda, S.; Hano, Y.; Nomura, T.; Tokuda, H.; Nishino, H. *J. Nat. Prod*. **1998**, *61*, 629−632.
(23) Kizu, H.; Habe, S.; Ishida, M.; Tomimori, T. *Yakugaku Zasshi* **1994**, *114*, 492−513.
(24) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc*. **1991**, *113*, 4092−4096.
(25) Cui, B.; Lee, Y. H.; Chai, H.-B.; Tucker, J. C.; Fairchild, C. R.; Raventos-Suarez, C.; Long, B.; Lane, K. E.; Menendez, A. T.; Beecher, C. W. W.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod*. **1999**, *62*, 1545−1550.
(26) Marsh, J. C.; Shoemaker, R. H.; Suffness, M. *Cancer Treat. Rep*. **1985**, *69*, 683−685.
(27) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M.; Ruangrungsi, N. *J. Nat. Prod*. **1993**, *56*, 30−38.
(28) Buttke, T. M.; McCubrey, J. A.; Owen, T. C. *J. Immunol. Meth*. **1993**, *157*, 233−240.

NP990528L