New Compounds with DNA Strand-Scission Activity from the Combined Leaf and Stem of *Uvaria hamiltonii*

Li Huang,[†] Monroe E. Wall,[*,†] Mansukh C. Wani,[*,†] Hernán Navarro,[†] Thawatchai Santisuk,[‡] Vichai Reutrakul,[§] Eun-Kyoung Seo,[⊥] Norman R. Farnsworth,[⊥] and A. Douglas Kinghorn[⊥]

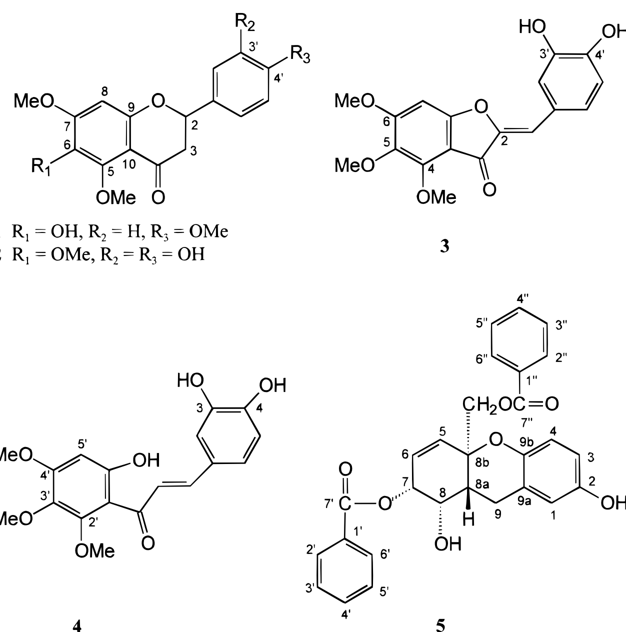
Chemistry and Life Sciences Group, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709, Royal Forest Herbarium, Bangkok 10900, Thailand, Department of Chemistry, Mahidol University, Bangkok 10400, Thailand, and Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

Received July 29, 1997

Two flavanones, hamiltones A (**1**) and B (**2**), an aurone, hamiltrone (**3**), a chalcone, hamilcone (**4**), and a tetrahydroxanthene, hamilxanthene (**5**), were isolated from *Uvaria hamiltonii* extracts guided initially by fractionation based on DNA strand-scission and/or 9KB cytotoxicity assays. Compounds **2**−**5** have not been reported previously, while **1** is new as a natural product. Structural assignments were made based on extensive spectroscopic measurements. Compounds **1**−**3** were inactive in the 9KB cytotoxicity assay, with compounds **4** and **5** having weak activity. In the DNA strand-scission assay, **3** was the most active compound found in the DNA strand-scission assay, being 10 times more potent than **1** or **2**. Compound **4** was only weakly active, and **5** was inactive.

In the course of our continuing search for natural products with potential for use in cancer chemotherapy, four flavonoids, of which three are new, were isolated from the combined leaf and stem extracts of *Uvaria hamiltonii* Hook. f et Th. (Annonaceae) by bioactivity-guided fractionation. In addition, a nonflavonoid compound was also isolated with the novel structure of a highly substituted analogue of Δ^5-tetrahydroxanthene. These compounds comprised the two flavanones, hamiltone A (6-hydroxy-5,7,4′-trimethoxyflavanone) (**1**) and hamiltone B (3′,4′-dihydroxy-5,6,7-trimethoxyflavanone) (**2**); the aurone, hamiltrone (3′,4′-dihydroxy-4,5,6-trimethoxyaurone) (**3**); the chalcone, hamilcone (3,4-dihydroxy-2′,3′,4′-trimethoxychalcone) (**4**), and the novel tetrahydroxanthene, hamilxanthene (**5**). The structures of compounds **1**−**5** were obtained by extensive NMR, MS, and UV analysis.

U. hamiltonii was collected in Thailand in 1993. There are few references to the ethnopharmacology or specific constituents of *Uvaria* species found in Thailand,[1−3] and only one paper referring specifically to flavonoids.[4] On the other hand, there is available considerable literature dealing with many aspects of the ethnopharmacology, biology, and constituents of East African *Uvaria* species. A number of references to specific types of flavonoids found in African *Uvaria* include chalcones and condensed chalcones;[5] dihydro- and benzyldihydro-chalcones;[6−12] and flavanones and benzylated flavanones.[13−18]



1 R_1 = OH, R_2 = H, R_3 = OMe
2 R_1 = OMe, R_2 = R_3 = OH

Results and Discussion

Compound **1** was isolated as a yellow solid. Although compound **1** was previously reported as a synthetic flavanone,[19] it was obtained as a natural product in the present investigation. The elemental formula of **1** was deduced as $C_{18}H_{18}O_6$ by HREIMS, which was in accord with a flavanone with one hydroxyl and three methoxyl substituents. The UV absorption at 225, 276, and 336 nm suggested a flavanone skeleton.[20] Its ^{1}H NMR signals at δ 2.76 (1H, dd, J = 2.9, 16.8 Hz), 3.04 (1H, dd, J = 13.3, 16.8 Hz), and 5.34 (1H, dd, J = 2.9, 13.3 Hz) were attributed to H_2-3 and H-2, respectively. The presence of two sets of doublets with an A_2B_2 splitting pattern in the aromatic region revealed that ring B had

* To whom correspondence should be addressed. Tel.: (919) 541-6672 (Wall); (919) 541-6685 (Wani). FAX: (919) 541-6499 (Wall and Wani).
† Research Triangle Institute.
‡ Royal Forest Herbarium.
§ Mahidol University.
⊥ University of Illinois at Chicago.

S0163-3864(97)00360-1 CCC: $15.00 © 1998 American Chemical Society and American Society of Pharmacognosy
Published on Web 03/19/1998

Table 1. ^{13}C NMR Spectral Data of Compounds **1**−**4**

position	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3-CD_3OD$)	**4** ($CDCl_3-CD_3OD$)
1				127.3
2	79.2	79.1	146.5	114.3
3	45.3	45.1		144.8
4			151.6	147.0
5	145.9	154.0	136.7	115.2
6	133.8	137.3	162.0	122.2
7	153.9	160.2	90.9	
8	96.3	96.5	164.1	
9	157.3	160.0	107.6	
10	108.4	108.8		
α				122.8
β				144.3
Ph−CH=			113.6	
1′	130.7	130.6	124.7	108.5
2′	127.7	113.4	118.2	154.7
3′	114.2	144.7	145.2	135.0
4′	159.9	144.2	147.6	159.5
5′	114.2	115.4	115.8	96.1
6′	127.7	119.0	125.4	161.6
C=O	189.7	191.0	181.6	192.8
OMe-4			62.3	
OMe-5	61.8	61.7	61.8	
OMe-6		61.4	56.8	
OMe-7	56.3	56.2		
OMe-2′				61.5
OMe-3′				60.9
OMe-4′	55.4			55.7

a 4′-substituent. Ring A had only one unsubstituted aromatic carbon as indicated by the ^{1}H NMR signal at δ 6.37 (s). The EIMS fragments at *m*/*z* 196 and 134 (retro-Diels−Alder fragments)[21] further suggested that one hydroxyl and two methoxyl groups were located in ring A, and one methoxyl was in ring B. In addition, the HMBC (Table 2) and HMQC NMR spectral data showed that the hydroxyl carbon in ring A was flanked by two methoxyl carbon atoms. Therefore, compound **1** was determined to be 6-hydroxy-5,7,4′-trimethoxyflavanone. The ^{13}C NMR assignments (Table 1) and ^{1}H NMR data are in good agreement with those reported for 6-hydroxy-5,7-dimethoxyflavanone derivatives.[22] The optical rotation of compound **1** was positive. A CD spectrum would be desirable to determine configuration of **1**,[23] but the very limited supply was exhausted. Its absolute configuration at C-2 has not been determined.

Similar to **1**, compound **2** displayed the characteristic UV spectrum of a flavanone.[20] The HREIMS gave a molecular ion at *m*/*z* 346.1055, which corresponded to the formula $C_{18}H_{18}O_7$. The EIMS fragment peaks observed at *m*/*z* 210 and 136 suggested that compound **2** had three methoxyl groups in ring A and two phenolic hydroxyl groups in ring B.[21] The ^{1}H NMR signals at δ 6.30 (s), 6.94 (d, J = 1.7 Hz), 6.88 (d, J = 8.1 Hz), and 6.80 (dd, J = 1.7, 8.1 Hz) were attributable to the protons at C-8, C-2′, C-5′, and C-6′, respectively. Moreover, the ^{13}C NMR (Table 1) and the HMQC and HMBC data (Table 2) of **2** supported the structure 3′,4′-dihydroxy-5,6,7-trimethoxyflavanone. Unlike compound **1**, which was optically active, compound **2** was obtained as a racemic mixture.

Isolated as a yellow solid, compound **3** had a UV spectrum with major peaks at 257, 331, and 405 nm, which was similar to those of aurones.[20] The HREIMS showed a molecular ion at *m*/*z* 344.0894, which corresponded to a molecular formula of $C_{18}H_{16}O_7$. The ^{1}H NMR signals at δ 4.23, 3.82, and 4.00, which were correlated to the ^{13}C NMR signals at δ 62.3 (C-4), 61.8 (C-5), and 56.8 (C-6), respectively, in its HMQC NMR spectrum, suggested the presence of three methoxyl groups. Therefore, this aurone compound must have dihydroxy-, trimethoxy-substitutions. The ^{1}H NMR signal at δ 6.70 (s) and the ^{13}C NMR resonance at δ 113.6 and 146.5 were assignable to a *cis*-olefinic group.[24] The signals at δ 7.46 (d, J = 2.0 Hz, H-2′), 6.89 (d, J = 8.4 Hz, H-5′), and 7.31 (dd, J = 2.0, 8.4 Hz, H-6′) were attributable to the protons at 3′,4′-dihydroxy-substituted aromatic ring, which was confirmed further by the HMBC correlation between the olefinic proton at δ 6.70 and ^{13}C NMR signals at δ 124.7 (C-1′) and 118.2 (C-2′). Moreover, the cross-peaks in the HMBC spectrum between the three methoxyl protons and their connected aromatic carbons (C-4, δ 151.6; C-5, δ 136.7; and C-6, δ 162.0), and between H-7 (δ 6.59) and carbons at δ 136.7 (C-5), δ 164.1 (C-8), and δ 107.6 (C-9) (Table 2), established the structure of **3** as 3′,4′-dihydroxy-4,5,6-trimethoxyaurone. The NOE correlation between the OMe-6 and H-7 also allowed for the unambiguous assignment of protons and carbons on the trimethoxyl aromatic system.

Compound **4**, which was isolated as yellow needles, displayed a characteristic chalcone UV spectrum with absorptions at 267, 316, and 387 nm.[20] The ^{1}H NMR signal at δ 13.76 (s) was due to a chelated phenolic hydroxyl adjacent to the carbonyl group. In addition, ^{1}H NMR signals at δ 7.73 (H-β) and 7.80 (H-α) with a large coupling constant (J = 15.5 Hz) and ^{13}C NMR signals at δ 122.8 (C-α) and 144.3 (C-β) could be assigned to a *trans*-α,β-olefinic moiety. The remaining ^{1}H and ^{13}C NMR signals (Table 1), plus the HMQC and HMBC (Table 2) patterns, were found to resemble those of compound **3** due to the presence of six oxygenated aromatic carbon atoms and three methoxyl groups. Moreover, the overall ^{1}H NMR data were found to relate closely to those reported for 6′-hydroxy-2′,3′-4′-trimethoxy-3,4-methylenedioxychalcone.[25] Therefore, the structure of 3,4,6′-trihydroxy-2′,3′,4′-trimethoxychalcone was assigned to **4**, in agreement with the molecular formula $C_{18}H_{18}O_7$ deduced by HREIMS.

The molecular formula of compound **5** was established as $C_{28}H_{24}O_7$ by HREIMS. Its IR spectrum showed ester carbonyl and hydroxyl absorptions at 1719 and 3585 cm^{-1}, respectively. The ^{1}H NMR of **5** indicated that there were two nonsubstituted phenyl groups with four protons as two doublet of doublets at δ 8.01 and 7.92, two protons as two triplets at δ 7.59 and 7.56, and four protons as multiplets at δ 7.46 to 7.31, which showed cross peaks with the ^{13}C NMR signals at δ 129.8 (C-2′ and C-6′), 129.7 (C-2″ and C-6″), 133.6 (C-4′), 133.2 (C-4″), 128.5 (C-3′ and C-5′), and 128.4 (C-3″ and C-5″), respectively, in the HMQC NMR spectrum. Signals of a trisubstituted aromatic ring containing two oxygenated carbons (δ 114.8, 6.58, 1H, d, J = 2.5 Hz, C-1; δ 149.8, C-2; δ 114.6, 6.61, 1H, dd, J = 2.5, 7.0 Hz, C-3; δ 117.6, 6.73, 1H, d, J = 7.0 Hz, C-4; δ 123.8, C-9a; δ 147.0, C-9b) were observed along with signals of a self-coupled OCH_2 (δ 67.8, 4.45, 4.53, two d, J = 11.6 Hz), a CH=CH (δ 132.4, 127.6, 6.01, 5.96, two d, J = 10.0 Hz, C-5, C-6), one quaternary oxygen carbon (δ 77.4, C-8b), and two carboxylic carbonyl carbons (δ 166.3 and 167.3). In addition, a $-OCHCH(O)CHCH_2-$ (δ 73.8, 5.64, dd,

Table 2. Summary of HMBC Spectral Correlations for Compounds **1**−**4**

position	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3-CD_3OD$)	**4** ($CDCl_3-CD_3OD$)
1				H-5
2	H-2′, H-6′	H-2′, H-3′, H-6′	Ph−CH=	H-6
3				H-5
4			OMe-4	H-2, H-6
5	OMe-5	OMe-5	OMe-5, H-7	
6	H-8	OMe-6, H-8	OMe-6	H-2
7	OMe-7	OMe-7, H-8		
8			H-7	
9	H-8	H-8	H-7	
10	H-8	H-8		
α				
β				H-2, H-6
Ph−CH=				
1′	H-3′, H-5′	H-5′	H-5′, Ph−CH=	H-5′
2′	H-6′	H-6′	H-6′, Ph−CH=	OMe-2′
3′	H-5′	H-2′, H-5′	H-5′	OMe-3′, H-5′
4′	OMe-4′, H-2′	H-5′	H-2′, H-6′	OMe-4′
5′	H-3′			
6′	H-2′	H-2′	H-2′	
C=O	H-3	H-3		

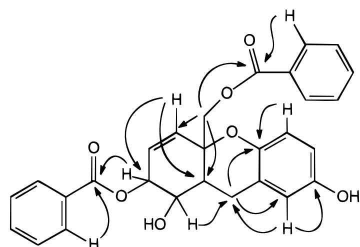


Figure 1. Important HMBC correlations of compound **5**.

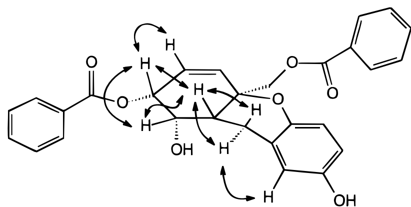


Figure 2. Important NOE correlations of compound **5**.

J = 6.5, 1.5 Hz, C-7; δ 71.8, 4.41, m, C-8; δ 38.0, 2.69, m, C-8a; δ 23.6, 3.10, dd, J = 14.5, 4.5 Hz, H-9α, and δ 2.66, m, H-9β) linkage was suggested based on the cross peaks apparent in the COSY spectrum. The HMBC/LRHETCOR spectra summarized in Figure 1 further indicated that compound **5** has a unique linear three-ring system of fused cyclohexene and dihydrobenzopyran, resembling a partially reduced xanthene derivative with a benzoyloxyl group attached at C-7, and a benzoyloxymethyl attached at C-8b. The spectral analysis, based on NOESY NMR (Figure 2), established the stereochemistry of compound **5** as 2,8(α)-dihydroxy-7(α)-benzoyloxy-8b(α)-benzoyloxymethyl-7,8,8a(β),8b-tetrahydroxanthene. The absolute stereochemistry of **5** has not been determined.

Compounds **1**, **2**, and **3** all demonstrated strand-scission activity in the DNA strand-scission assay.[26] Compound **4** was considered weakly active, and **5** was inactive (Table 3). The order of activity was 3 ≫ (1 = 2) > 4. Compound **3** showed activity at one-tenth the dose of **1**,**2**,**4**, and **5**. A 3′,4′-catechol group is present in compounds **2**−**4**, and this type of functionality appears to be important for DNA strand-scission activity in compounds, as in our previous reports.[27,28] However, compound **1**, which does not have a catechol group, was able to cause DNA breakage. It must be emphasized that the DNA strand-scission activity of these compounds is weak compared to the bleomycin standard (cf. Experimental Section and Table 3).

Table 3. DNA Strand Scission and 9KB Cytotoxicity of Compounds **1**−**5**

compound	% relaxation of supercoiled DNA	bleomycin units[b]	9KB[c]
1	88	1.1	>20
2	76	1.0	>20
3	83[a]	10.0[a]	>20
4	47	0.6	8.0
5	negative	negative	10.0

[a] Assayed at 2.5 μg/mL. [b] For explanation of terms, see text. [c] Data expressed as IC_{50} values (μg/mL).

The 9KB growth inhibition activity of all the compounds was weak or negative. Compound **4** displayed an IC_{50} of 8.0 μg/mL; compound **5**, 10.0 μg/mL; compounds **1**−**3** are considered negative with IC_{50} values > 20 μg/mL (cf. Table 3).

Experimental Section

General Experimental Procedures. Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR experiments were performed on a Bruker AMX 250 MHz instrument operating at 250 MHz for proton, and 62.5 MHz for carbon, respectively. For HMQC, HMBC, and NOE experiments, a Bruker AMX 500 spectrometer was utilized. $CDCl_3$ or $CDCl_3-CD_3OD$ was used as a solvent and TMS as internal standard. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. UV and IR spectra were recorded on a Varian Cary 3G UV vis spectrophotometer and a Shimadzu IR-460 spectrometer, respectively. EIMS were recorded on VGZAB-E magnetic sector instrument. Column chromatography was carried out either on Si gel 60 (70−230 mesh, Merck, Darmstadt, Germany), or on prepacked Si gel columns utilizing equipment for flash chromatography (Biotage, Charlottesville, VA), or on Sephadex LH-20 (Sigma, St. Louis, MO). Fractions were monitored by TLC (Si gel 60 F_{254}, 0.25-mm thickness) with visualization under UV (254 and 365 nm) and with 5% phosphomolybdic acid in EtOH.

Preparative TLC was carried out on 1-mm thickness Si gel 60 F_{254} plates (Merck, Darmstadt, Germany or Analtech, Newark, DE).

Plant Material. The leaves and stems of *U. hamiltonii* were collected at Phu Naa Yoi National Park, Ubonratchatani, Thailand, in October 1993. Voucher specimens (A3024 and A3025) are on deposit in the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, IL, and represent collections of the leaves and stems of this species, respectively.

Extraction and Isolation. Air-dried samples of *U. hamiltonii* leaves (584 g) and stems (715 g) were separately extracted by percolation for 24 h with MeOH (2 ×). The MeOH samples were concentrated and partitioned between $CHCl_3$−MeOH (4:1) and H_2O. The $CHCl_3$ fraction was washed several times with dilute saline and then concentrated. The $CHCl_3$ fractions from *U. hamiltonii* leaf (23.5 g) and stem (2.1 g) were combined because both fractions had similar DNA strand-scission and 9KB cytotoxicity activity.

Chromatography of the combined leaf and stem $CHCl_3$ fractions (25.6 g) on Si gel (500 g) using initially a solvent gradient of hexanes−EtOAc (from 100:0 to 1:1) and then $CHCl_3$−MeOH (95:5 to 0:100) yielded a total of 13 fractions. The fraction 7 (2.6 g) eluted by 1:1 hexanes−EtOAc was active in 9KB. DNA strand-scission activity was negative. Further purification of the above fraction 7 by Sephadex LH-20 (200 g, MeOH) chromatography yielded a 9KB-active fraction, which was subjected to preparative TLC (10% MeOH−$CHCl_3$) and chromatography on a reversed-phase C_{18} (5 g, 60 → 70% MeOH−H_2O) column (J. T. Baker, Inc., Phillipsburg, NJ). A pure compound (**5**, 23 mg) was obtained as a white solid from MeOH.

Fraction 8, from the initial Si gel chromatography, eluted with $CHCl_3$−MeOH (95:5), was further chromatographed on Sephadex LH-20 (200 g, MeOH) and then subjected to preparative TLC (10% MeOH−$CHCl_3$). Two pure flavonoids, **1** (7.5 mg) and **2** (20 mg), were thus obtained.

Fraction 9 (3.1 g) from the initial Si gel chromatography, eluted with $CHCl_3$−MeOH gradient (95:5 → 92:8), was further subjected to Sephadex LH-20 (200 g, MeOH) and flash chromatography (2% MeOH−$CHCl_3$). The latter procedure yielded three pure compounds, **2** (42 mg), **3** (34 mg), and **4** (17 mg).

Hamiltone A (1): yellow solid (10% MeOH−$CHCl_3$); mp 85−87 °C; $[\alpha]^{21}_D$ +3.3° (*c* 0.36 75% MeOH−$CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 225 (4.04), 276 (3.73), 336 (3.53) nm; IR (KBr) ν_{max} 3435, 2930, 1606, 1249 cm^{-1}; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.39 (2H, dd, J = 1.9, 6.7 Hz, H-2′ and H-3′), 6.95 (2H, dd, J = 1.9, 6.7 Hz, H-3′ and H-5′), 6.37 (1H, s, H-8), 5.34 (1H, dd, J = 2.9, 13.3 Hz, H-2), 3.95 (3H, s, OMe-5), 3.91 (3H, s, OMe-7), 3.84 (3H, s, OMe-4′), 3.04 (1H, dd, J = 13.3, 16.8 Hz, H-3), and 2.76 (1H, dd, J = 2.9, 16.8 Hz, H-3); ^{13}C NMR data, see Table 1; EIMS *m/z* 330 [M]$^+$ (40), 196 (100), 134 (25); HREIMS *m/z* [M]$^+$ 330.1105 (calcd for $C_{18}H_{18}O_6$, 330.1103).

Hamiltone B (2): yellow solid (MeOH); mp 200 °C dec; $[\alpha]^{21}_D$ 0° (*c* 0.67, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 221 (4.25), 232 (4.25), 278 (4.08), 322 (3.53) nm; IR (KBr) ν_{max} 3420, 2930, 1598, 1262, 1105 cm^{-1}; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.94 (1H, d, J = 1.7 Hz, H-2′), 6.88 (1H, d, J = 8.1 Hz, H-5′), 6.80 (1H, dd, J = 1.7, 8.1 Hz, H-6′), 6.30 (1H, s, H-8), 5.22 (1H, dd, J = 3.0, 13.0 Hz, H-2), 3.93 (3H, s, OMe-5), 3.84 (3H, s, OMe-7), 3.80 (3H, s, OMe-6), 2.94 (1H, dd, J = 13.0, 16.8 Hz, H-3), 2.70 (1H, dd, J = 3.0, 16.8 Hz, H-3); ^{13}C NMR data, see Table 1; EIMS *m/z* 346 [M]$^+$ (100), 210 (85), 195 (80), 167 (30), 136 (7); HREIMS *m/z* [M]$^+$ 346.1055 (calcd for $C_{18}H_{18}O_7$, 346.1052).

Hamiltrone (3): yellow solid (MeOH); mp 222−225 °C; UV (MeOH) λ_{max} (log ϵ) 257 (3.96), 331 (3.96), 278 (4.08), 405 (4.33) nm; IR (KBr) ν_{max} 3400, 3000, 1600, 1256 cm^{-1}; ^{1}H NMR ($CDCl_3$−CH_3OD, 250 MHz) δ 7.46 (1H, d, J = 2.0 Hz, H-2′), 7.31 (1H, dd, J = 2.0, 8.4 Hz, H-6′), 6.89 (1H, d, J = 8.4 Hz, H-5′), 6.70 (1H, s, Ph−CH=), 6.59 (1H, s, H-7), 4.23 (3H, s, OMe-4), 4.00 (3H, s, OMe-6), 3.82 (3H, s, OMe-5); ^{13}C NMR data, see Table 1; EIMS *m/z* 344 [M]$^+$ (85), 329 (100), 315 (13), 210 (85), 167 (13); HREIMS *m/z* [M]$^+$ 344.0894 (calcd for $C_{18}H_{16}O_7$, 344.0896).

Hamilcone (4): yellow needles (MeOH); mp 160−162 °C; UV (MeOH) λ_{max} (log ϵ) 207 (4.58), 267 (3.98), 316 (4.01), 387 (4.37) nm; IR (KBr) ν_{max} 3415, 1614, 1274, 1251, 1195 cm^{-1}; ^{1}H NMR ($CDCl_3$−CD_3OD, 250 MHz) δ 13.76 (1H, s, OH-6′), 7.80 (1H, d, J = 15.5 Hz, H-α), 7.73 (1H, d, J = 15.5 Hz, H-β), 7.19 (1H, d, J = 2.0 Hz, H-2), 7.08 (1H, dd, J = 2.0, 8.2 Hz, H-6), 6.86 (1H, d, J = 8.2 Hz, H-5), 5.93 (1H, s, H-5′), 3.93 (3H, s, OMe-2′), 3.91 (3H, s, OMe-4′), 3.84 (3H, s, OMe-3′); ^{13}C NMR data, see Table 1; EIMS *m/z* 346 [M]$^+$ (100), 210 (83), 195 (93), 167 (42); HREIMS *m/z* [M]$^+$ 346.1055 (calcd for $C_{18}H_{18}O_7$, 346.1052).

Hamilxanthene A (5): white solid (MeOH); mp 92−95 °C; $[\alpha]^{21}_D$ 105° (*c* 0.77, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 (4.46), 282 (3.47) nm; IR ($CHCl_3$) ν_{max} 3585, 1719, 1492, 1268, 1111 cm^{-1} ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.01 (2H, dd, J = 7.0, 1.5 Hz, H-2′ and H-6′), 7.92 (2H, dd, J = 7.0, 1.5 Hz, H-2″ and H-6″), 7.59 (1H, t, J = 8.0, 7.5 Hz, H-4′), 7.56 (1H, t, J = 8.0, 7.5 Hz, H-4″), 7.46−7.31 (4H, m, H-3′ and H-5′, H-3″ and H-5″), 6.73 (1H, d, J = 7.0 Hz, H-4), 6.61 (1H, dd, J = 2.5, 7.0 Hz, H-3), 6.58 (1H, d, J = 2.5, Hz, H-1), 6.01, 5.96 (each 1H, each d, J = 10.0 Hz, H-5 and H-6), 5.64 (1H, dd, J = 6.5, 1.5 Hz, H-7), 4.53, 4.45 (each 1H, each d, J = 11.5 Hz, OCH_2), 4.41 (1H, m, H-8), 3.10 (1H, dd, J = 14.5, 4.5 Hz, H-9α), 2.69 (1H, m, H-8a), 2.66 (1H, m, H-9β); ^{13}C NMR ($CDCl_3$, 250 MHz) δ 167.3 (C-7′), 166.3 (C-7″), 149.8 (C-2), 147.0 (C-9b), 133.6 (C-4′), 133.2 (C-4″), 132.4 (C-5), 129.8 (C-2′ and C-6′), 129.7 (C-2″ and C-6″), 129.5 (C-1″), 129.3 (C-1′), 128.5 (C-3′ and C-5′), 128.4 (C-3″ and C-5″), 127.6 (C-6), 123.8 (C-9a), 117.6 (C-4), 114.8 (C-1), 114.6 (C-3), 77.4 (C-8b), 73.8 (C-7), 71.8 (C-8), 67.8 (OCH_2), 38.0 (C-8a), 23.6 (C-9); EIMS *m/z* 472 [M]$^+$ (17), 350 (2), 332 (7), 319 (2), 228 (30), 211 (20), 122 (20), 105 (100), 77 (24); HREIMS *m/z* [M]$^+$ 472.1528 (calcd for $C_{28}H_{24}O_7$, 472.1522).

DNA Strand-Scission Assay. The DNA strand-scission assay was performed based on a modified Hecht procedure,[26] which has been described previously.[27,28] In brief, test compounds were evaluated in duplicate at a concentration of 25 or 2.5 (compound **3**) μg/mL, and the results (Table 3) were compared with a positive control, bleomycin sulfate assayed at 0.025 μg/mL.

The DNA-cleaving activity of each test sample is reported as % relaxation of supercoiled DNA, and also as units of bleomycin activity (defined as the % reduc-

tion in supercoiled $DNA_{test\ sample}$/% reduction in supercoiled $DNA_{bleomycin}$) to maintain consistency with earlier papers.[27,28] Thus, a test substance calculated to have 0.5 units of bleomycin activity relaxed half as much supercoiled DNA as 0.025 μg/mL bleomycin.

9KB Cytotoxicity Assay. The KB cell line was purchased from the American Type Culture Collection (Rockville, MD). The assay was carried out in 96-well microtiter plates as described previously[29] (cf. Table 3).

Acknowledgment. The research reported in this paper was supported by NCl Grant No. U19 CA52956. We wish to thank Drs. J. Burgess and K. Chen for NMR experiments and Ms. S. Spaulding and Ms. Y. Brackeen for technical assistance.

References and Notes

(1) Kodpinid, M.; Sadavongivivad, C.; Thebtaranonth, C., Thebtaranonth, Y. *Tetrahedron Lett.* **1983**, *24*, 2019−2022.
(2) Kodpinid, M.; Sadavongivivad, C.; Thabtaranonth, C.; Thabtaranonth, Y. *Phytochemistry* **1984**, *23*, 199−200.
(3) Chayunkiat, A.; Wiriyachitra, P.; Taylor, W. *J. Sci. Soc. Thailand* **1984**, *10*, 239−245.
(4) Lojanaptwatna, V.; Promsuwansiri, K.; Suwannatip, B.; Wiriyachitra, P. *J. Sci. Soc. Thailand* **1981**, *7*, 83−86.
(5) Nkunya, M. H. H.; Achenbach, H.; Renner, C.; Waibel, R.; Weenen, H. *Phytochemistry* **1990**, *29*, 1261−1264.
(6) Okorie, D. A. *Phytochemistry* **1977**, *16*, 1591−1594.
(7) Hufford, C. D.; Oguntimein, B. O. *Phytochemistry* **1980**, *19*, 2036−2038.
(8) Hufford, C. D.; Oguntimein, B. O. *J. Nat. Prod.* **1982**, *45*, 337−342.
(9) Hufford, C. D.; Oguntimein, B. O.; Shoolery, J. N. *J. Org. Chem.* **1987**, *52*, 5286−5288.
(10) Nkunya, M. H. H.; Waibel, R.; Achenbach, H. *Phytochemistry* **1993**, *34*, 853−856.
(11) Nkunya, M. H. H.; Weenen, H.; Renner, C.; Achenbach, H. *Phytochemistry* **1993**, *32*, 1297−1300.
(12) Achenbach, H.; Hohn, M.; Waibel, R.; Nkunya, M. H. H.; Jonker, S. A.; Muhie, S. *Phytochemistry* **1997,** *44*, 359−364.
(13) Hufford, C. D.; Lasswell, W. L., Jr. *J. Org. Chem.* **1976**, *41*, 1297−1298.
(14) Hufford, C. D.; El Sohly, H. N. *Lloydia* **1978**, *41*, 652.
(15) Hufford, C. D.; Lasswell, W. L., Jr.; Hirotsu, K.; Clardy, J. *J. Org. Chem.* **1979**, *44*, 4709−4710.
(16) El Sohly, H. N.; Lasswell, W. L., Jr.; Hufford, C. D. *J. Nat. Prod.* **1979**, *42*, 264−270.
(17) Hufford, C. D.; Oguntimein, B. O.; Baker, J. K. *J. Org. Chem.* **1981**, *46*, 3073−3078.
(18) Chantrapromma, K.; Pakawatchai, C.; Skelton, B. W.; White, A. H.; Worapatamasri, S. *Aust. J. Chem.* **1989**, *42*, 2289−2293.
(19) Stout, M. G.; Reich, H.; Huffman, M. N. *J. Pharm. Sci.* **1964**, *53*, 192−195.
(20) Markham, K. R.; Mabry, T. J. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, H., Eds. Academic: New York, 1975; pp 44−77.
(21) Mabry, T. J.; Markham, K. R. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, H., Eds. Academic: New York, 1975; pp 78−126.
(22) Iinuma, M.; Matsuura, S., Tanaka, T. *Chem. Pharm. Bull.* **1984**, *32*, 1472−1476.
(23) Gaffield, W. *Tetrahedron* **1970**, *26*, 4093−4108.
(24) Agrawal, P. K.; Bansal, M. C. In *Carbon-13 NMR of Flavonoids*; Agrawal, P. K., Ed. Elsevier: Amsterdam, 1989; pp 243−250.
(25) Quijano, L.; Calderon, J. S.; Gomez, F.; Rios, T. *Phytochemistry* **1982**, *21*, 2575−2579.
(26) Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kikuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869−877.
(27) Chaudhuri, S. K.; Huang, L.; Fullas, F.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Tucker, J. C.; Beecher, C. W. W.; Kinghorn, A. D. *J. Nat. Prod.* **1995**, *58*, 1966−1969.
(28) Huang, L.; Fullas, F.; McGivney, R. J.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Tucker, J. C.; Beecher, C. W. W.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1996**, *59*, 290−292.
(29) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M.; Ruangrungsi, N. *J. Nat. Prod.* **1993**, *56*, 30−38.

NP9703609