Five New Diprenylated Flavonols from the Leaves of *Broussonetia kazinoki*

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Five new diprenylated flavonols, broussonol A (**1**), broussonol B (**2**), broussonol C (**3**), broussonol D (**4**), and broussonol E (**5**), along with two known compounds, were isolated from an ethanolic extract of the leaves of *Broussonetia kazinoki*. Their structures were elucidated by chemical and spectral methods. Cytotoxic activities were evaluated against several different cell lines.

Broussonetia kazinoki Sieb (Moraceae) is distributed throughout China, Korea, and Japan. Its branches, leaves, and fruits have been used as a diuretic, a tonic, and a suppressant for edema in Chinese folk medicine. Previous investigations of this plant revealed the presence of prenylated flavans, prenylated flavonoids,[1] prenylated 1,3-diphenylpropanes,[2,3] and alkaloids.[4,5] Prenylated flavonoids have shown potent inhibition against human hepatoma PLC/PRF/5 and epidermoid carcinoma KB cells in vitro.[6] In a search for biologically active compounds a chemical study on this plant revealed five new diprenylated flavonols, named broussonol A (**1**), broussonol B (**2**), broussonol C (**3**), broussonol D (**4**), and broussonol E (**5**), along with two known 1,3-diphenylpropanes compounds, kazinol F[3] and kazinol J.[7] Cytotoxic activities of **1**−**5**, kazinol F, and kazinol J were evaluated against several different cell lines.

Results and Discussion

Broussonol A (**1**) was obtained as a yellow powder, mp 162−164 °C, exhibiting a positive ferric chloride test (dark green) and magnesium hydrochloric acid test (orange). The IR spectrum indicated hydroxyl, hydrogen-bonded carbonyl, and aromatic groups. The HREIMS of **1** showed a molecular ion peak at *m*/*z* 436.1546 corresponding to molecular formula $C_{25}H_{24}O_7$. The UV spectrum of **1** showed absorption maxima at 250, 266 (Band II), and 376 (Band I) nm in MeOH, and bathochromic shifts of 62 nm with $A1C1_3$ and $A1C1_3$ + HC1 in Band I. These data revealed that **1** was a flavonol without *ortho*-dihydroxyl groups.

The ^{1}H NMR spectrum of **1** indicated the presence of a 1,1-dimethylallyl at δ 1.68 (6H, s, 2 CH_3), 4,88 (1H, d, J = 10.0 Hz), 4.93 (1H, d, J = 17.5 Hz), and 6.35 (1H, dd, J = 10.0, 17.5 Hz) and a 2,2-dimethylchromene ring at δ 1.46 (6H, s, 2 CH_3), 5.82 (1H, d, J = 9.6 Hz), and 6.47 (1H, d, J = 9.6 Hz), as well as three aromatic proton signals at δ 6.33 (1H, s, H-6), 7.71 (1H, d, J = 2.1 Hz, H-2′), and 7.51 (1H, d, J = 2.1 Hz, H-6′) and four phenolic hydroxyl proton signals at δ 12.52, 9.37, and 7.80 (2H). EIMS of **1** showed a molecular ion peak at *m*/*z* 436 $[M]^+$ and significant fragments at *m*/*z* 421 $[M - CH_3]^+$ and 353 $[M - CH_3 - C_5H_8]^+$.

The ^{13}C NMR data (Table 1) showed 25 signals, suggesting a flavonoid nucleus and two prenyl groups. The chemical shift values of C-2 to C-10 were similar to those of the corresponding data for 8-isoprenyl-5,7-dihydroxylflavonol.[5,7] The 1,1-dimethyallyl group was confirmed at the C-8 position by the ^{1}H−^{13}C long-range correlation of the methyl protons at δ 1.68 with C-8 at δ 111.8. The substitution patterns of a hydroxyl and 2,2-dimethylchromene ring in ring B were determined at the C-3′, C-4′, and C-5′ positions by the detailed analysis of HMQC, HMBC, and NOESY spectra (Figure 1) combined with the observation of two *meta*-coupled protons in ring B. All these results indicated structure **1** as shown.

Table 1. ^{13}C NMR Data (δ) for Compounds **1**−**5** (Acetone-d_6)

position	**1**	**2**	**3**	**4**	**5**
2	147.4	146.0	146.6	146.9	146.8
3	136.6	136.7	136.3	136.4	136.5
4	176.7	176.5	176.4	176.6	176.3
5	160.1	162.6	162.5	159.7	158.8
6	100.3	94.3	94.2	98.7	111.6
7	163.4	165.7	165.6	161.9	162.5
8	111.8	113.4	113.3	107.1	93.7
9	156.2	152.6	152.6	154.7	155.5
10	105.1	104.6	104.6	104.0	103.9
11	41.8	44.3	44.3	22.1	21.9
12	150.9	91.3	91.3	123.1	123.3
13	109.6	26.2	26.2	132.1	131.5
14	30.4	21.8	21.7	18.0	17.8
15	30.4	14.4	14.5	25.8	25.7
1′	124.2	124.4	122.7	123.1	122.8
2′	116.9	115.7	113.5	113.6	113.2
3′	146.0	146.3	144.8	144.9	144.9
4′	142.7	142.7	146.2	146.2	146.2
5′	122.0	122.3	128.0	129.0	129.0
6′	118.9	118.0	120.7	121.3	121.7
7′	122.6	122.5	28.7	28.9	28.9
8′	132.2	132.3	122.8	123.1	123.3
9′	78.0	78.1	133.1	133.0	132.8
10′	28.1	28.1	17.9	17.8	17.8
11′	28.1	28.1	26.1	25.8	25.8

Broussonol B (**2**) was obtained as a yellow powder, mp 210−212 °C, exhibiting a positive ferric chloride test (dark green) and magnesium hydrochloric acid test (orange). The IR spectrum again indicated hydroxyl, hydrogen-bonded carbonyl, and aromatic groups. The HREIMS of **2** showed a molecular ion peak at *m*/*z* 436.1523 corresponding to molecular formula $C_{25}H_{24}O_7$. The UV spectrum also revealed that **2** was also a flavonol without *ortho*-dihydroxyl groups.

The ^{1}H NMR spectrum of **2** demonstrated the presence of a 2,2-dimethylchromene ring at δ 1.46 (6H, s, 2 CH_3), 5.83 (1H, d, J = 9.6 Hz), and 6.48 (1H, d, J = 9.6 Hz), as well as three aromatic proton signals at δ 6.19 (1H, s, H-6), 7.67 (1H, d, J = 2.1 Hz, H-2′), and 7.52 (1H, d, J = 2.1 Hz, H-6′) and three phenolic hydroxyl proton signals at δ 12.43, 8.09 (2H). In addition, resonances for a doublet methyl at δ 1.41, two singlet methyls at δ 1.62 and 1.34, and a

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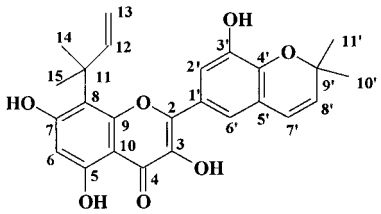

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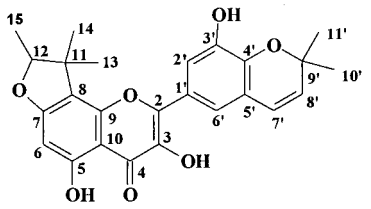

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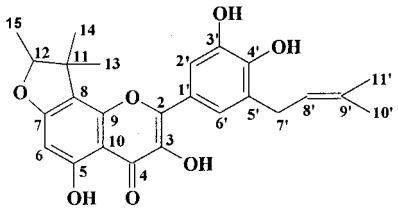

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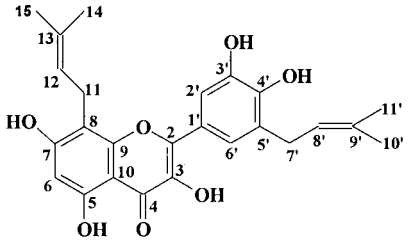

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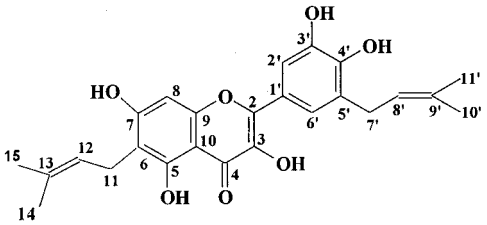

5

methine at δ 4.57 suggested the presence of a 2-methyl-3,3-dimethyl dihydrofuran ring.

The ^{13}C NMR data (Table 1) indicated that **2** was also a flavonol with two prenyl groups. The carbon signals in rings B and C were similar to those of the corresponding signals in **1**. It was evident from the HMBC spectrum (Figure 2) that a hydroxyl and a 2,2-dimethyl chromene ring were at the C-3′, C-4′, and C-5′ positions. Furthermore, the methyl at δ 1.41 was correlated with C-12 at δ 91.3, and the two methyl protons at δ 1.34 (s) and 1.62 (s) were correlated with the C-8 signal at δ 113.4 in the HMBC spectrum. Our conclusion was that a 2-methyl-3,3-dimethyl dihydrofuran ring is fused at positions C-7 and C-8, as indicated in structure **2**.

Broussonol C (**3**) was obtained as a yellow powder, mp 174−176 °C, that exhibited a positive ferric chloride test and magnesium hydrochloric acid test. The IR spectrum of **3** was analogous to that of **2**. The HREIMS of **3** showed

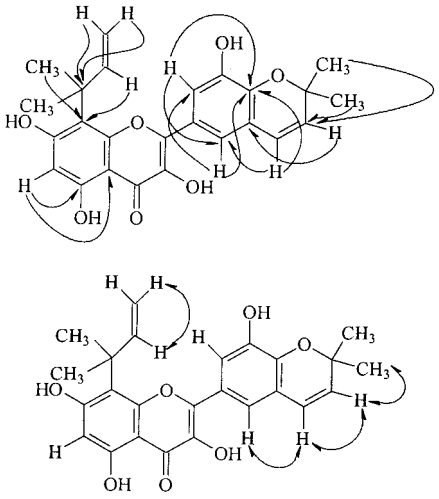

Figure 1. HMBC and NOESY correlations for **1**.

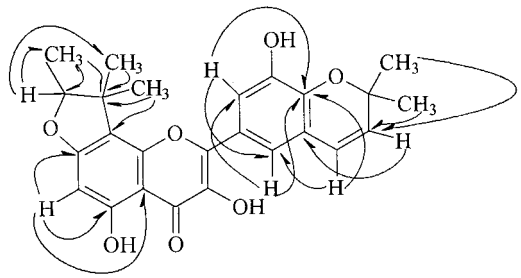

Figure 2. HMBC correlation for **2**.

a molecular ion peak at *m/z* 438.1633 corresponding to molecular formula $C_{25}H_{26}O_7$. The UV spectrum of **3** showed absorption maxima at 259 (Band II) and 382 (Band I) nm in MeOH and bathochromic shifts of 89 and 56 nm with $A1C1_3$ and $A1C1_3$ + HC1 in Band I, respectively. These data revealed that **3** was a flavonol with *ortho*-dihydroxyl groups.

The ^{1}H NMR spectrum of **3** showed the presence of a 2-methyl-3,3-dimethyl dihydrofuran ring at δ 1.34 (3H, s, CH_3), 1.62 (3H, s, CH_3), 4.57 (1H, q, J = 6.6 Hz), and 1.41 (1H, d, J = 6.6 Hz) and a 3-methyl-2-butenyl at δ 1.75 (3H, s, CH_3), 1.76 (3H, s, CH_3), 3.43 (2H, d, J = 7.5 Hz), and 5.42 (1H, m), as well as three aromatic proton signals at δ 6.18, 7.80, and 7.56 and four phenolic hydroxyl proton signals at δ 12.46, 8.86, 7.94, and 7.87.

The ^{13}C NMR data (Table 1) suggested that **3** was also a flavonol that has two prenyl groups. The carbon signals of rings A and C were similar to those of the corresponding carbon signals in **2**. Further indication of the substitution pattern in ring A came from the HMBC spectrum, which exhibited a correlation of the methyl protons at δ 1.34 and 1.62 with C-8 at δ 113.3. These data suggested that a 2-methyl-3,3-dimethyl dihydrofuran ring also was fused at the C-7 and C-8 positions in ring A. The only difference was the presence of signals from a 3-methyl-2-butenyl unit in **3** instead of the signals assigned to a 2,2-dimethyl chromene ring in **2**. In the HMBC spectrum, the methylene protons at δ 3.43 (H-7′) were correlated with C-4′ at δ 146.2, C-5′ at δ 128.0, and C-6′ at δ 120.7, and the proton at δ 7.56 (H-6′) was correlated with C-7′ at δ 28.7. The existence of two *meta*-coupled protons in ring B combined with the above evidence uniquely defined the substitution pattern in ring B as 3′,4′-dihydroxy-5′-(3-methyl-2-butenyl). Thus, the structure of broussonol C is **3**.

Broussonol D (**4**) was obtained as a yellow powder, mp 187−189 °C, exhibiting a positive ferric chloride test and

Table 2. Cytotoxicity ($ED_{50}\mu g$/ML) of Compounds **1**−**5**, Kazinol F, and Kazinol J against Some Human Tumor Cell Lines by the MTT Method

compound	A549	HCT-8	KB
1	8.74	9.10	>10
2	5.52	8.80	>10
3	7.77	9.63	>10
4	>10	>10	4.15
5	>10	>10	>10
6	>10	>10	>10
7	>10	>10	>10

magnesium hydrochloric acid test. The IR spectrum of **4** revealed the presence of hydroxyl, hydrogen-bonded carbonyl, and aromatic groups. The HREIMS of **4** showed a molecular ion peak at *m*/*z* 438.1687 corresponding to molecular formula $C_{25}H_{26}O_7$. The UV spectrum also suggested that **4** was a flavonol with *ortho*-dihydroxyl groups.

The ^{1}H NMR spectrum of **4** contained resonances for three aromatic protons and five phenolic hydroxyl protons. The presence of two 3-methyl-2-butenyl groups was suggested by the proton signals at δ 1.49 (3H, s), 1.74 (3H, s), 3.55 (2H, d, J = 6.9 Hz), and 5.32 (1H, m) and δ 1.74 (3H, s), 1.79 (3H, s), 3.42 (2H, d, J = 7.5 Hz), and 5.39 (1H, m) and mass fragments at *m*/*z* 383 $[M - C_4H_7]^+$ and 328 $[M - 2C_4H_7]^+$.

The ^{13}C NMR data (Table 1) indicated that **4** was also a flavonol with two prenyl groups. The ^{13}C signals of rings B and C were similar to those of the corresponding data for **3**. The substitution patterns of rings B and C in **4** were the same as those in **3**. Another 3-methyl-2-butenyl was attached to C-8, instead of the C-6 of the flavone skeleton, which was confirmed by a comparison with the spectral data for broussoflavonol C[8] and an HMBC spectrum of **4** in which the methylene protons at δ 3.55 (2H, d, J = 6.9 Hz, H-11) were correlated with C-8 at δ 107.1 (quaternary carbon) and C-9 at δ 154.7. Thus, the structure of broussonol D was elucidated as **4**.

Broussonol E (**5**) was obtained as a yellow powder, mp 192−193 °C. It was analogous to **4** in the Mg−HCl color reaction and UV and IR spectra. The HREIMS of **5** indicated a molecular formula $C_{25}H_{26}O_7$. The ^{1}H NMR spectrum of **5** also revealed the presence of two 3-methyl-2-butenyl units and showed three aromatic proton signals at δ 6.55 (H-8), 7.70 (H-2′), and 7.60 (H-6′) and five phenolic hydroxyl proton signals. The ^{13}C NMR data of **5** were identical with those of **4** except for ring A. In the ^{13}C NMR spectrum, C-6 was shifted downfield to δ 111.6 (quaternary carbon). In the HMBC spectrum, the methylene protons at δ 3.35 (2 H, d, J = 6.9 Hz, H-11) were correlated with C-7 at δ 162.5 and C-5 at δ 158.8, and H-8 at δ 6.55 was correlated with C-9 at δ 155.5 and C-7 at δ 162.5, indicating the 3-methyl-2-butenyl in ring A was linked to C-6 in **5**, instead of C-8 as in **4**. Thus, the structure of broussonol E was elucidated as **5**.

Bioassay experiments using the MTT method[9] revealed that compounds **1**−**3** were weakly cytotoxic against A549 and HCT-8 human tumor cell lines, and **4** was weakly cytotoxic against KB human tumor cells. Compound **5**, kazinol F, and kazinol J exhibited no cytotoxicity at 10 μg/mL (Table 2).

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert Nr-229 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter in CH_3OH. UV spectra were determined with a Hitachi UV-240 spectrophotometer. IR spectra were taken on a Perkin-Elmer 683 (KBr) spectrometer. EIMS spectra were recorded on a VG ZAB-2F spectrometer. HREIMS spectra were performed on an Autospec-UltimaETOF spectrometer. NMR spectra were recorded on a Bruker instrument at 300 MHz for ^{1}H and 75 MHz for ^{13}C.

Plant Material. The leaves of *Broussonetia kazinoki* Sieb were collected from Jiangxi province of the People's Republic of China in May 1999. The plant material was identified by Professor Ce-ming Tan. A voucher specimen (No. 99) has been deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Media, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China.

Extraction and Isolation. The dried leaves *of Broussonetia kazinoki* Sieb (4 kg) were exhaustively extracted with 95% EtOH at reflux. The EtOH extract was then concentrated under reduced pressure to give a residue (180 g), which was suspended in H_2O, and the suspension was then extracted with EtOAc. The EtOAc extract was evaporated in a vacuum to give a residue (70 g), which was chromatographed over silica gel. The column was eluted starting with petroleum ether, and seven fractions were produced by gradually increasing the polarity with EtOAc and MeOH. The first two fractions (nonpolar compounds) and the MeOH fraction were not investigated further. Fraction 3 (11 g) was chromatographed over silica gel, eluting with petroleum ether−acetone to give **2** (25 mg). Fraction 4 (9.5 g) was chromatographed over silica gel, eluting with petroleum ether−acetone to give **1** (80 mg). Fraction 5 (8.4 g) was chromatographed over silica gel, eluting with petroleum ether−acetone to give **3** (20 mg), **4** (40 mg), and kazinol F (100 mg). Fraction 6 (10 g) was chromatographed over silica gel, eluting with petroleum ether−acetone to give **5** (125 mg) and kazinol J (80 mg).

Broussonol A (1): yellow powder; mp 162−164 °C; UV (MeOH) λ_{max} (log ϵ) 209 (4.58), 250 (4.47), 266 (4.46), 376 (4.31) nm, λ_{max} ($AlCl_3$) 211, 259, 275, 438 nm, λ_{max} ($AlCl_3$ + HCl) 212, 259, 276, 438 nm; IR (KBr) ν_{max} 3430, 3269, 1651, 1589, 1549, 1520 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ 1.68 (6H, s, H-14, H-15), 4,88 (1H, d, J = 10.0 Hz, H-13), 4.93 (1H, d, J = 17.5 Hz, H-13), 6.35 (1H, dd, J = 10.0, 17.5 Hz, H-12), 1.46 (6H, s, H-10′, H-11′), 5.82 (1H, d, J = 9.6 Hz, H-8′), 6.47 (1H, d, J = 9.6 Hz), 6.33 (1H, s, H-6), 7.71 (1H, d, J = 2.1 Hz, H-2′), 7.51 (1H, d, J = 2.1 Hz, H-6′), 12.52 (1H, s, 5-OH), 9.37 (1H, s, 7-OH), 7.80 (2H, s, 3, 3′-OH); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 436 $[M]^+$ (75), 421 (100), 393 (7), 353 (18); HREIMS *m*/*z* 436.1546 (calcd for $C_{25}H_{24}O_7$, 436.1522).

Broussonol B (2): yellow powder; mp 210−212 °C; $[\alpha]^{25}_D$ 0° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.55), 250 (4.60), 266 (4.58), 381 (4.37) nm, λ_{max} (NaOAc) 207, 251, 266, 385 nm, λ_{max} ($AlCl_3$) 213, 258, 277, 446 nm, λ_{max} ($AlCl_3$ + HCl) 212, 258, 276, 447 nm; IR (KBr) ν_{max} 3410, 3035, 1655, 1603, 1574, 1520 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ 1.41 (3H, d, J = 6.6 Hz, H-15), 1.62 (3H, s, H-13), 1.34 (3H, s, H-14), 4.57 (1H, q, J = 6.6 Hz, H-12), 1.46 (6H, s, H-10′, H-11′), 5.83 (1H, d, J = 9.6 Hz, H-8′), 6.48 (1H, d, J = 9.6 Hz, H-7′), 6.19 (1H, s, H-6), 7.67 (1H, d, J = 2.1 Hz, H-2′), 7.52 (1H, d, J = 2.1 Hz, H-6′), 12.43 (1H, s, 5-OH), 8.09 (2H, s, 3, 3′-OH); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 436 $[M]^+$ (75), 421 (100), 203 (18), 189 (10); HREIMS *m*/*z* 436.1523 (calcd for $C_{25}H_{24}O_7$, 436.1522).

Broussonol C (3): yellow powder; mp 174−176 °C; $[\alpha]^{25}_D$ 0° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (4.61), 259 (4.30), 382 (4.19) nm, λ_{max} ($AlCl_3$) 208, 275, 471 nm, λ_{max} ($AlCl_3$ + HCl) 209, 270, 438 nm; IR (KBr) ν_{max} 3388, 1653, 1603, 1572, 1520 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ 1.41 (3H, d, J = 6.6 Hz, H-15), 1.62 (3H, s, H-13), 1.34 (3H, s, H-14), 4.57 (1H, q, J = 6.6 Hz, H-12), 1.75 (3H, s, H-10′), 1.76 (3H, s, H-11′), 3.43 (2H, d, J = 7.5 Hz, H-7′), 5.42 (1H, m, H-8′), 6.18 (1H, s, H-6), 7.80 (1H, d, J = 2.1 Hz, H-2′), 7.56 (1H, d, J = 2.1 Hz, H-6′), 12.46 (1H, s, 5-OH), 8.86 (H, s, 4′-OH), 7.94 (H, s, 3-OH), 7.87 (H, s, 3′-OH); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 438 $[M]^+$ (70), 423 (100), 367 (20), 189 (30); HREIMS *m*/*z* 438.1633 (calcd for $C_{25}H_{26}O_7$, 438.1679).

Broussonol D (4): yellow powder; mp 187−189 °C; UV (MeOH) λ_{max} (log ϵ) 207 (4.72), 259 (4.37), 381 (4.28) nm, λ_{max} ($AlCl_3$) 208, 274, 466 nm, λ_{max} ($AlCl_3$ + HCl) 206, 269, 438 nm; IR (KBr) ν_{max} 3356, 1653, 1600, 1560, 1514 cm^{-1}; ^1H NMR (CD_3COCD_3, 300 MHz) δ 1.49 (3H, s, H-14), 1.74 (3H, s, H-15), 3.55 (2H, d, J = 6.9 Hz, H-11), 5.32 (1H, m, H-12), 1.74 (3H, s, H-10′), 1.79 (3H, s, H-11′), 3.42 (2H, d, J = 7.5 Hz, H-7′), 5.39 (1H, m, H-8′), 7.83 (1H, d, J = 2.1 Hz, H-2′), 7.75 (1H, d, J = 2.1 Hz, H-6′), 6.33 (1H, s, H-6), 12.09 (1H, s, 5-OH), 9.59 (1H, s, 7-OH), 8.82 (1H, s, 4′-OH), 7.92 (2H, s, 3,3′-OH); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 438 $[M]^+$ (100), 423 (28), 421 (30), 383 (35), 367 (20), 328 (30), 314 (28); HREIMS *m*/*z* 438.1687 (calcd for $C_{25}H_{26}O_7$, 438.1679).

Broussonol E (5): yellow powder; mp 192−193 °C; UV (MeOH) λ_{max} (log ϵ) 209 (4.78), 259 (4.43), 375 (4.44) nm, λ_{max} ($AlCl_3$) 207, 273, 468 nm, λ_{max} ($AlCl_3$ + HCl) 207, 268, 440 nm; IR (KBr) ν_{max} 3363, 1651, 1608, 1564, 1522 cm^{-1}; ^1H NMR (CD_3COCD_3, 300 MHz) δ 1.64 (3H, s, H-14), 1.75 (3H, s, H-15), 3.35 (2H, d, J = 6.9 Hz, H-11), 5.26 (1H, m, H-12), 1.74 (3H, s, H-10′), 1.77 (3H, s, H-11′), 3.40 (2H, d, J = 7.5 Hz, H-7′), 5.39 (1H, m, H-8′), 6.55 (1H, s, H-8), 7.70 (1H, d, J = 2.1 Hz, H-2′), 7.60 (1H, d, J = 2.1 Hz, H-6′), 12.42 (1H, s, 5-OH), 9.62 (1H, s, 7-OH), 8.75 (1H, s, 4′-OH), 7.85 (2H, s, 3′-OH), 7.91 (H, s, 3-OH); ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 438.1680 (calcd for $C_{25}H_{26}O_7$, 438.1679).

Kazinol F: colorless needles; mp 108−109 °C; IR, EIMS, and ^1H and ^{13}C NMR data were in agreement with literature values.[3]

Kazinol J: colorless needles; mp, IR, EIMS, and ^1H and ^{13}C NMR data were in agreement with literature values.[7]

Cytotoxicity Experiments. Cytotoxicity against human tumor cell lines was measured in a 5-day MTT test for KB human epidermoid carcer cells, HCT-8 human ileocecal carainoma, and A549 human lung carcinoma.[9] Briefly, 1×10^3 cells/100 μL were seeded in 96-well microplates and preincubated for 24 h to allow cell attachment. This medium was then aspirated, and 100 μL of fresh medium containing various concentrations of test drug were added to the cultures. The cells were incubated with each drug for 5 days. Cell survival was evaluated by adding 50 μL of MTT reagent (5 mg MTT/mL in RPMI 1640 medium) to each well. After 4 h reincubation at 37 °C, 100 μL of DMSO was added to dissolve the precipitate of reduced MTT. Microplates were agitated on a rotation platform at room temperature for 15 min, and the absorbance of the reaction mixtures was determined at 570 nm with a multiwell scanning spectrophotometer.

References and Notes

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