Tannins, Flavonol Sulfonates, and a Norlignan from *Phyllanthus virgatus*

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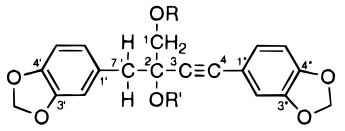
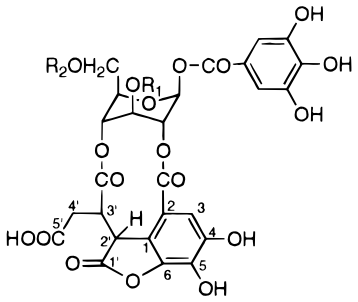
Investigation of the constituents of *Phyllanthus virgatus* has led to the isolation of five new compounds, including a norlignan, 2-(3,4-methylenedioxybenzyl)-4-(3,4-methylenedioxyphenyl)-3-butyne-1,2-diol named virgatyne (**1**); a hydrolyzable tannin, virganin (**2**); and three flavonoid sulfonates, galangin-8-sulfonate (**4**), galangin-3-*O*-*β*-D-glucoside-8-sulfonate (**5**), and kaempferol-8-sulfonate (**6**). Their structures were established by spectral and chemical methods.

Phyllanthus virgatus Forst. f. (Euphorbiaceae) is an annual plant in Taiwan, and it is traditionally used to protect the liver.[1] In previous work on *P. virgatus*, several lignans and indole-3-carboxylic acid were identified from the whole plant.[2] Continuing our investigation on *P. virgatus*, we obtained five additional new compounds including a norlignan, 2-(3,4-methylenedioxybenzyl)-4-(3,4-methylenedioxyphenyl)-3-butyne-1,2-diol (virgatyne, **1**); a hydrolyzable tannin (virganin, **2**); and three flavonoid sulfonates, galangin-8-sulfonate (**4**), galangin-3-*O*-*β*-D-glucoside-8-sulfonate (**5**), and kaempferol-8-sulfonate (**6**). Twenty-four known compounds—(−)-lirioresinol-B;[3] glochidone;[4] indole-3-carboxaldehyde;[5] kaempferol; quercetin; quercitrin; astragalin; isoquercitrin; rutin; myricitrin; 5;7-dihydroxy-4′-methoxyflavonol; quercetin-3-*O*-*β*-D-glucosyl-(1→6)-*β*-D-glucoside; gallic acid; methyl gallate; corilagin;[6] 1-*O*-galloyl-*β*-D-glucose; 1,6-di-*O*-galloyl-*β*-D-glucose;[7] 1,4,6-tri-*O*-galloyl-*β*-D-glucose;[8] 1,3,4,6-tetra-*O*-galloyl-*β*-D-glucose;[9] geraniin;[10] furosin;[11] brevifolin;[12] methyl brevifolincarboxylate;[13] and potassium brevifolincarboxylate[14]—were also recognized. The known compounds were identified by comparison of their spectral data with those in the literature or by comparison with authentic samples. The structures of new compounds **1**, **2**, and **4**−**6** were established by spectral and chemical methods, and the details are presented herein.

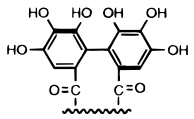


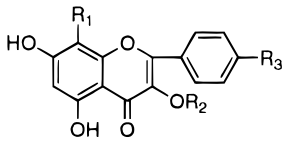
1: R = R′ = H
1a: R = Ac, R′ = H
1b: R = R′ = Ac



2: $R_1 = R_2 = H$
3: R_1, R_2 = HHDP

HHDP :





4: $R_1 = SO_3Na$, $R_2 = H$, $R_3 = H$
4a: $R_1 = H$, $R_2 = H$, $R_3 = H$
5: $R_1 = SO_3Na$, R_2 = glucosyl, $R_3 = H$
6: $R_1 = SO_3Na$, $R_2 = H$, R_3 =OH
6a: $R_1 = H$, $R_2 = H$, $R_3 = OH$

Results and Discussion

Compound **1** was obtained as white needles, mp 123−125 °C. The UV spectrum showed absorption bands at λ_{max} 294, 265, and 228 nm. The IR spectrum exhibited bands at 3322 (OH), 2236 (C≡C), 1606 and 1496 (aromatic rings), and 932 (O−CH_2−O) cm^{-1}. The HRMS of **1** displayed a molecular ion peak at *m*/*z* 340.0950 corresponding to $C_{19}H_{16}O_6$. The ^{13}C NMR and DEPT-NMR spectra of **1** showed 19 carbons, including four methylene, six methine, and nine quaternary carbons. In the ^1H NMR and ^1H−^{13}C COSY spectra of **1**, six aromatic proton signals between *δ* 6.44 and 6.61 could be analyzed into a pair of ABX patterns. The rest of the signals were assigned to two 7′ benzylic protons at *δ* 2.69 (1H, d, *J* = 13.4 Hz) and 2.75 (1H, d, *J* = 13.4 Hz), two 1-hydroxymethylene protons at *δ* 3.33 (2H, m), and two methylenedioxyl groups at *δ* 5.64 (2H, s) and 5.68 (2H, s). Consistent with the ^1H NMR data, the carbon signals of four methylene groups were attributed to two methylenedioxyl (*δ* 100.2 and 100.8) groups, one benzylic carbon (*δ* 43.3, C-7′), and one CH_2O carbon (*δ* 68.0, C-1). Among the quaternary resonances, the signals at *δ* 85.5 (C-4) and 88.1 (C-3) could be directly assigned to an alkyne group. The structure of **1** was further established using HMBC experiments (Figure 1). Two 7′ benzylic methylene protons at *δ* 2.69 and 2.75 were correlated with C-1 (*δ* 68.0), C-3 (*δ* 88.1), C-2′ (*δ* 110.7), and C-6′ (*δ* 123.3). The proton signal at *δ* 3.33 (H-1) was correlated

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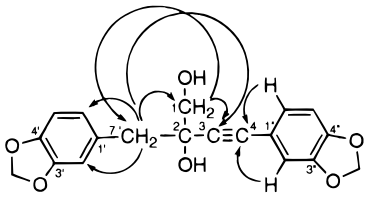

Figure 1. $^1H-^{13}C$ long-range correlations observed in the HMBC spectrum of 1.

with C-7′ (δ 43.3) and C-3 (δ 88.1). These HMBC experimental data suggested that C-2 was located between C-3 and C-7′. Moreover, the carbon signal at δ 85.5 (C-4) was correlated with aromatic proton signals at δ 6.52 (H-2″) and 6.61 (H-6″), indicating that one of alkynyl carbons was linked to the phenyl group. The presence of two hydroxyl groups in **1** was supported by acetylation of **1** with acetic anhydride in pyridine to afford a monoacetate (**1a**) and a diacetate (**1b**). Thus, the structure of **1** was determined to be 2-(3,4-methylenedioxybenzyl)-4-(3,4-methylenedioxyphenyl)-3-butyne-1,2-diol, which we named virgatyne.

Compound **2** gave a positive color test with ferric chloride (a dark blue). The ^1H NMR spectral features of **2** were very similar to those of euphormisin M_2 (**3**),[15] except for the absence of an hexahydroxydiphenoyl (HHDP) group and existence of three upfield-shifted signals at δ 4.93 (glc H-3), 4.16 (glc H-6), and 4.01 (glc H-6) assigned with the aid of $^1H-^1H$ and $^1H-^{13}C$ COSY. Comparing the ^{13}C NMR spectrum of **2** with that of **3**, only five carbonyl signals at δ 164.7, 165.2, 171.6, 172.1, and 176.1 were observed in the former compound, which supported the absence of the HHDP group in the structure of **2**. Additionally, the FABMS of **2** showed a molecular ion peak at *m/z* 622 ($C_{26}H_{22}O_{18}$) corresponding to the loss of an HHDP group (302 mass units) from **3** (*m/z* 924, $C_{40}H_{28}O_{26}$). The glc H-2 and glc H-4 signals resonated at a lower field (δ 5.13 and 4.93, respectively) than other glucose proton signals, indicating that the hydroxyl groups at glc C-2 and glc C-4 were acylated. The ABXY-type signals at δ 1.88 (1H, dd, J = 3.2, 17.0 Hz), 2.15 (1H, dd, J = 11.0, 17.0 Hz), 3.63 (dd, J = 2.9, 11.0 Hz), and 4.93 (overlapped with glc H-3, 4) attributed to two methylene (H-4′) and two methine (H-3′ and H-2′) protons, respectively, and an aromatic proton signal at δ 7.26 (1H, s) were due to the acyl moiety at O-2/O-4 of the glucose core. The glucopyranose ring in **2** was indicated from the coupling pattern of the sugar proton signals to adopt the $^1C_4$-conformation.[15] Accordingly, this compound has the structure represented by **2** and was named virganin.

Compounds **4**−**6** were characterized as flavonol-8-sulfonates on the basis of spectral analyses and chemical evidence. Compound **4** was obtained as a yellow powder. Its UV spectrum showed absorption bands at λ_{max} 355, 316, 268, and 213 nm. The negative FABMS of **4** displayed an ion peak at *m/z* 371 $[M - H]^-$ corresponding to the formula $C_{15}H_9O_8SNa$. In addition, peaks at *m/z* 349 $[M - Na]^-$ and 269 $[M - Na - SO_3]^-$ suggested the presence of a sodium sulfate or sodium sulfonate in the structure of **4**, and Na^+ was also confirmed by atomic absorption spectroscopy. The aryl proton signal of the A-ring of compound **4** was observed at δ 6.19 (1H, s), and ^{13}C NMR spectral data indicated that compound **4** was a flavonol with a 5,6,7- or 5,7,8-trisubstituted structure. The ^1H and ^{13}C NMR spectra of **5** were similar to those of **4** except for the presence of signals of a sugar residue, which indicated that compound **5** was a glycoside of **4**. This observation was consistent with the negative FABMS spectrum of **5**, which displayed an ion peak at *m/z* 533 $[M - H]^-$, 163 units higher than that of **4**.

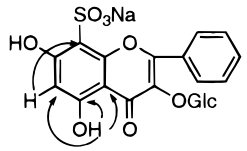

Figure 2. $^1H-^{13}C$ long-range correlations observed in the HMBC spectrum of 5.

In the ^{13}C NMR spectrum of **5,** the C-3 signal appeared ca. 3 ppm upfield and the C-2 signal (δ 155.2) appeared ca. 9 ppm downfield from those of **4** (δ 145.9),[16] suggesting that the location of this sugar moiety was at C-3. It was further confirmed by the observation that its anomeric proton signal (δ 5.56) showed a cross-peak with the carbon signal at δ 134.6 (C-3) in the HMBC spectrum of **5**. The configuration of the anomeric center was determined to be β on the basis of the coupling constant of the anomeric proton signal (δ 5.56, 1H, d, J = 7.6 Hz). In DEPT and HMBC spectra of **5** (Figure 2), the proton signal at δ 12.51 (chelated 5-OH) showed a cross-peak by two-bond coupling with the carbon signal at δ 160.8 and also showed cross-peaks by three-bond coupling with the carbon signals at δ 110.0 (quaternary carbon) and 98.6 (tertiary carbon). The assignment of C-5 (δ 160.8), C-10 (δ 110.0), and C-6 (δ 98.6) was thus confirmed. Therefore, the signal at δ 6.19 was assignable as H-6, which correlated with the C-6 signal at δ 98.6. Moreover, the H-6 signal showed cross-peaks with the carbon signals at δ 110.0 (C-10) and δ 104.3, allowing us to assign the latter to C-8. Acid hydrolysis[17] of compounds **4** and **5** yielded **4a**, which was identified as galangin (5,7-dihydroxyflavonol) by comparison of its ^{13}C NMR spectral data with those reported in the literature.[18] The hydrolysate gave a white precipitate on addition of barium chloride, which also confirmed the presence of the sulfonate or the sulfate group.[19,20] In addition, the sugar in the hydrolysate of **5** was identified as glucose by Si gel TLC comparison[21] with authentic sample. Comparing the ^1H NMR spectra of **4** and **4a**, the signal of ring A changed from a singlet (**4**, δ 6.16, H-6) into a pair of meta-coupling doublets (**4a**, δ 6.17 and 6.42, each 1H, J = 1.9 Hz), indicating the substituent at C-8 was a sulfonate group. This supposition was supported by the presence of the ion peaks at *m/z* 371 $[M - H]^-$ and *m/z* 269 $[M - Na - SO_3]^-$ in the FABMS spectrum of compound **4**. Furthermore, the C-8 (δ 103.2) signal in the ^{13}C NMR spectrum of **4** appeared ca. 10 ppm downfield from that (δ 93.6) of **4a**. This spectral characteristic was in agreement with that of disodium epicatechin-(4β,5′)-disulfonate,[22] which showed that the aromatic carbon carrying the sulfonyl group shifts downfield ca. 12 ppm compared to its aglycon.[23] In general, sulfation of a phenolic hydroxy group exhibits upfield displacement (3.5−5.0 ppm) for the *ipso* carbon.[19−20,24] Based on the above data, compound **4** was identified as galangin-8-sulfonate. Therefore, compound **5** was established as galangin-3-*O*-β-D-glucoside-8-sulfonate.

The ^1H and ^{13}C NMR spectra of compound **6** indicated that its structure differed from that of compound **4** by the presence of a hydroxy substitute on the B-ring, in agreement with a molecular ion at *m/z* 387 $[M - H]^-$ (16 units larger than **4**) and $[M - Na]^-$ peaks at *m/z* 365 and *m/z* 285 $[M - Na - SO_3]^-$ in the negative FABMS spectrum. Moreover, acid hydrolysis of compound **6** gave kaempferol, identified by direct comparison (co-TLC, ^1H NMR, and ^{13}C NMR spectra) with an authentic sample. Accordingly, compound **6** was determined to be kaempferol-8-sulfonate.

Although flavonoid sulfates and $C-O-SO_3^-$ linkage, are known to be present in a number of plant families,[19,20] only a relative few sulfonates such as sodium quercetin-5′-

sulfonate, sodium apigenin-3′-sulfonate,[25,26] and sodium procyanidin sulfonate[22] have been characterized. These three compounds (**4**−**6**) represent the first known examples of flavonoid-8-sulfonates from a natural source.

Experimental Section

General Experimental Procedures. IR spectra were obtained on a Bio-Rad FTS-7 spectrometer. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. UV spectra were recorded on a JASCO model 7800 UV/vis spectrometer. Atomic absorption analysis was performed by a Hitachi 180−30 atomic absorption spectrometer. EIMS and FABMS spectra were obtained using a JEOL JMS-D100 and a JEOL SX-102A spectrometer, respectively. ^{1}H NMR and ^{13}C NMR spectra were measured with a Bruker AM-300 spectrometer and a Varian Gemini-200 spectrometer.

Plant Material. The whole plants of *P. virgatus* were collected at Pintoung, Taiwan, in July 1993. A voucher specimen was deposited in the National Research Institute of Chinese Medicine.

Extraction and Isolation. In a previous paper,[2] it was reported that an EtOH extract of the dried whole plants of *P. virgatus* (3.0 kg) was chromatographed on a Si gel column eluting with gradient solvent systems of *n*-hexane−EtOAc (10:1 to 0:1) and CH_2Cl_2−MeOH (10:1 to 0:1) to yield 38 fractions. Fractions 27−30 belonged to the portion eluted with EtOAc. From fraction 27, indole-3-carboxylic acid was isolated.[2] Further separation of fraction 27 through a Si gel column eluted with CH_2Cl_2−MeOH (15:1) gave mixtures A and B. Mixture A was further separated by Sephadex LH-20 (MeOH−H_2O 3:1) and preparative silica-TLC (CH_2Cl_2−Me_2-CO 10:1) to afford virgatyne (**1**, 34 mg), indole-3-carboxaldehyde (17 mg), and (−)-lirioresinol-B (12 mg). Mixture B was separated by preparative silica-TLC (CH_2Cl_2−MeOH 6:1) to give methyl gallate (13 mg). Fractions 28 and 30 were repeatedly rechromatographed over Sephadex LH-20, eluting with MeOH, to afford gallic acid (18 mg) from fraction 28, as well as methyl brevifolincarboxylate (35 mg) and quercitrin (17 mg) from fraction 30. Fractions 31 and 32 were purified by elution from Sephadex LH-20 with aqueous MeOH (50%, 75%, 90%). The 75% MeOH eluate was further purified by GPC on Sephadex LH-20 (MeOH−H_2O 3:1) and MPLC (Cosmosil C-18, 0−25% aqueous MeOH) to obtain isoquercitrin (33 mg), astragalin (26 mg), 1,6-di-*O*-galloyl-*β*-D-glucose (18 mg), corilagin (47 mg), myricitrin (16 mg), and furosin (7 mg). Similarly, the 90% MeOH eluate gave virganin (**2**, 5 mg), 1,4,6-tri-*O*-galloyl-*β*-D-glucose (17 mg), and 1,3,4,6-tetra-*O*-galloyl-*β*-D-glucose (19 mg). Fraction 34 was subjected to the same procedure as fractions 31 and 32. The 50% MeOH eluate was purified on Sephadex LH-20 (MeOH−H_2O 3:1) to give 1-*O*-galloyl-*β*-D-glucose (12 mg), 5,7-dihydroxy-4′-methoxyflavonol (25 mg), quercetin-3-*β*-*O*-D-glucosyl-(1→6)-*β*-D-glucoside (32 mg), galangin-3-*O*-*β*-D-glucoside-8-sulfonate (**5**, 42 mg), and rutin (35 mg). The 75% MeOH eluate was similarly chromatographed to afford kaempferol (18 mg), quercetin (15 mg), galangin-8-sulfonate (**4**, 37 mg), and kaempferol-8-sulfonate (**6**, 25 mg). The 90% MeOH eluate was further purified by MPLC with aqueous MeOH (10% to 25%) to afford geraniin (28 mg). Rechromatography of fraction 35 over Sephadex LH-20 with MeOH−H_2O (3:1) furnished potassium brevifolincarboxylate (34 mg) and brevifolin (16 mg).

Virgatyne (1): needles from *n*-hexane; mp 123−125°; $[\alpha]^{25}_D$ +30° (*c* 1.0, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ϵ) 294 (3.67), 265 (3.86), 228 (3.98) nm; IR (KBr) ν_{max} 3322 (OH), 2236 (C≡C), 1606 and 1496 (aromatic rings), 932 (O−CH_2−O) cm^{-1}; EIMS (70 eV) *m*/*z* 340 $[M]^+$ (18), 322 (13), 205 (98), 187 (30), 159 (44), 136 (100), 77 (20); HREIMS 340.0950, calcd for $C_{19}H_{16}O_6$, 340.0947; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 2.32 (1H, br s, −OH), 2.69 (1H, d, *J* = 13.4 Hz, H-7′), 2.75 (1H, d, *J* = 13.4 Hz, H-7′), 3.19 (1H, br s, −OH), 3.33 (2H, m, H-1), 5.64 (2H, s, O−CH_2−O), 5.68 (2H, s, O−CH_2−O), 6.44 (1H, d, *J* = 8.1 Hz, H-5′), 6.46 (1H, d, *J* = 7.8 Hz, H-5″), 6.52 (1H, d, *J* = 1.6 Hz, H-2″), 6.56 (1H, dd, *J* = 1.6, 8.1 Hz, H-6′), 6.61 (1H, dd, *J* = 1.6, 7.8 Hz, H-6″), 6.65 (1H, d, *J* = 1.6 Hz, H-2′); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 43.3 (C-7′), 68.0 (C-1), 71.5 (C-2), 85.5 (C-4), 88.1 (C-3), 100.2 and 100.8 (O−CH_2−O), 107.1, (C-5″), 107.8 (C-5′), 110.7 (C-2′), 111.0 (C-2″), 115.4 (C-1′), 123.3 (C-6′), 125.7 (C-6″),129.7 (C-1″), 146.0 (C-4′), 146.7 (C-3″), 146.9 (C-3′), 147.5 (C-4″).

Acetylation of Virgatyne (1). Virgatyne (**1**, 5 mg) in pyridine (0.5 mL) and Ac_2O (1 mL) was allowed to stand at room-temperature overnight. Usual workup and purification by preparative TLC (*n*-hexane−EtOAc 5:1) afforded monoacetate **1a** (1.5 mg) and diacetate **1b** (3.0 mg). Compound **1a**: ^{1}H NMR ($CDCl_3$, 200 MHz) *δ* 2.15 (3H, s, OCOMe-1), 3.00 (2H, br s, H_2-7′), 4.13 (1H, d, *J* = 11.2 Hz, H-1), 4.36 (1H, d, *J* = 11.2 Hz, H-1), 5.96 (2H, s, O−CH_2−O), 5.98 (2H, s, O−CH_2−O), 6.73−6.91 (6H, m, aromatic protons). Compound **1b**: ^{1}H NMR ($CDCl_3$, 200 MHz) *δ* 2.05 (3H, s, OCOMe-2), 2.14 (3H, s, OCOMe-1), 3.14 (1H, d, *J* = 13.6 Hz, H-7′), 3.34 (1H, d, *J* = 13.6 Hz, H-7′), 4.21 (1H, d, *J* = 11.5 Hz, H-1), 4.61 (1H, d, *J* = 11.5 Hz, H-1), 5.93 (2H, s, O−CH_2−O), 5.95 (2H, s, O−CH_2−O), 6.69−6.94 (6H, m, aromatic protons).

Virganin (2): brown amorphous powder; $[\alpha]^{25}_D$ −43° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 279 (4.38), 220 (4.66) nm; HRFABMS *m*/*z* 622.0845 $[M]^-$, calcd for $C_{26}H_{22}O_{18}$, 622.0806; ^{1}H NMR (Me_2CO-d_6 + D_2O, 200 MHz) *δ* 1.88 (1H, dd, *J* = 3.2, 17.0 Hz, H-4′), 2.15 (1H, dd, *J* = 11.0, 17.0 Hz, H-4′), 3.63 (1H, dd, *J* = 2.9, 11.0 Hz, H-3′), 4.01 (1H, dd, *J* = 6.1, 11.2 Hz, glc H-6), 4.16 (1H, dd, *J* = 6.7, 11.2 Hz, glc H-6), 4.36 (1H, br t, glc H-5), 4.93 (3H, m, H-2′, glc H-3, 4), 5.13 (1H, dd, *J* = 2.2, 3.8 Hz, glc H-2), 6.25 (1H, d, *J* = 2.2 Hz, glc H-1), 7.15 (2H, s, galloyl-H), 7.26 (1H, s, H-3); ^{13}C NMR (Me_2CO-d_6 + D_2O, 50 MHz) *δ* 30.6 (C-4′), 45.2 (C-3′), 47.8 (C-2′), 60.5 (glc C-3), 62.9 (glc C-6), 72.2 (glc C-4), 72.7 (glc C-2), 78.8 (glc C-5), 92.5 (glc C-1), 110.1 (galloyl C-2, 6), 114.1 (C-3), 117.5, 118.4, 120.6, 135.9, 139.4, 146.1 (2 × C), 147.7, 164.7, 165.2, 171.6, 172.1, 176.1.

Galangin-8-sulfonate (4): yellow powder; mp 186−188 °C; UV (MeOH) λ_{max} (log ϵ) 213 (4.54), 268 (4.32), 316 (4.12), 355 (4.22) nm; IR (KBr) ν_{max} 3432 (OH), 2918, 1654 (C═O), 1562, 1459, 1424, 1385, 1223 (S═O), 1122 cm^{-1}; negative FABMS *m*/*z* 371 $[M - H]^-$ (30), 349 $[M - Na]^-$ (100), 269 $[M - Na - SO_3]^-$ (17); HRFABMS 348.9987 $[M - Na]^-$, calcd for $C_{15}H_9O_8S$, 349.0018; ^{1}H NMR (DMSO-d_6, 300 MHz) *δ* 6.16 (1H, s, H-6), 7.45−7.56 (3H, m, H-3′,4′,5′), 8.50 (2H, dd, *J* = 1.9, 8.1 Hz, H-2′, 6′), 12.50 (1H, br s, 5-OH); ^{13}C NMR (DMSO-d_6, 75 MHz) *δ* 98.0 (C-6), 103.2 (C-8), 109.9 (C-10), 128.0 (C-2′, 6′), 128.2 (C-3′, 5′), 129.7 (C-4′), 131.0 (C-1′), 137.3 (C-3), 145.9 (C-2), 153.0 (C-9), 160.5 (C-5, 7), 176.1 (C-4).

Acid Hydrolysis of Galangin-8-sulfonate (4). Compound **4** (10 mg) in 2 N HCl was heated for 2 h in a boiling H_2O bath to yield galangin (**4a)** (2.3 mg), EIMS *m*/*z* $[M]^+$ 270 (100). The hydrolysate gave a white precipitate with $BaCl_2$, which was assumed to be $BaSO_4$.[17]

Galangin-3-*O*-*β*-D-glucoside-8-sulfonate (5): yellow powder; mp 215−217 °C; $[\alpha]^{25}_D$ −6° (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.65), 267 (4.47), 304 (4.24) nm; negative FABMS *m*/*z* 533 $[M - H]^-$ (29), 511 $[M - Na]^-$ (100), 371 [M − glucosyl]$^-$ (49); ^{1}H NMR (DMSO-d_6, 300 MHz) *δ* 3.09−3.34 (5H, m, glucosyl protons), 3.54 (1H, d, *J* = 12.0 Hz, H-6″), 5.56 (1H, d, *J* = 7.6 Hz, H-1″), 6.19 (1H, s, H-6), 7.49−7.54 (3H, m, H-3′,4′,5′), 8.49 (2H, dd, *J* = 2.0, 7.6 Hz, H-2′, 6′), 12.51 (1H, s, 5-OH); ^{13}C NMR (DMSO-d_6, 75 MHz) *δ* 60.6 (C-6″), 69.8, 74.2, 76.4, 77.5 (glucosyl carbons), 98.6 (C-6), 100.8 (C-1″), 104.3 (C-8), 110.0 (C-10), 128.1 (C-3′, 5′), 129.3 (C-2′, 6′), 130.2 (C-1′), 130.9 (C-4′), 134.6 (C-3), 153.1 (C-9), 155.2 (C-2), 160.8 (C-5), 161.0 (C-7), 177.7 (C-4).

Acid Hydrolysis of Galangin-3-*O*-*β*-D-glucoside-8-sulfonate (5). Compound **5** (10 mg) in 20% H_2SO_4 was heated for 1 h in a boiling H_2O bath to yield **4a** (3.2 mg). After neutralization, the hydrolysate was analyzed by Si gel TLC [Kieselgel 60 (Merck Art. 5554), *i*PrOH−Me_2CO−H_2O (5:3:1)].[21] It showed a brown spot (R_f 0.46) on TLC over spraying anilinephthalate solution and heating, which was coincident with that of glucose.

Kaempferol-8-sulfonate (6): yellow powder; mp 204−206 °C; UV (MeOH) λ_{max} (log ϵ) 268 (4.28), 366 (4.41) nm; negative

FABMS *m/z* 387 $[M - H]^-$ (40), 365 $[M - Na]^-$ (100), 285 $[M - Na - SO_3]^-$ (11); HRFABMS 364.9955 $[M - Na]^-$, calcd for $C_{15}H_9O_9S$, 364.9967; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.13 (1H, s, H-6), 6.89 (2H, d, J = 8.8 Hz, H-3′, 5′), 8.37 (2H, d, J = 8.8 Hz, H-2′, 6′), 12.44 (1H, s, 5-OH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 97.9 (C-6), 103.0 (C-8), 109.8 (C-10), 115.2 (C-3′, 5′), 121.8 (C-1′), 130.1 (C-2′, 6′), 135.6 (C-3), 147.2 (C-2), 152.6 (C-9), 159.2 (C-4′), 160.3 (C-5), 160.6 (C-7), 175.7 (C-4).

Acid Hydrolysis of Kaempferol-8-sulfonate (6). Compound **6** (5 mg) in 2 N HCl was heated for 2 h in a boiling H_2O bath to yield kaempferol (1.6 mg). The hydrolysate gave a white precipitate with $BaCl_2$, which was assumed to be $BaSO_4$.

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