Allelopathic Prenylflavanones from the Fallen Leaves of *Macaranga tanarius*

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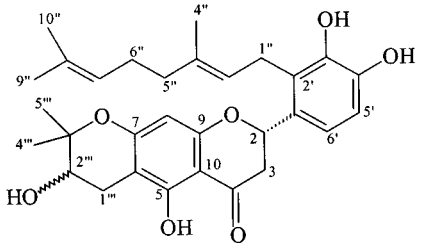
Two new prenylflavanones, tanariflavanones A (**1**), and B (**2**), and one known compound, (−)-nymphaeol-C (**3**), were isolated from the fallen leaves of *Macaranga tanarius*. Compounds **1** and **2** showed inhibition of radicle growth of lettuce seedlings at 200 ppm. Their structures were elucidated primarily by NMR, circular dichroism, and mass spectroscopic methods.

The genus *Macaranga* is one of the largest genera of the Euphorbiaceae, with approximately 300 species.[1] Previously reported components from the species of this genus include diterpenoids, triterpenoids, steroids,[2,3] and hydrolyzable tannins[4] from *M. tanarius*, a prenyl stilbene, vedelianin,[5] and a geranyl flavonol[6] from *M. vedeliana*, antibacterial prenylated flavanones[7] from *M. pleiostemona*, chromenoflavones[8] from *M. indica*, and cytotoxic geranyl stilbenes[9] from *M. schweinfurthii*. *M. tanarius* is a common tropical tree distributed from southern Asia to northern Australia.[10] The allelopathic potential of the fallen leaves of *M. tanarius* was indicated from field observations and bioassays, prompting us to study its chemical components. A methanol extract of the fallen leaves was suspended in H_2O and then partitioned with ethyl acetate. The ethyl acetate solubles were fractionated to yield two new prenylflavanones, tanariflavanones A (**1**) and B (**2**), and the known (−)-nymphaeol-C (**3**).[11] This paper describes the structural elucidation of **1** and **2** and their inhibitory effect on radicle growth of germinating lettuce seeds.



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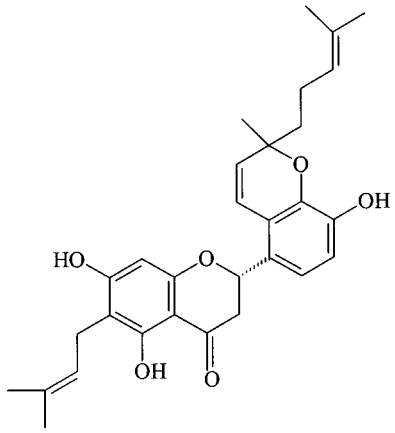

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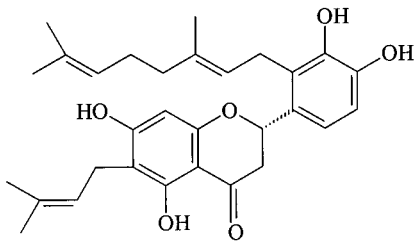

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The molecular formula ($C_{30}H_{36}O_7$) of **1**, showing one more oxygen atom than **3**, was obtained from its HREIMS and ^{13}C NMR data. The IR spectrum of **1** indicated the presence of hydroxyl (3422 cm^{-1}), conjugated carbonyl (1647 cm^{-1}), and aromatic (1610 and 1500 cm^{-1}) groups. Its UV spectrum exhibited maxima at 231 (sh) and 293 nm. The ^1H NMR spectrum of **1** exhibited signals for a phenolic OH at δ 12.35 (s, OH-5), which was a hydrogen strongly bonded to the 4-carbonyl group. An ABX system at δ 5.48 (1H, dd, J = 12.8, 2.8 Hz), 2.73 (1H, dd, J = 17.2, 2.8 Hz), and 3.12 (1H, dd, J = 17.2, 12.8 Hz) was diagnostic for H-2 and H-3 of a flavanone nucleus.[11] The appearance of three olefinic methyl groups (δ 1.57, 1.64, 1.74), four multiplet methylene protons (δ 2.06), two benzylic methylene protons (δ 3.42, br d, J = 6.8 Hz), and two triplet vinyl protons (δ 5.02, 5.17) indicated the presence of a geranyl group.[11] The presence of a 2,2-dimethylchromane moiety with a secondary hydroxy group was revealed by signals at δ 1.31 and 1.35 (3H each, s), 2.64 (1H, dd, J = 16.8, 5.2 Hz, H_a-1‴), 2.87 (1H, dd, J = 16.8, 5.2 Hz, H_b-1‴), and 3.83 (1 H, t, J = 5.2 Hz, H-2‴). The geranyl group was placed at C-2′ due to H_2-1″ and H-2 having NOESY correlation. There was also a significant MS fragment at m/z 384 [M^+ − 124].[11] The absence of a bathochromic shift in the UV spectrum of **1**, after the addition of aluminum chloride, indicated a substituent at C-6.[7] The methylene protons at δ 2.64 and 2.87 were assigned to a benzylic methylene. The coupling constant between H-1‴ and H-2‴ clearly indicated that the hydroxyl group at C-2‴ occupied an axial position. Therefore, 2-hydroxy-3,3-dimethylchromane must fuse on ring A. The *ortho*-coupled signals at δ 6.80 and 6.96 (J = 8.4 Hz, H-5′, -6′) as well as H-6′ and H-3 having NOESY correlation suggested that C-1′, -2′, -3′, and -4′ positions in ring B were substituted. Therefore, two phenolic hydroxyl groups (δ 5.56 and 5.89, exchangeable) were assigned to C-3′ and C-4′. Thus, compound **1** was deduced to

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be 2′-geranyl- 5,3′,4′-trihydroxy-6,7-(2,2-dimethyl-3-hydroxychromano)flavanone. The absolute stereochemistry at C-2 of **1** was established as *S* based upon a positive extremum at 344 nm ($\theta = +13\,590$) and a negative extremum at 291 nm ($\theta = -34\,010$) in its circular dichroism spectrum.[12]

Compound **2** had the molecular formula $C_{30}H_{34}O_6$ on the basis of HREIMS and ^{13}C NMR data. The IR spectrum of **2** showed bands attributed to hydroxyl (3374 cm^{-1}), conjugated carbonyl (1641 cm^{-1}), and aromatic (1606 and 1449 cm^{-1}) groups. Its UV spectrum exhibited maxima at 227 (sh), 274, and 285 nm. Comparison of ^{13}C NMR data of **2** with those of known (−)-nymphaeol-C (**3**) suggested that **2** possessed the same skeletal structure as **3**. Closer inspection of ^1H, ^{13}C, and DEPT NMR spectra of **2** revealed the presence of three methyls (δ_H 1.41, 1.55, and 1.65; δ_C 17.6, 25.6, and 26.1), two methylenes (δ_C 40.7 and 22.7), one oxygenated quaternary carbon (δ_C 79.0), and two double bonds (δ_C 118.9 and 130.9; 123.7 and 132.1). In the ^1H NMR spectrum of **2**, vicinally coupled protons at δ 5.65 and 6.58 ($J = 10.0$ Hz) indicated that an oxygen atom at C-3′ must have cyclized onto C-3″ to form a pyran ring as shown in the structure **2**. This arrangement was supported by the observation of a fragment at *m/z* 407 $[M - CH_2\text{-}CH_2CH{=}C(Me)_2]^+$ in the EIMS of **2**.[13] The ^1H NMR spectrum [δ 1.74, 1.80 (each 3H, s), 3.32 (2H, br d, $J = 5.8$ Hz, H-1‴), 5.23 (H, br t, H-2‴)] further showed the presence of one 1,1-dimethylallyl group. This group was placed at C-6, as **2** also showed no bathchromic shift on the addition of aluminum chloride. Compound **2** had the same absolute configuration (2 *S*) as **1** and **3** on the basis of the positive Cotton effect at 340 nm ($\theta = +12\,390$) and the negative Cotton effect at 287 nm ($\theta = -16\,570$). The structure of **2** was thus assigned as shown.

The tentative phytotoxic activity of **1** and **2** was evaluated by determining their effect on the radicle elongation of germinating lettuce. At 200 ppm, **2** inhibited radicle length of lettuce up to 30% as compared to the distilled H_2O control, while **1** caused 11% growth inhibition. These results suggest that compounds **1** and **2** play an allelopathic role in *M. tanarius*.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 983G spectrophotometer. ^1H and ^{13}C NMR spectra were run on a Varian Unity Plus 400 spectrometer. EIMS and specific rotations were taken on a JEOL JMS-HX 300 mass spectrometer and a JASCO DIP-1000 digital polarimeter, respectively. Extracts were chromatographed on silica gel (MERK 70-230, 230−400 mesh, ASTM).

Plant Material. The fallen leaves of *M. tanarius* were collected from the campus of National Taiwan University, Taiwan, in 1998. A voucher specimen (voucher no. 01566) has been deposited at the Herbarium of the Department of Mathematics and Science Education, Taipei Municipal Teachers College, Taipei, Taiwan.

Extraction and Isolation. The dried fallen leaves of *M. tanarius* were ground to powder, and 4 kg of the powder was extracted with MeOH (100 L) at room temperature (7 days × 2). The extract was evaporated in vacuo to yield a residue, which was suspended in H_2O (1 L), and this was partitioned with ethyl acetate (1 L × 3). The combined ethyl acetate layer afforded a black syrup (250 g) that was subsequently chromatographed over silica gel using a hexane/EtOAc gradient solvent system. Crude compounds **1**, **2**, and **3** were all eluted with 30% EtOAc in hexane. Further purification by HPLC (Merck LichroCART 250-10 Cat. 1.50179 Lichrosorb Si 60 (7 μm)) gave **1** (30 mg), **2** (7 mg), and **3** (500 mg) using 30% EtOAc/hexane.

Bioassays. A 200 ppm solution of each pure isolated compound in MeOH was prepared for bioassay. The solutions were spread onto the silica TLC sheets (1 cm × 5 cm), allowing the MeOH to evaporate completely in a laminar flow hood. Prior to bioassay, the TLC sheets were placed in Petri dishes and moistened with distilled H_2O, then surrounded by wet sponge without contact. Lettuce (*Lactuca sativa* L. var. *longifolia* Lam.) seeds were imbibed in distilled H_2O for 2 h, then placed on the TLC sheets containing the pure compound or on untreated control sheets. The Petri dishes were sealed with M Parafilm (American National Can), then placed in an incubator at 25 °C for 48 h in dark.

Tanariflavanone A (1): greenish oil; $[\alpha]^{24.6}_D$ +26.8° (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 231 sh (4.34), 293 (4.29) nm; IR (KBr) ν_{max} 3422, 1647, 1610, 1500, 1457 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.31, 1.35, 1.57, 1.64, 1.74 (each 3H, s), 2.06 (4H, m, H-5″,-6″), 2.64 (1H, dd, $J = 16.8$, 5.2 Hz, H_a-1‴), 2.73 (1H, dd, $J = 17.2$, 2.8 Hz, H_{eq}-3), 2.87 (1H, dd, $J = 16.8$, 5.2 Hz, H_b-1‴), 3.12 (1H, dd, $J = 17.2$, 12.8 Hz, H_{ax}-3), 3.42 (2H, br d, $J = 6.8$ Hz, H-1″), 3.83 (1H, t, $J = 5.2$ Hz, H-2‴), 5.02 (1H, br t, $J = 6.8$ Hz, H-7″), 5.17 (1H, br t, $J = 6.6$ Hz, H-2″), 5.48 (1H, dd, $J = 12.8$, 2.8 Hz, H-2), 5.94 (1H, s, H-8), 6.80, 6.96 (each 1H, d, $J = 8.4$ Hz, H-5′, -6′), 5.56, 5.89, 12.35 (each 1H, s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4 (s, C-4), 161.9 (s, C-9), 161.5 (s, C-7), 160.8 (s, C-5), 144.8 (s, C-4′), 142.5 (s, C-3′), 138.9 (s, C-3″), 132.2 (s, C-8″), 128.3 (s, C-1′), 126.4 (s, C-2′), 123.7 (d, C-7″), 121.3 (d, C-2″), 118.9 (d, C-6′), 112.9 (d, C-5′), 102.7 (s, C-6), 99.9 (s, C-10), 96.2 (d, C-8), 78.6 (s, C-3‴), 76.9 (d, C-2), 68.8 (d, C-2‴), 42.4 (t, C-3), 39.6 (t, C-5″), 26.3 (t, C-6″), 25.7 (q, C-10″), 25.4 (t, C-1″), 25.0 (q, C-4‴), 24.9 (t, C-1‴), 22.0 (q, C-5‴), 17.7 (q, C-9″), 16.2 (q, C-4″); EIMS *m/z* 508 $[M]^+$ (33), 490 (10), 423 (50), 384 (29), 237 (100); HREIMS *m/z* 508.2446 (calcd for $C_{30}H_{36}O_7$, 508.2451).

Tanariflavanone B (2): brownish oil; $[\alpha]^{24.6}_D$ +28.2° (*c* 0.5, $CHCl_3$); IR ν_{max} 3374, 1641, 1606, 1449, 1156 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 227 (4.83), 274 (4.47), 285 (4.49) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 1.41, 1.55, 1.65, 1.74, 1.80 (each 3H, s), 2.08 (4H, m, H-5″, -6″), 2.73 (1H, dd, $J = 17.2$, 2.8 Hz, H_{eq}-3), 3.11 (1H, dd, $J = 17.2$, 13.2 Hz, H_{ax}-3), 3.32 (2H, br d, $J = 5.8$ Hz, H-1‴), 5.07 (H, br t, $J = 5.7$, H-7″), 5.23 (H, br t, $J = 5.8$ Hz, H-2‴), 5.48 (1H, dd, $J = 13.2$, 2.8 Hz, H-2), 5.65 (1H, d, $J = 10.0$ Hz, H-2″), 5.97 (1H, s, H-8), 6.58 (1H, d, $J = 10.0$ Hz, H-1″), 6.81 (1H, d, $J = 8.4$ Hz, H-5′), 6.89 (1H, d, $J = 8.4$ Hz, H-6′); 5.66, 6.32, 12.38 (each 1H, s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3 (s, C-4), 163.7 (s, C-7), 161.3 (s, C-9), 161.0 (s, C-5), 145.1 (s, C-4′), 139.7 (s, C-3′), 135.3 (s, C-3‴), 132.1 (s, C-8″), 130.9 (d, C-2″), 124.8 (s, C-1′), 123.7 (d, C-7″), 121.5 (d, C-2‴), 118.9 (d, C-1″), 118.9 (d, C-6′), 118.8 (s, C-2′), 114.5 (d, C-5′), 107.2 (s, C-6), 102.9 (s, C-10), 95.5 (d, C-8), 79.0 (s, C-3″), 76.0 (d, C-2), 42.5 (t, C-3), 40.7 (t, C-5″), 26.1 (q, C-4″), 25.8 (q, C-4‴), 25.6 (q, C-10″), 22.7 (t, C-6″), 21.1 (t, C-1‴), 17.8 (q, C-5‴), 17.6 (q, C-9″); EIMS *m/z* 490 $[M]^+$ (17), 407 (100), 219 (26); HREIMS *m/z* 490.2374 (calcd for $C_{30}H_{34}O_6$, 490.2346).

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