New Prenylflavones from the Leaves of *Epimedium sagittatum*

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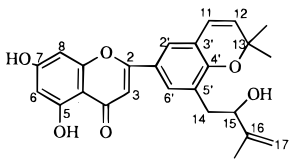
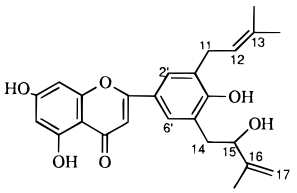
Five new prenylflavones, yinyanghuo A (**1**), yinyanghuo B (**2**), yinyanghuo C (**3**), yinyanghuo D (**4**), and yinyanghuo E (**5**), along with six known flavonoids, chrysoeriol, quercetin, apigenin, apigenin 7,4′-dimethyl ether, kaempferol, and luteolin, were isolated from the leaves of *Epimedium sagittatum*. Their structures were determined from spectral analysis. Compounds **1** and **2** showed significant antiplatelet induced by arachidonic acid.

In our previous paper,[1] we reported three new 2-phenoxychromones isolated from the Me_2CO extract of the aerial part of *Epimedium sagittatum* (Chinese name "Yinyanghuo", Berberidaceae). Further studies on the constituents of this drug resulted in the isolation of five new prenylfavones, yinyanghuo A (**1**), yinyanghuo B (**2**), yinyanghuo C (**3**), yinyanghuo D (**4**), and yinyanghuo E (**5**), along with six known flavonoids, chrysoeriol,[2] quercetin,[3] apigenin,[4] apigenin 7,4′-dimethyl ether,[5] kaempferol,[6] and luteolin.[7] All of these known compounds were identified by spectral analysis and comparison of their data (EIMS, IR, NMR) with published reports.[2−7] Herein, we report the structure elucidation of the five new compounds and the antiplatelet aggregation of compounds **1** and **2**.



1



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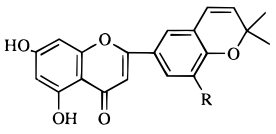
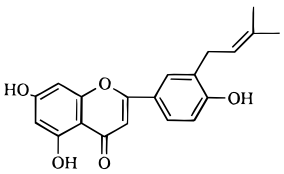


3 R = H
5 R = OH



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Yinyanghuo A (**1**) was shown to have the molecular formula $C_{25}H_{24}O_6$ by HREIMS. The unambiguous assignments of ^1H NMR signals of **1** were achieved on the basis of one-dimensional (1D) and two-dimensional (2D) NMR techniques such as $^1H-^1H$ COSY. The signals at δ 1.42 (6H, s), 5.85 (1H, d, J = 9.8 Hz), and 6.50 (1H, d, J = 9.8 Hz) were derived from one *gem*-dimethylchromene ring,[8,9] and signals at δ 1.75 (3H, s), 2.58 (1H, m) 2.84 (1H, m), 4.18 (1H, br, s), 4.70 (1H, s) and 4.80 (1H, s) were derived from the 2-hydroxy-3-methyl-3-butenyl group. The signals at δ 6.18 (1H, d, J = 2.1 Hz), 6.47 (1H, d, J = 2.1 Hz), and 6.76 (1H, s) were attributed to H-6, H-8, and H-3, respectively. The presence of only a pair of downfield doublets (J = 2.0 Hz) at δ 7.67 and 7.71 showed a 2′,6′-unsubstituted system in the B ring. Irradiation of the signal of H-3 (δ 6.76) gave 6% NOE effects on the H-2′ and H-6′ signals at δ 7.67 and 7.71, respectively. Furthermore, irradiation of the H-11 (δ 6.50) signal caused 3% and 4% NOE effects at δ 5.85 (H-12) and 7.67 (H-2′), respectively. This clearly indicated that the *gem*-dimethylchromene moiety was fused to C-3′ and C-4′. Thus, the 2-hydroxy-3-methyl-3-butenyl group was attached to C-5′. From the above spectral evidence, we propose formula **1** for the structure of yinyanghuo A.

Yinyanghuo B (**2**) was shown to have the molecular formula $C_{25}H_{26}O_6$ by HREIMS. In the ^1H NMR spectrum, the presence of a γ,γ-dimethylallyl group was evidenced by signals at δ 1.65 (6H, s), 3.40 (2H, d, J = 7.1 Hz), and 5.38 (1H, m).[1,8−11] As in the case of **1**, compound **2** also displayed the signals of a 2-hydroxy-3-methyl-3-butenyl group at δ 1.80 (3H, s), 2.58, 2.84 (each 1H, m), 4.47 (1H, m), and 4.83, 5.02 (each 1H, s). Similarly, the signals at δ 6.24, 6.49 (each 1H, d, J = 2.1 Hz), and 6.60 (1H, s) were assigned to H-6, H-8, and H-3, respectively; the two downfield doublet (each 1H, J = 2.2 Hz) signals at δ 7.69 and 7.71 were assigned to H-2′ and H-6′. From these results, the structure of yinyanghuo B is concluded to be formula **2**.

Yinyanghuo C (**3**) was shown to have the molecular formula $C_{20}H_{16}O_5$ by HREIMS. In the ^1H NMR spectrum, the *gem*-dimethylchromene ring was observed as one 6H singlet at δ 1.45 (*gem*-dimethyl) and two 1H doublets due to olefinic protons at δ 5.85 (1H, d, J = 10.1 Hz) and 6.52 (1H, d, J = 10.1 Hz).[8,9] The aromatic proton signals at δ 6.24, 6.53 (each 1H, d, J = 1.8 Hz), and 6.64 (1H, s) were assigned to H-6, H-8, and H-3, respectively. Furthermore, a typical ABX system at δ 6.89 (1H, d, J = 8.5 Hz, H-5′), 7.76 (1H, d, J = 2.1 Hz, H-2′), and 7.81 (1H, dd, J = 2.1 and 8.5 Hz, H-6′) showed the presence of three aromatic protons in the B ring. Irradiation of the H-2′ (δ 7.76) signal caused 6% and 4% NOE effects at δ 6.64 (H-3) and 6.52 (H-11),

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respectively. This clearly indicated that the *gem*-dimethylchromene moiety was fused to C-3′ and C-4′. On the basis of these results, we propose formula **3** for the structure of yinyanghuo C.

Yinyanghuo D (**4**) was shown to have the molecular formula $C_{20}H_{18}O_5$ by HREIMS. The presence of a γ,γ-dimethylallyl group was evidenced by signals at δ 1.75 (6H, s), 3.40 (2H, d, J = 7.3 Hz), and 5.38 (1H, m).[1,8−11] The aromatic proton signals of **4** were very similar to those of **3**. The γ,γ-dimethylallyl group was suggested to be attached at C-3′ due to the lower field signal [δ 7.80 (1H, d, J = 2.2 Hz)] of H-2′. From these results, the structure of yinyanghuo D is concluded to be formula **4**.

Yinyanghuo E (**5**) was shown to have the molecular formula $C_{20}H_{16}O_6$ by HREIMS. In the ^{1}H NMR spectrum, signals for a *gem*-dimethylchromene ring appeared at δ 1.46 (6H, s), 5.85 (1H, d, J = 10.0 Hz), and 6.50 (1H d, J = 10.0 Hz).[8,9] The two downfield doublet signals at δ 7.33 (1H, J = 2.0 Hz) and 7.39 (1H, J = 2.0 Hz) were assigned to H-6′ and H-2′. Irradiation of the H-11 (δ 6.50) signal caused 3% and 4% NOE effects at δ 5.85 (H-12) and 7.39 (H-2′), respectively. This clearly indicated that the *gem*-dimethylchromene ring was linked to C-3′ and C-4′ and one hydroxyl group was attached to C-5′. On the basis of these results, the structure of yinyanghuo E is represented by the formula **5**.

The antiplatelet effects of compounds **1** and **2** were tested *in vitro* by turbidimetry.[12,13] Both compounds showed significant antiplatelet aggregation induced by arachidonic acid; the IC_{50} values for **1** and **2** were determined as 7.14 and 1.67 μM, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. The IR spectra were recorded on a JASCO-IR-100 spectrometer. ^{1}H NMR spectra were taken on Bruker AM-300 WB (300 MHz) FT-NMR. HREIMS and EIMS spectra were recorded on JEOL SX-102A and JEOL JMS-HX100 spectrometers, respectively.

Plant Material. Plant material "Yinyanghuo" used in this study was purchased from a market in Taipei and was identified as the leaves of *Epimedium sagittatum* by Mr. M. T. Kao, National Research Institute of Chinese Medicine, where a voucher sample was deposited.

Extraction and Separation. In the previous paper,[1] it was reported that the Me_2CO extract of the leaves of *E. sagittatum* (15 kg) was chromatographed on Si gel, eluting with a gradient of *n*-hexane, *n*-hexane−Me_2CO (10:1 → 1:1) to give several fractions. The fraction eluted with *n*-hexane−Me_2CO (5:1) was rechromatographed on a Si gel column and subjected to preparative TLC (PTLC) to give three new 2-phenoxychromones. Further examination of this fraction with PTLC (*n*-hexane−EtOAc = 2:1 or CH_2Cl_2−MeOH = 18:1) gave yinyanghuo A (**1**; 200 mg), yinyanghuo B (**2**; 15 mg), yinyanghuo C (**3**; 10 mg) yinanghuo D (**4**; 5 mg), yinyanghuo E (**5**; 3 mg), and apigenin 7,4′-dimethylether (10 mg).

The fraction eluted with *n*-hexane−Me_2CO (1:1) in the above column chromatography was evaporated, and the residue was further chromatographed on a Si gel column (230−400 mesh) with *n*-hexane−Me_2CO (2:1) as an eluent to give five known flavonoids, chrysoeriol (17 mg), quercetin (45 mg), apigenin (100 mg), kaempferol (23 mg), and luteolin (34 mg).

Yinyanghuo A (**1**) was obtained as yellow needles: mp 230−232 °C; R_f 0.25 [*n*-hexane−EtOAc (2:1)]; $[\alpha]^{25}_D$ −1 (*c* 1.0, DMSO); EIMS (70 eV) *m/z* $[M]^+$ 420 (41), 405 (58), 350 (100), 335 (92), 153 (48); HREIMS 420.1587, calcd for $C_{25}H_{24}O_6$, 420.1573; IR (KBr) ν max (3500−3000 (br), 1650, 1615, 1570 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.42 (6H, s, 2 × CH_3-13), 1.75 (3H, s, CH_3-16), 2.58, 2.84 (each 1H, m, CH_2-14), 4.18 (1H, m, H-15), 4.70, 4.80 (each 1H, s, H-17), 5.85 (1H, d, J = 9.8 Hz, H-12), 6.18 (1H, d, J = 2.1 Hz, H-6), 6.47 (1H, d, J = 2.1 Hz, H-8), 6.50 (1H, d, J = 9.8 Hz, H-11), 6.76 (1H, s, H-3), 7.67 (1H, d, J = 2.0 Hz, H-2′), 7.71 (1H, d, J = 2.0 Hz, H-6′), 12.91 (1H, s, 5-OH); ^{13}C NMR, DEPT, and C−H COSY (DMSO-d_6) δ 161.4 (s, C-2), 103.3 (d, C-3), 181.5 (s, C-4), 163.4 (s, C-5), 99.0 (d, C-6), 164.7 (s, C-7), 94.0 (d, C-8), 157.3 (s, C-9), 103.4 (s, C-10), 122.0 (s, C-1′), 122.7 (d, C-2′), 120.6 (s, C-3′), 153.7 (s, C-4′), 127.3 (s, C-5′), 129.3 (d, C-6′), 121.4 (d, C-11), 131.3 (d, C-12), 77.2 (s, C-13), 35.7 (t, C-14), 73.5 (d, C-15), 148.1 (s, C-16), 109.8 (t, C-17), 27.8 and 28.0 (each q, 2 × CH_3-13), 17.8 (q, CH_3-16).

Yinyanghuo B (**2**) was obtained as yellow needles: mp 100−102 °C; R_f 0.38 [*n*-hexane−EtOAc (2:1)]; $[\alpha]^{25}_D$ −2.3 (*c* 1.3, Me_2CO); EIMS (70 eV) *m/z* $[M]^+$ 422 (53), 352 (100), 309 (84), 296 (49), 153 (66); HREIMS 422.1731, calcd for $C_{25}H_{26}O_6$, 422.1729; IR (KBr) ν max 3500−3000 (br), 1650, 1610, 1580 cm^{-1}; ^{1}H NMR (Me_2CO-d_6) δ 1.65 (6H, s, 2 × CH_3-13), 1.80 (3H, s, CH_3-16), 2.58, 2.84 (each 1H, m, CH_2-14), 3.40 (2H, d, J = 7.1 Hz, CH_2-11), 4.47 (1H, m, H-15), 4.83, 5.02 (each 1H, s, H-17), 5.38 (1H m, H-12), 6.24 (1H, d, J = 2.1 Hz, H-6), 6.49 (1H, d, J = 2.1 Hz, H-8), 6.60 (1H, s, H-3), 7.69 (1H, d, J = 2.2 Hz, H-2′), 7.71 (1H, d, J = 2.2 Hz, H-6′), 13.02 (1H, s, 5-OH); ^{13}C NMR, DEPT, and C−H COSY (Me_2CO-d_6) δ 163.4 (s, C-2), 104.0 (d, C-3), 183.0 (s, C-4), 164.9 (s, C-5), 99.7 (d, C-6), 165.5 (s, C-7), 94.7 (d, C-8), 158.9 (s, C-9), 105.3 (s, C-10), 123.0 (s, C-1′), 127.4 (d, C-2′), 127.7 (s, C-3′), 158.8 (s, C-4′), 130.9 (s, C-5′), 128.7 (d, C-6′), 25.9 (t, C-11), 123.4 (d, C-12), 133.0 (s, C-13), 39.5 (t, C-14), 77.6 (d, C-15), 147.8 (s, C-16), 111.0 (t, C-17), 17.9 and 25.9 (each q, 2 × CH_3-13), 18.4 (q, CH_3-16).

Yinyanghuo C (**3**) was obtained as yellow needles: mp 228−230 °C; R_f 0.49 [*n*-hexane−EtOAc (2:1)]; EIMS (70 eV) *m/z* $[M]^+$ 336 (21), 321 (100), 169 (12), 161 (14), 153 (8); HREIMS 336.0986, calcd for $C_{20}H_{16}O_5$, 336.0997; IR (KBr) ν max 3500−3000 (br), 1650, 1600, 1585 cm^{-1}; ^{1}H NMR (Me_2CO-d_6) δ 1.45 (6H, s, 2 × CH_3), 5.85 (1H, d, J = 10.1 Hz, H-12), 6.24 (1H, d, J = 1.8 Hz, H-6), 6.52 (1H, d, J = 10.1 Hz, H-11), 6.53 (1H, d, J = 1.8 Hz, H-8), 6.64 (1H, s, H-3), 6.89 (1H, d, J = 8.5 Hz, H-5′), 7.76 (1H, d, J = 2.1 Hz, H-2′), 7.81, (1H, dd, J = 2.1, 8.5 Hz, H-6′), 12.93 (1H, s, 5-OH).

Yinyanghuo D (**4**) was obtained as yellow needles: mp 98−100 °C; R_f 0.36 [CH_2Cl_2−MeOH (18:1)]; EIMS (70 eV) *m/z* $[M]^+$ 338 (100), 323 (14), 295 (33), 283 (60), 153 (36); HREIMS 338.1155, calcd for $C_{20}H_{18}O_5$, 338.1154; IR (KBr) ν max 3500−3000 (br), 1655, 1615, 1580 cm^{-1}; ^{1}H NMR (Me_2CO-d_6) δ 1.75 (6H, s, 2 × CH_3-13), 3.40 (2H, d, J = 7.3 Hz, CH_2-11), 5.38 (1H, m, H-12), 6.24 (1H, d, J = 2.1 Hz, H-6), 6.50 (1H, d, J = 2.1 Hz, H-8), 6.58 (1H, s, H-3), 7.02 (1H, d, J = 8.5 Hz, H-5′), 7.75

(1H, dd, J = 2.2 and 8.5 Hz, H-6′), 7.80 (1H, d, J = 2.2 Hz, H-2′), 13.01 (1H, s, 5-OH).

Yinyanghuo E (**5**) was obtained as yellow needles: mp 88−90 °C; R_f 0.45 [CH_2Cl_2−MeOH (18:1)]; EIMS (70 eV) m/z $[M]^+$ 352 (37), 337 (100), 169 (10), 153 (12); HREIMS 352.0930, calcd for $C_{20}H_{16}O_6$, 352.0946; IR (KBr) ν max 3500−3000 (br), 1650, 1615, 1570 cm^{-1}; ^1H NMR (Me_2-CO-d_6) δ 1.46 (6H, s, 2 × CH_3-13), 5.85 (1H, d, J = 10.0 Hz, H-12), 6.25 (1H, d, J = 1.8 Hz, H-6), 6.50 (1H, d, J = 10.0 Hz, H-11), 6.54 (1H, d, J = 1.8 Hz, H-8), 6.62 (1 H, s, H-3), 7.33 (1H, d, J = 2.0 Hz, H-6′), 7.39 (1H, d, J = 2.0 Hz, H-2′), 12.96 (1H, s, 5-OH).

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