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CONSTITUENTS OF LEAVES OF *PHELLODENDRON CHINENSE* VAR. *GLABRIUSCULUM*

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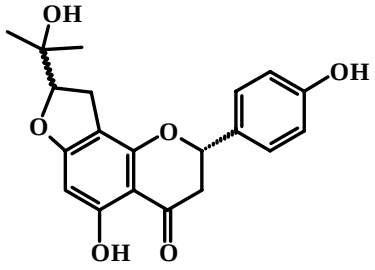
Abstract —The leaves of *Phellodendron chinense* var. *glabriusculum* yielded three new flavanones, phellodensin D (**1**), phellodensin E (**2**), and phellodensin F (**3**) along with five known compounds, amurensin (**4**), phellamurin (**5**), methyl caffeate (**6**), β-sitosterol (**7**), and pheophytin-a (**8**). The structural assignment of new compounds was based on spectroscopic studies.

INTRODUCTION

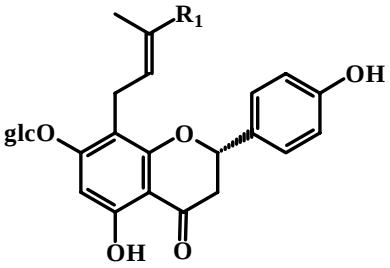
Phellodendron chinense var. *glabriusculum* (Rutaceae) is a deciduous tree that occurred widely in south-western China.[1] The bark of the plants of genus *Phellodendron* has found application in Chinese traditional medicine for various diseases like meningitis, bacillary dysentery, pneumonia, tuberculosis, and liver cirrhosis.[1-3] Examination of a sample of the leaves of this plant collected in Yunnan, mainland China, has now led to the isolation and characterization of three new flavanones, phellodensin D (**1**), phellodensin E (**2**), and phellodensin F (**3**), as well as five known compounds.

RESULTS AND DISCUSSION

A methanol extract of leaves of *P. chinense* var. *glabriusculum* was partitioned between chloroform and water. Chloroform solubles were column chromatographed on silica gel to give phellodensin D (**1**). Aqueous layer on column chromatography over silica gel afforded phellodensin E (**2**) and phellodensin F (**3**).



Phellodensin D (1)



$R_1 = CH_2OH$ Phellodensin E (2)
$R_1 = CH_3$ Phellodensin F (3)

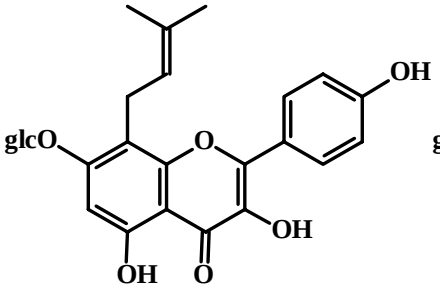
Phellodensin D (**1**) was isolated as yellow amorphous powder and was assigned a molecular formula of $C_{20}H_{20}O_6$ from its HRFABMS. The IR spectrum showed absorption bands at 3381 and 1647 cm^{-1}, consistent with the presence of a hydroxyl group and a conjugated carbonyl group in the molecule. The UV spectrum exhibited characteristic absorption peaks of the flavanone moiety at 243, 294, and 336 nm.[4] The presence of a flavanone structure was also indicated by an AMX system with resonances at δ 5.49 (1H, dd, *J* = 12.7, 2.9 Hz, H-2), 3.18 (1H, dd, *J* = 17.2, 12.7 Hz, H-3α), and 2.75 (1H, dd, *J* = 17.2, 2.9 Hz, H-3β) and the resonance occurring at δ 196.8 for C-4. In the ^{1}H NMR and ^{1}H-^{1}H COSY spectra, a pair of A_2B_2 doublets at δ 7.39 (2H, *J* = 8.4 Hz, H-2', -6') and 6.89 (2H, *J* = 8.4 Hz, H-3', -5'), and a broad singlet at 8.55 (1H, 4'-OH) were typical of a *p*-hydroxy substituted B-ring. A downfield signal at δ 12.43, exchangeable with D_2O, was attributed to the chelated hydroxyl at C-5. A sharp singlet at δ 5.87 was assigned to H-6 as it showed HMQC correlation with a carbon at δ 91.5 and HMBC correlation with C-5 at δ 165.8. The ^{1}H NMR signals of **1** at δ 1.18 (3H, s), 1.25 (3H, s), 2.97 (1H, dd, *J* = 15.2, 9.6 Hz), 3.05 (1H, dd, *J* = 15.2, 7.7 Hz), 4.74 (1H, dd, *J* = 9.6, 7.7 Hz), and 3.76 (1H, br s) revealed the presence of an α-(1-hydroxy- 1-methylethyl)dihydrofuran moiety in **1**.[5] The fusion of this group at C-7 and C-8 was confirmed by the ^2J and ^3J correlations observed between H-1"/C-7, -8, and -9 in its HMBC spectrum. In the CD spectrum of **1**, characteristic Cotton effects based on n-π* transition of flavanone, a positive absorption at 313 nm and a negative absorption at 292 nm were observed, from which the absolute

configuration of **1** was deduced as 2*S*.[6] Thus, the structure of phellodensin D was elucidated as **1**.

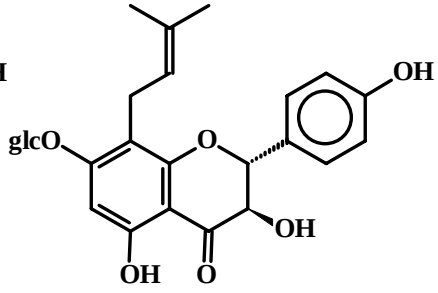
Phellodensin E (**2**), obtained as yellow amorphous powder, showed $[M+H]^+$ at *m/z* 519.1865 in its HR-FABMS corresponding to the pseudomolecular formula $C_{26}H_{31}O_{11}$ and a prominent fragment at *m/z* 341 ($[M+H-162]^+$) indicating the presence of an hexosyl moiety. The IR spectrum of **2** displayed a hydroxyl absorption band at 3368 cm^{-1} and a conjugated carbonyl absorption band at 1630 cm^{-1}. The UV absorption maxima at 226, 287, and 340 nm were typical of a flavanone derivative.[4] The ^1H NMR spectrum revealed a D_2O exchangeable downfield signal at δ 12.08 (5-OH), a broad singlet at δ 8.49 (4'-OH), an A_2B_2 system of proton signals at δ 7.46 (d, *J* = 8.6 Hz, H-2', -6') and 6.89 (d, *J* = 8.6 Hz, H-3', -5'), a sharp singlet at δ 6.29 (H-6), and an AMX system of protons at δ 5.48 (1H, dd, *J* = 12.6, 2.8 Hz, H-2), 3.17 (1H, dd, *J* = 17.1, 12.6 Hz, H-3α), and 2.78 (1H, m, H-3β). Additionally, characteristic signals for 4-hydroxy-3-methyl-2-butenyl group at δ 3.23 (1H, dd, *J* = 14.4, 7.6 Hz, H-1"), 3.41 (1H, d, *J* = 14.4, 7.6 Hz, H-1"), 5.43 (1H, m, H-2"), 3.86 (2H, br s, H-4"), 4.29 (1H, br s, 4"-OH), and 1.64 (3H, s, 5"-CH_3) and an anomeric proton doublet at δ 5.06 (*J* = 7.6 Hz) were also observed. Considering these informations, **2** was thus identified as a flavanone, phellodensin derivative with glucosyl and 4-hydroxy-3-methyl-2-butenyl substituents. The placement of glucose moiety was determined by analysis of HMBC spectrum in which the anomeric proton signal showed long range correlation with the carbon at δ 164.5 (C-7) indicating that the glucosyl moiety is linked to C-7 of **2**. The coupling constant (*J* = 7.6 Hz) of the anomeric proton signal inferred the β-configuration of the glucopyranosyl moiety. The position of 4-hydroxy-3-methyl-2- butenyl group whose *E*-configuration was inferred by the NOESY correlations between H-2" / H-4" was deduced to be at C-8 from the HMBC correlations of H-1" with C-7, 8, and 9. The absolute configuration at C-2 was confirmed as *S* by a positive Cotton effect at 336 nm and a negative Cotton effect at 289 nm in CD spectrum.[6] Thus the structure of phellodensin E was established as shown in **2**.

Phellodensin F (**3**) was obtained as yellow amorphous powder that was analyzed for the molecular formula $C_{26}H_{30}O_{10}$ from its HRFABMS spectrum. The IR spectrum suggested the presence of hydroxyl group (3416 cm^{-1}) and conjugated carbonyl group (1641 cm^{-1}). The UV spectrum exhibited absorption

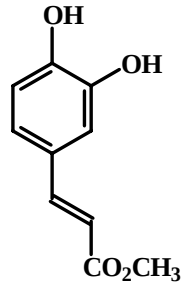
maxima at 288 and 344 nm characteristic of a flavanone derivative.[4] In its ^{1}H and ^{13}C NMR spectra, data corresponding to flavanone and glucosyl moieties were almost superimposible to those of phellodensin E. Also, characteristic prenyl proton signals were observed at δ 3.18 (1H, dd, *J* = 13.9, 7.5 Hz, H-1"), 3.36 (1H, dd, *J* = 13.9, 7.5 Hz, H-1"), 5.20 (1H, t, *J* = 7.5 Hz, H-2"), 1.60 (3H, s, 4"-CH_3), and 1.64 (3H, s, 5"-CH_3). These results indicated that **3** has a flavanone skeleton with two hydroxyl groups, one glucose unit, and one prenyl subunit. The position of these functional groups were determined unambiguously as C-5 and C-4' for two hydroxyls, C-7 for glucosyl, and C-8 for prenyl, respectively, using the HMBC NMR technique. The absolute configuration at C-2 was assigned as *S* by CD spectral data comparison with literature values for a group of flavanones.[6] Accordingly, the structure of the phellodensin F was elucidated as shown in **3**.



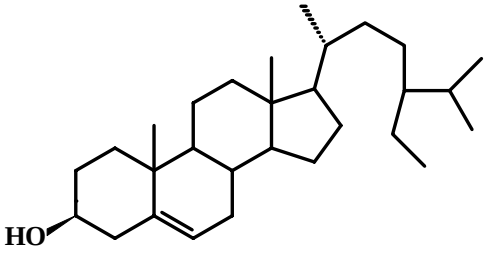
Amurensin (4)



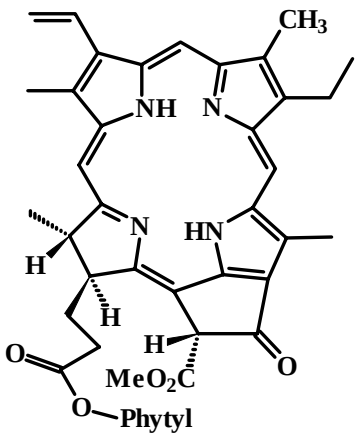
Phellamurin (5)



Methyl caffeate (6)



β-Sitosterol (7)



Pheophytin-a (8)

Five known compounds, amurensin (**4**),[7] phellamurin (**5**),[7] methyl caffeate (**6**),[8] β-sitosterol (**7**),[9] and pheophytin-a (**8**)[10] were also isolated from the leaves of *P. chinense* var. *glabriusculum*. These compounds were identified by comparison with the authentic samples.

EXPERIMENTAL

General Experimental Procedures. Melting points were measured on Yanaco MP-S3 melting point apparatus without correction. UV spectra were recorded on a Hitachi UV-3210 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. IR spectra were recorded on a Shimadzu FT-IR DR-8011 spectrophotometer with KBr discs. ^{1}H and ^{13}C NMR spectra were determined on Varian Unity plus 400 spectrometer. Chemical shifts are shown in δ values (ppm) with tetramethyl-silane (TMS) as internal standard. FAB and HRFABMS spectra were measured on a VG-70-250S spectrometer by a direct inlet system. CD spectra were recorded with Jasco J-720 spectropolarimeter.

Plant Material. The leaves of *P. chinense* var. *glabriusculum* were collected from Kunming, Yunnan, in 1997. A herbarium specimen (Wu 19970009) is deposited at the National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The air-dried leaves of *P. chinense* var. *glabriusculum* (75 g) was ground and extracted with methanol (500 mL × 6) under reflux for 8 hours to give an extract (8.6 g), which as partitioned between chloroform and water. The chloroform soluble fraction (4 g) was subjected to silica gel column chromatography using chloroform-methanol gradients to give 6 fractions. Fraction 5 was subjected to column chromatography with chloroform to get **7** (1.5 mg) and **8** (2.5 mg). Similarly, fraction 6 gave **1** (8.3 mg) with chloroform-methanol (19:1) solvent system. The water soluble fraction (4.6 g) was chromatographed over reversed-phase Diaion HP-20 using water-methanol gradients which afforded 8 fractions. Fractions 7 and 8 were separately subjected to column chromatography over silica gel with chloroform-acetone-methanol (4:1:1) and chloroform-methanol (9:1) solvent systems to give **2** (13.7 mg), **5** (3.6 mg), and **6** (2.2 mg), and **3** (237.8 mg) and **4** (5.4 mg), respectively.

Phellodensin D（**1**） $C_{20}H_{20}O_6$: Yellow powder; mp 88-89℃ (MeOH); $[\alpha]_D$+35.6° (*c* 0.083, MeOH); UV λ_{max}^{MeOH} nm (log ε)：219 (4.35), 243 (3.93), 294 (4.15), 336 (3.56); IR ν_{max} cm^{-1}：3381, 1647, 1611, 1384, 1252. ^{1}H-NMR (400 MHz, Acetone-d_6): δ 1.18 (3H, s, CH_3), 1.25 (3H, s, CH_3), 2.75 (1H, dd, *J* = 17.2, 2.9 Hz, H-3β), 2.97 (1H, dd, *J* = 15.2, 9.6 Hz, H-1”), 3.05 (1H, dd, *J* = 15.2, 7.7 Hz, H-1”), 3.18 (1H, dd, *J* = 17.2, 12.7 Hz, H-3α), 3.76 (1H, br s, 3”-OH), 4.74 (1H, dd, *J* = 9.6, 7.7 Hz, H-2”), 5.49 (1H,

dd, *J* = 12.7, 2.9 Hz, H-2), 5.87 (1H, s, H-6), 6.89 (2H, d, *J* = 8.4 Hz, H-3', -5'), 7.39 (2H, d, *J* = 8.4 Hz, H-2', -6'), 8.55 (1H, br s, 4'-OH), and 12.43 (1H, s, 5-OH). ^{13}C-NMR (100 MHz, Acetone-d_6): δ 25.5 (CH_3), 25.8 (CH_3), 27.0 (C-1"), 43.4 (C-3), 71.4 (C-3"), 79.9 (C-2), 91.5 (C-6), 92.7 (C-2"), 103.4 (C-10), 105.8 (C-8), 116.2 (C-3', -5'), 129.0 (C-2', -6'), 130.7 (C-1'), 158.2 (C-4'), 158.7 (C-9), 165.8 (C-5), 169.9 (C-7), and 196.8 (C-4). CD (MeOH: *c* = 0.00005): $[\theta]_{313}$ +8458, $[\theta]_{306}$ 0, $[\theta]_{292}$ -33350, $[\theta]_{268}$ 0, $[\theta]_{240}$ +10110, $[\theta]_{217}$ +45230. FAB-MS *m/z* (*rel. int.* %): 357 ($[M+H]^+$, 50), 297 (17), 237 (26). HRFAB-MS *m/z* 357.1337 $[M+H]^+$ (Calcd for $C_{20}H_{21}O_6$: 357.1338).

Phellodensin E（2） $C_{26}H_{30}O_{11}$: Yellow powder; mp 235-236℃ (MeOH); $[\alpha]_D$+35.8° (*c* 0.094, MeOH); UV λ_{max}^{MeOH} nm (log ε)：226 (4.36), 287 (4.15), 327 (3.50); IR ν_{max} cm^{-1}：3368, 2922, 1630, 1520, 1177. ^{1}H-NMR (400 MHz, Acetone-d_6): δ 1.64 (3H, s, CH_3), 2.78 ~ 2.84 (1H, m, H-3β), 3.17 (1H, dd, *J* = 17.1, 12.6 Hz, H-3α), 3.23 (1H, dd, *J* = 14.4, 7.6 Hz, H-1"), 3.41 (1H, dd, *J* = 14.4, 7.6 Hz, H-1"), 3.43 ~ 3.65 (4H, m, H-2"'~ H-5"'), 3.65 ~ 3.75 (1H, m, H-6"'), 3.84 ~ 3.90 (1H, m, H-6"'), 3.86 (2H, br s, H-4"), 4.29 (1H, br s, OH), 4.39 (1H, br s, OH), 4.51 (1H, br s, OH), 5.06 (1H, d, *J* = 7.6 Hz, H-1"'), 5.43 ~ 5.48 (1H, m, H-2"), 5.48 (1H, dd, *J* = 12.6, 2.8 Hz, H-2), 6.29 (1H, s, H-6), 6.89 (2H, d, *J* = 8.6 Hz, H-3', -5'), 7.40 (2H, d, *J* = 8.6 Hz, H-2', -6'), 8.49 (1H, br s, 4'-OH), and 12.08 (1H, s, 5-OH). ^{13}C-NMR (100 MHz, Acetone-d_6): δ 13.9 (CH_3), 22.0 (C-1"), 43.6 (C-3), 62.2 (C-6"'), 68.4 (C-4"), 71.2 (C-4"'), 74.7 (C-2"'), 78.0&78.1 (C-3"'&-5"'), 79.8 (C-2), 96.3 (C-6), 101.5 (C-1"'), 104.4 (C-10), 110.0 (C-8), 116.2 (C-3', -5'), 123.6 (C-2"), 128.9 (C-2', -6'), 130.8 (C-1'), 135.6 (C-3"), 158.7 (C-4'), 160.3 (C-9), 163.1 (C-5), 164.5 (C-7), and 198.3 (C-4). CD (MeOH: *c* = 0.00005): $[\theta]_{336}$ +7766, $[\theta]_{311}$ 0, $[\theta]_{298}$ -39860, $[\theta]_{264}$ 0, $[\theta]_{253}$ +5232, $[\theta]_{218}$ +52690. FAB-MS *m/z* (*rel. int.* %):519 ($[M+H]^+$, 7), 462 (23), 397 (13), 357 (11), 338 (24), 321 (22), 320 (27), 319 (26), 312 (35). HRFAB-MS *m/z* 519.1865 $[M+H]^+$ (Calcd for $C_{26}H_{31}O_{11}$: 519.1866).

Phellodensin F（3） $C_{26}H_{30}O_{10}$: Yellow powder; mp 221-222℃ (MeOH); $[\alpha]_D$+18.2° (*c* 0.036, MeOH); UV λ_{max}^{MeOH} nm (log ε)：288 (4.37), 344 (3.76); IR ν_{max} cm^{-1}：3416, 2918, 1641, 1599, 1364. ^{1}H-NMR (400 MHz, Acetone-d_6): δ 1.60 (6H, s, 2 × CH_3), 2.70 ~ 2.90 (1H, m, H-3β), 3.18 (1H, dd, *J* = 17.0, 12.6 Hz, H-3α), 3.18 (1H, dd, *J* = 13.9, 7.5 Hz, H-1"), 3.36 (1H, dd, *J* = 13.9, 7.5 Hz, H-1"), 3.44 ~ 3.51 (1H,

m, H-4’’’), 3.51 ~ 3.57 (2H, m, H-2’’’, -3’’’), 3.57 ~ 3.62 (1H, m, H-5’’’), 3.71 (1H, m, H-6’’’), 3.90 (1H, m, H-6’’’), 4.27 (1H, br s, OH), 4.37 (1H, br s, OH), 4.45 (1H, br s, OH), 5.05 (1H, d, *J* = 7.5 Hz, H-1’’’), 5.20 (1H, t, *J* = 7.3 Hz, H-2”), 5.47 (1H, dd, *J* = 12.6, 3.0 Hz, H-2), 6.29 (1H, s, H-6), 6.89 (2H, d, *J* = 8.6 Hz, H-3’, -5’), 7.40 (2H, d, *J* = 8.6 Hz, H-2’, -6’), 8.49 (1H, br s, 4’-OH), and 12.08 (1H, s, 5-OH). ^{13}C-NMR (100 MHz, Acetone-d_6): δ 17.9 (CH_3), 22.5 (C-1”), 25.9 (CH_3), 43.6 (C-3), 62.6 (C-6’’’), 71.3 (C-4’’’), 74.7 (C-2’’’), 78.0&78.1 (C-3’’’&-5’’’), 79.8 (C-2), 96.3 (C-6), 101.5 (C-1’’’), 104.4 (C-10), 110.3 (C-8), 116.2 (C-3’, -5’), 123.8 (C-2”), 128.9 (C-2’, -6’), 130.9 (C-1’), 131.2 (C-3”), 158.6 (C-4’), 160.3 (C-9), 163.0 (C-5), 164.4 (C-7), and 198.3 (C-4). CD (MeOH: *c* = 0.00005): $[\theta]_{336}$ +12300, $[\theta]_{308}$ 0, $[\theta]_{289}$ -68250, $[\theta]_{262}$ 0, $[\theta]_{254}$ +7161, $[\theta]_{217}$ +83570. FAB-MS *m/z* (*rel. int.* %): 503 ($[M+H]^+$, 8), 428 (11), 341 (21), 340 (14), 339 (13), 326 (15), 289 (13), 285 (10). HRFAB-MS *m/z* 503.1916 $[M+H]^+$ (Calcd for $C_{26}H_{31}O_{10}$: 503.1917).

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