Flavonoids and Coumarins from Leaves of *Phellodendron chinense*

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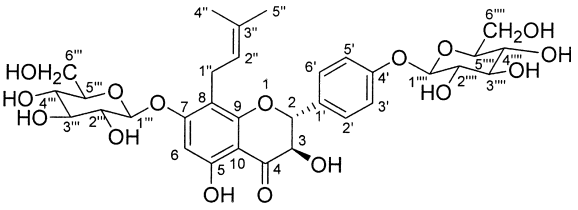
Abstract

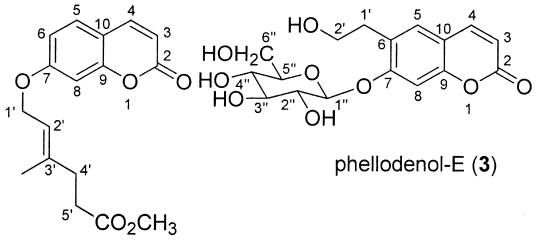
Three new compounds, phellodensin G, phellodenols D and E have been isolated from the leaves of *Phellodendron chinense* Schneid (Rutaceae), together with thirteen known compounds. Their structures were established by means of spectroscopic analysis, including extensive 2D NMR and mass spectra.

Phellodendron chinense Schneid (Rutaceae) is a deciduous tree found widely in southwestern China. It has been used for the treatment of meningitis, bacillary dysentery, pneumonia, tuberculosis, and liver cirrhosis in traditional Chinese medicine [1]. Previous phytochemical work on this plant has reported the isolation of berberine- and aporphine-type alkaloids and some triterpenoids [2], [3]. During the course of our investigation on the bioactive chemical components of the Rutaceous plants, we examined the leaves of *P. chinense* and report here the isolation and structural elucidation of a new flavanonol diglucoside, phellodensin G (**1**), two new coumarins, phellodenols D (**2**) and E (**3**), in addition to thirteen known compounds (**4 – 16**).

Phellodensin G (**1**) was determined to be $C_{32}H_{40}O_{16}$ from its HR-FAB-MS. The UV and IR spectral data of **1** were typical of a flavanonol derivative [4]. The ^{1}H- and ^{13}C-NMR spectra of **1** showed characteristic signals due to H-2 (C-2), H-3 (C-3), and 3-OH of a 2,3-*trans*-flavanonol derivative [5]. A broad D_2O exchangeable singlet, a set of A_2B_2 doublets, a 1H singlet, and a set of prenyl proton signals were similar to those of phellamurin [6], except for the signals due to the sugar moiety. The presence of an anomeric proton doublet at δ = 6.29 (J = 6.4 Hz) integrated for two protons and the carbon signals at δ = 100.6, 77.5, 77.3, 76.9, 73.5, 70.1 and 60.0 suggested the presence of two glucosyl moieties with the β-configuration. From ROESY studies, glucose residues in **1** were found to be linked to C-7 and C-4′ as NOEs of anomeric protons with H-6, and with H-3′, H-5′, respectively, were observed. The stereochemistry of C-2 and C-3 in **1** was deduced to be 2*R*, 3*R* from the results of CD measurements and the *trans* diaxial coupling between H-2 and H-3 [7]. Thus the structure of phellodensin G (**1**) was elucidated as shown.



Phellodensin-G (**1**)



phellodenol-E (**3**)

phellodenol-D (**2**)

Phellodenol D (**2**) had the molecular formula $C_{17}H_{18}O_5$ as derived from the HR-FAB-MS. The UV and IR absorptions were typical of a 7-oxygenated coumarin [8]. In the aromatic region, a pair of doublets and an ABX pattern signals were consistent with a 7-substituted coumarin skeleton. A set of signals consisting of a 2H doublet at δ = 4.59 (J = 6.4 Hz), a 1H broad triplet at δ = 5.48 (J = 6.4 Hz), two multiplets at δ = 2.40 – 2.44 and 2.45 – 2.50, and two 3H singlets at δ = 1.77 and 3.66 were accounted for a $-OCH_2CH=C(CH_3)CH_2CH_2CO_2CH_3$ side chain, whose *E*-configuration was deduced from the ROESY correlations of H-1′/CH_3 – 3′, and H-2′/H-4′. The unambiguous location of this side chain on C-7 was determined based on strong cross-peaks between H-1′ and H-6, H-8 in the ROESY experiment. Thus the structure of phellodenol D was established as **2**.

Phellodenol E (**3**) was determined to be $C_{17}H_{20}O_9$ by HR-FAB-MS. The UV and IR spectral data were also similar to those of a 7-oxygenated coumarin [8]. The chemical shifts and multiplicities of aromatic proton signals in ^{1}H-NMR spectrum indicated the presence of a 6,7-disubstituted coumarin skeleton. The ^{1}H-NMR spectrum also showed signals for a hydroxyethyl side chain. The H-1′ multiplet showed HMBC correlations with C-6, C-5, and C-7 and, thus the hydroxyethyl group was placed at C-6. The ^{1}H- and ^{13}C-NMR spectra also displayed signals for the presence of a β-glucopyranoside moiety. The location of glucosidation was determined to be C-7 based on the ^3J-correlation of H-1″ with C-7 in the HMBC spectrum. Thus, phellodenol E is represented by structure **3**.

Furthermore, fifteen known compounds, friedelin (**4**) ($[\alpha]_D^{25}$: –25.5°, *c* 0.01, $CHCl_3$) [9], aurapten (**5**) [9], 7-[(*E*)-7′-hydroxy-3′,7′-dimethyl-2′,5′-octadienyloxy]coumarin (**6**) [9], clerosterol (**7**) ($[\alpha]_D^{25}$: –45.2°, *c* 0.01, $CHCl_3$) [10], pheophytin-a (**8**) [6], xanthyletin (**9**) [9], pheophytin-b (**10**) [11], (*R*)-(+)-7-hydroxy-6-(2-hydroxy-3-methyl-3-butenyl)-2*H*-1-benzopyran-2-one (**11**) ($[\alpha]_D^{25}$: + 26.3°,

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c 0.021, $CHCl_3$) [12], methyl pheophorbide-a (**12**) [11], methyl caffeate (**13**) [6], 3-hydroxy-4-methoxycinnamic acid (**14**) [13], phellamurin (**15**) ($[\alpha]_D^{25}$: +64.9°, *c* 0.017, MeOH) [6], and amurensin (**16**) ($[\alpha]_D^{25}$: +50.8°, *c* 0.016, MeOH) [6] were identified by comparison with published physical and spectral data.

Materials and Methods

The leaves of *P. chinense* Schneid (Rutaceae) were collected from Kunming, Yunnan, in August, 1997. A voucher specimen (Wu 19970011) was deposited in the herbarium of the National Cheng Kung University. The shade-dried and powdered leaves (105 g) of *P. chinense* were extracted with hot MeOH (500 mL×3) and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extract (6 g) was subjected to column chromatography (CC) over silica gel (230–400 mesh, 150 g) eluting with a step gradient of $CHCl_3$-MeOH (19:1 to 0:1, 6×200 mL each) to afford fractions I - V. Compound **4** (26 mg) was recrystallized from fractions I and II in MeOH. Fraction III was separated on silica gel CC (230–400 mesh, 100 g) repeatedly, using *n*-hexane-acetone (9:1, 300 mL) as eluent to yield **5** (1 mg), **6** (0.9 mg), and **7** (15.8 mg). Work-up of fraction IV by silica gel CC (230–400 mesh, 100 g) with *n*-hexane-$CHCl_3$-acetone as eluent (20:10:1, 400 mL) followed by TLC purification of subfractions afforded **8** (10 mg) and **9** (1 mg). Fraction V on CC over silica gel (230–400 mesh, 100 g) using a gradient of $CHCl_3$-MeOH (1:0 to 0:1, 6×100 mL each) gave four subfractions (sub frs. 1–4). Subfraction 1 was recrystallized with MeOH to obtain **2** (5.8 mg). Subfractions 2–4 were purified by CC over silica gel (230–400 mesh) with $CHCl_3$-acetone (4:1, 320 mL), followed by TLC ($CHCl_3$-acetone, 3:1, 60 mL) to furnish **10** (9.0 mg), **11** (1 mg), and **12** (4.5 mg).

The aqueous extract (41 g) was applied over Diaion HP-20 gel CC (500 g) using H_2O-MeOH (1:0 to 0:1, 6×4 L each) gradient to give 4 fractions (fr. 1–4). Fraction 1 gave compound **3** (25.5 mg) on CC over silica gel (70–230 mesh, 250 g) with $CHCl_3$-MeOH (6:1, 2 L) as eluent followed by recrystallization in acetone. Fraction 2 was subjected to CC over silica gel (230–400 mesh, 200 g) with $CHCl_3$-MeOH (6:1, 500 mL) as eluent to yield **1** (9.5 mg), **13** (1 mg), and **14** (1.5 mg), successively. Fraction 3 on CC over silica gel (70–230 mesh, 250 g) with $CHCl_3$: acetone : MeOH (6:1:1, 2 L) as eluent afforded compound **15** (8.1 g). Fraction 4 on recrystallization in MeOH yielded compound **16** (551 mg).

Phellodensin G (**1**): colorless powder, m.p. 266–267 °C; $[\alpha]_D^{25}$: +66.7° (*c* 0.023, MeOH); UV (MeOH): λ_{max} (log ε) = 221 (4.46), 291 (4.16), 345 (3.51) nm; IR (KBr): ν_{max} = 3377, 2914, 1640, 1583, 1234 cm^{-1}; FAB-MS: *m/z* = 680 $[M]^+$ (0.6), 679 (2), 289 (9), 154 (100); HR-FAB-MS: *m/z* = 680.2311 $[M]^+$ (calcd. for $C_{32}H_{40}O_{16}$: 680.2316); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 1.47 (3H, s, 5″-CH_3), 1.57 (3H, s, 4″-CH_3), 2.99 (1H, dd, *J* = 13.2, 5.1 Hz, H-1″), 3.12–3.20 (2H, m, H-4‴, -4⁗), 3.20–3.30 (3H, m, H-1′, -2‴, -2⁗), 3.30–3.40 (4H, m, H-3‴, -3⁗, -5‴, -5⁗), 3.40–3.47 (2H, m, H-6‴, -6⁗), 3.66–3.69 (2H, m, H-6‴, -6⁗), 4.54 (1H, t, *J* = 6.0 Hz, D_2O exchangeable, OH), 4.56 (1H, dd, *J* = 12.8, 7.2 Hz, H-3), 4.57–4.61 (1H, m, D_2O exchangeable, OH), 4.90 (2H, d, *J* = 6.4 Hz, H-1‴, -1⁗), 4.99–5.03 (2H, m, D_2O exchangeable, 2×OH), 5.06–5.13 (2H, m, D_2O exchangeable, 2×OH), 5.09 (1H, m, H-2″), 5.10 (1H, d, *J* = 12.8 Hz, H-2), 5.28 (1H, d, *J* = 4.8 Hz, D_2O exchangeable, OH), 5.31 (1H, d, *J* = 4.8 Hz, D_2O exchangeable, OH), 5.85 (1H, d, *J* = 7.2 Hz, D_2O exchangeable, OH), 6.29 (1H, s, H-6), 7.05 (2H, d, *J* = 8.8 Hz, H-3′, -5′), 7.42 (2H, d, *J* = 8.8 Hz, H-2′, -6′), 11.81 (1H, s, D_2O exchangeable, 5-OH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ = 17.9 (5″-CH_3), 21.6 (C-1″), 25.7 (4″-CH_3), 61.0 (C-6‴, -6⁗), 70.1 (C-4‴, -4⁗), 72.2 (C-3), 73.5 (C-2‴, -2⁗), 76.9 & 77.3 & 77.5 (C-3‴, -3⁗, -5‴, -5⁗), 82.8 (C-2), 85.9 (C-6), 100.6 (C-1‴, -1⁗), 102.1 (C-10), 109.4 (C-8), 116.2 (C-3′, -5′), 122.6 (C-2″), 129.2 (C-2′, -6′), 130.5 (C-1′), 130.9 (C-3″), 157.8 (C-4′), 158.8 (C-9), 161.2 (C-5), 163.5 (C-7), 198.9 (C-4); CD (MeOH: *c* = 0.0002): $[\Theta]_{335}$ = +4022, $[\Theta]_{311}$ = 0, $[\Theta]_{290}$ = −19060, $[\Theta]_{263}$ = 0, $[\Theta]_{235}$ = +12810.

Phellodenol-D (**2**): colorless powder, m.p. 88–89 °C; UV (MeOH): λ_{max} (log ε) = 203 (3.61), 252 (2.09), 294 (2.88, sh), 323 (3.16) nm; IR (KBr): ν_{max} = 2941, 1734, 1614, 1279, 1126 cm^{-1}; EI-MS: *m/z* = 302 $[M]^+$ (6), 301 (11), 292 (12), 214 (25), 181 (47), 141 (43), 133 (100); HR-FAB-MS: *m/z* = 303.1233 $[M + H]^+$ (calcd. for $C_{17}H_{19}O_5$: 303.1232); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.77 (3H, s, CH_3), 2.40–2.44 (2H, m, H-4′), 2.45–2.50 (2H, m, H-5′), 3.66 (3H, s, OCH_3), 4.59 (2H, d, *J* = 6.4 Hz, H-1′), 5.48 (1H, br t, *J* = 6.4 Hz, H-2′), 6.24 (1H, d, *J* = 9.6 Hz, H-3), 6.80 (1H, d, *J* = 2.4 Hz, H-8), 6.83 (1H, dd, *J* = 8.8, 2.4 Hz, H-6), 7.35 (1H, d, *J* = 8.8 Hz, H-5), 7.63 (1H, d, *J* = 9.6 Hz, H-4).

Phellodenol-E (**3**): colorless powder, m.p. 166–167 °C; $[\alpha]_D^{25}$: −49.5° (*c* 0.025, MeOH); UV (MeOH): λ_{max} (log ε) = 221 (4.27), 251 (3.48), 294 (3.94), 325 (4.13) nm; IR (KBr): ν_{max} = 3422, 2897, 1703, 1626, 1385, 1273, 1126 cm^{-1}; FAB-MS: *m/z* = 369 $[M + H]^+$ (8), 327 (17), 241 (10), 207 (13), 185 (100); HR-FAB-MS: *m/z* = 369.1190 $[M + H]^+$ (calcd. for $C_{17}H_{21}O_9$: 369.1186); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 2.73–2.81 (2H, m, H-1′), 3.14–3.18 (1H, m, H-4″), 3.27–3.31 (2H, m, H-2″, -3″), 3.38–3.47 (2H, m, H-5″, -6″), 3.57–3.62 (2H, m, H-2′), 3.70–3.74 (1H, m, H-6″), 4.60 (1H, t, *J* = 5.2 Hz, 2′-OH), 4.64 (1H, t, *J* = 5.2 Hz, 6″-OH), 4.98 (1H, d, *J* = 6.6 Hz, H-1″), 5.07 (1H, d, *J* = 5.4 Hz, 4″-OH), 5.13 (1H, d, *J* = 4.8 Hz, OH), 5.35 (1H, d, *J* = 5.1 Hz, 2′-OH), 6.28 (1H, d, *J* = 9.5 Hz, H-3), 7.07 (1H, s, H-8), 7.48 (1H, s, H-5), 7.95 (1H, d, *J* = 9.5 Hz, H-4); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ = 33.0 (C-1′), 60.6 (C-2′), 60.9 (C-6″), 64.9 (C-4″), 73.5 (C-2″), 76.7 (C-3″), 77.4 (C-5″), 100.6 (C-1″), 102.2 (C-8), 112.9 (C-10), 113.2 (C-3), 125.6 (C-6), 129.7 (C-5), 144.5 (C-4), 153.8 (C-9), 158.6 (C-7), 160.7 (C-2).

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