Two Tannins from *Phyllanthus tenellus*

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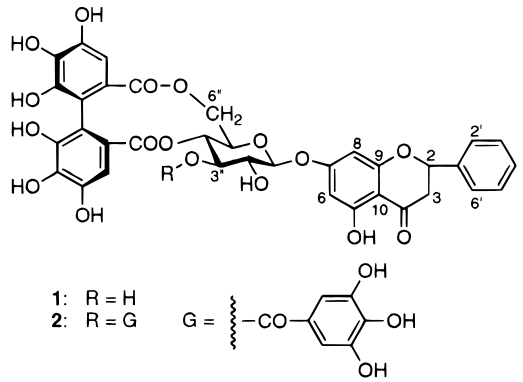
Received September 19, 1997

Two novel tannins, pinocembrin-7-*O*-[4″,6″-(*S*)-hexahydroxydiphenoyl]-*β*-D-glucose (**1**) and pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-(*S*)-hexahydroxydiphenoyl]-*β*-D-glucose (**2**) were isolated from *Phyllanthus tenellus*. The structures of new tannins were established on the basis of spectral and chemical evidence.

Phyllanthus tenellus Roxb. (Euphorbiaceae) is an introduced plant in Taiwan, and its appearance is very similar to that of *Phyllanthus niruri* Linn.[1] and *Phyllanthus virgatus* Forster f.[2] In our investigation on the constituents of *P. tenellus*, no lignans were found, but they were abundant in the other two plants mentioned above. Chromatographic separation of a MeOH extract of *P. tenellus* resulted in the isolation of two new tannins, pinocembrin-7-*O*-[4″,6″-(*S*)-hexahydroxydiphenoyl]-*β*-D-glucose (**1**) and pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-(*S*)-hexahydroxydiphenoyl]-*β*-D-glucose (**2**). Six known compounds including quercitrin, myricitrin, astragalin, gallic acid, corilagin, and geraniin were also obtained and identified by direct comparison with authentic samples (co-TLC, ^{1}H NMR, and ^{13}C NMR spectra). In the present paper we report the isolation and structure elucidation of **1** and **2**.



Compound **1** was obtained as a yellow powder, $[\alpha]^{25}_D$ −122° (*c* 0.5, MeOH), and it gave a strongly positive test for phenols (dark blue) with ferric chloride reagent. The UV spectrum of **1** showed absorption bands at λ_{max} 281 and 212 nm. It had a molecular formula of $C_{35}H_{28}O_{17}$ based on its negative FABMS, $[M - H]^-$ at *m*/*z* 719. In the ^{1}H NMR spectrum of **1**, the presence of two one-proton singlets at *δ* 6.55 and 6.68 suggested the occurrence of one hexahydroxydiphenoyl (HHDP) group in the structure of **1**. This was further supported by the ^{13}C NMR of **1**, which showed two ester carbonyl signals at *δ* 169.6 and 169.9. Acid hydrolysis of **1** yielded glucose and an aglycon, which was identified (^{1}H and ^{13}C NMR) as the known compound pinocembrin (5,7-dihydroxyflavanone) by comparison with literature data.[3,4] The large coupling constant (d, J = 7.6 Hz) of the anomeric proton signal (*δ* 5.01), which was assigned on the basis of the ^{1}H−^{1}H and ^{1}H−^{13}C COSY, implied that the configuration of the anomeric center was *β*. The site of the sugar linkage to the aglycon in **1** was considered to be C-7 from results of the HMBC experiments. The carbon signal at *δ* 166.7 showed cross peaks by two-bond coupling with H-6 (*δ* 6.20) and H-8 (*δ* 6.23), allowing assignment of the carbon signal for C-7 (*δ* 166.7), which also showed a correlation with the anomeric proton signal at *δ* 5.01 through oxygen. Correlations among the signals of H-4″ (*δ* 4.83), H-6″ (*δ* 3.79 and *δ* 5.22), and the two carboxyl signals (*δ* 169.6 and *δ* 169.9) of HHDP were also observed, suggesting that the HHDP group was at C-4″/C-6″ of the glucose core. This was consistent with the appearance of two lowfield-shift signals at *δ* 4.83 (1H, t, J = 9.3 Hz, H-4″) and 5.22 (1H, dd, J = 6.2, 13.2 Hz, H-6″) in the ^{1}H NMR spectrum of **1**. In addition, the CD spectrum of **1** showed a negative Cotton effect at 266 nm ([*θ*] −50 808) and a positive effect at 238 nm ([*θ*] +120 094), indicating an *S*-configuration of this HHDP group.[6−8] Based on the above data, compound **1** was established as pinocembrin-7-*O*-[4″,6″-(*S*)-HHDP]-*β*-D-glucose.

Compound **2** was obtained as yellow powder, $[\alpha]^{25}_D$ −56° (*c* 0.6, MeOH). Its UV spectrum showed absorption bands at λ_{max} 282 and 215 nm. The ^{1}H NMR spectrum of **2** was very similar to that of **1**, except for the appearance of an additional galloyl signal at *δ* 7.01 (2H, s), which was further confirmed by an ion peak $[M - H]^-$ at *m*/*z* 871, 152 amu more than that of **1**, in the negative FABMS of **2**. The HMBC experiments of **2** were similar to the results of **1** and determined the position of the sugar moiety and the HHDP groups. The galloyl group was shown to be at C-3″ of its glucose core due to a downfield shift of the H-3″ signal (*δ* 5.40) compared to the H-3″ (*δ* 3.68) of **1**, and there was an HMBC correlation between the H-3″ signal and the carboxyl signal (*δ* 168.0) of the galloyl group. The CD properties of **2** were quite similar to those of **1**, and both compounds showed a negative Cotton effect at 269 nm ([*θ*] −51 735) and a positive effect at 238 nm ([*θ*] +129 529). Thus, compound **2** was established as pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-(*S*)-HHDP]-*β*-D-glucose.

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S0163-3864(97)00428-X CCC: $15.00
Published on Web 04/08/1998

Experimental Section

General Experimental Procedures. IR spectra were taken on a Bio-Rad FTS-7 spectrometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a HITACHI model U-3200 spectrometer. FABMS spectra were obtained using a JEOL JMS−HX110 spectrometer. CD spectra were recorded on a JASCO J-715 spectrometer. ^{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. tenellus* Roxb. were collected at Taipei, Taiwan, in July 1994. A voucher specimen is deposited in the National Research Institute of Chinese Medicine.

Extraction and Isolation. The air-dried whole plants of *P. tenellus* (835 g) were extracted with MeOH. After evaporation of the solvent, the residue was chromatographed on a Si gel column eluting with gradient solvent systems of *n*-hexane−EtOAc (10:1 to 5:1) and CH_2Cl_2−MeOH (15:1 to 0:1) to yield 10 fractions. Fraction 10 was rechromatographed over Sephadex LH-20, eluting with MeOH, to give mixtures A−H. Mixture B was similarly chromatographed to afford 13 fractions. Of these fractions 7, 8−9, and 12−13 were individually further purified by Sephadex LH-20 eluting with MeOH−H_2O (3:1) to give gallic acid (13 mg) from fraction 7, astragalin (8 mg), quercitrin (23 mg), and myricitrin (13 mg) from fractions 8−9, and pinocembrin-7-*O*-[4″,6″-(*S*)-HHDP]-*β*-D-glucose (**1**, 47 mg) from fractions 12−13. In addition, mixtures D and E were further purified by the same procedure as mixture B to afford corilagin (22 mg) and geraniin (26 mg) from mixture D, as well as pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-(*S*)-HHDP]-*β*-D-glucose (**2**, 32 mg) from mixture E.

Pinocembrin-7-*O*-[4″,6″-(*S*)-HHDP]-*β*-D-glucose (1): yellow powder; $[\alpha]^{25}_D$ −122° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 281 (4.37), 212 (4.60) nm; IR (KBr) ν_{max} 3420 (OH), 1734, 1640 (C=O), 1579, 1447, 1347 (s), 1232, 1173, 1046, 1020 cm^{-1}; CD (*c* 5.2 × 10^{-4} M, MeOH) 238 ([θ] +120 094), 266 ([θ] −50 808) nm; negative FABMS *m*/*z* 719 [M − H]$^-$ (100), 720 [M]$^-$ (48), 301 (45); HRFABMS 720.1325 [M]$^-$, calcd for $C_{35}H_{28}O_{17}$, 720.1326; ^{1}H NMR (CD_3OD, 300 MHz) δ 2.80 (1H, dd, *J* = 3.0, 17.2 Hz, H-3), 3.14 (1H, dd, *J* = 12.8, 17.2 Hz, H-3), 3.56 (1H, br t, H-2″), 3.68 (1H, t, *J* = 9.3 Hz, H-3″), 3.79 (1H, br d, *J* = 13.2 Hz, H-6″), 4.03 (1H, dd, *J* = 6.2, 9.3 Hz, H-5″), 4.83 (1H, t, *J* = 9.3 Hz, H-4″), 5.01 (1H, d, *J* = 7.6 Hz, H-1″), 5.22 (1H, dd, *J* = 6.2, 13.2 Hz, H-6″), 5.46 (1H, dd, *J* = 3.0, 12.8 Hz, H-2), 6.20 (1H, d, *J* = 2.2 Hz, H-6), 6.23 (1H, d, *J* = 2.2 Hz, H-8), 6.55 and 6.68 (each 1H, s, HHDP−H), 7.35−7.45 (3H, m, H-3′, H-4′, H-5′), 7.47−7.52 (2H, m, H-2′, 6′); ^{13}C NMR (CD_3OD, 75 MHz) δ 44.3 (C-3), 64.3 (C-6″), 73.1 (C-3″, 5″), 75.2 (C-2″), 75.6 (C-4″), 80.8 (C-2), 96.8 (C-8), 98.1 (C-6), 101.4 (C-1″), 105.0 (C-10), 108.4 and 108.6 (HHDP C-3, 3′), 116.7 and 116.9 (HHDP C-1, 1′), 126.4 and 126.5 (HHDP C-2, 2′), 127.4 (C-2′, 6′), 129.8 (C-3′, 4′, 5′), 137.4 and 137.6 (HHDP C-5, 5′), 140.1 (C-1′), 145.0 (HHDP C-6, 6′), 145.8 (HHDP C-4, 4′), 164.4 (C-9), 165.0 (C-5), 166.7 (C-7), 169.6 and 169.9 (−COO−), 198.1 (C-4).

Acid Hydrolysis of Pinocembrin-7-*O*-[4″,6″-(*S*)-HHDP]-*β*-D-glucose (1). Compound **1** (15 mg) in 20% H_2SO_4 was refluxed for 2 h. After filtration, the precipitate was dissolved in MeOH subjected to Sephadex LH-20 column chromatography with MeOH to give the aglycon (4 mg), it was identified as pinocembrin by comparison of its ^{1}H and ^{13}C NMR spectra with literature data.[3,4] The filtrate was neutralized with $Ba(OH)_2$ and then analyzed by Si gel TLC [Kieselgel 60 (Merck Art 5554), *i*PrOH−Me_2CO−H_2O (5:3:1)]. It showed a brown spot (R_f 0.44) on TLC over spraying anilinephthalate solution and heating, which was coincident with that of glucose.

Pinocembrin: ^{1}H NMR (DMSO-d_6, 200 MHz,) δ 2.76 (1H, dd, *J* = 3.2, 17.1 Hz, H-3), 3.25 (1H, dd, *J* = 12.5, 17.1 Hz, H-3), 5.58 (1H, dd, *J* = 3.2, 12.5 Hz, H-2), 5.88 (1H, d, *J* = 2.1 Hz, H-6), 5.92 (1H, d, *J* = 2.1 Hz, H-8), 7.37−7.45 (3H, m, H-3′, 4′, 5′), 7.47−7.53 (2H, m, H-2′, 6′); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 42.1 (C-3), 78.4 (C-2), 95.1 (C-8), 96.0 (C-6), 101.8 (C-10), 126.7 (C-2′, 6′), 128.6 (C-3′, 4′, 5′), 138.7 (C-1′), 162.8 (C-9), 163.5 (C-5), 166.8 (C-7), 196.0 (C-4).

Pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-(*S*)-HHDP]-*β*-D-glucose (2): yellow powder; $[\alpha]^{25}_D$ −56° (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 282 (4.67), 215 (4.94) nm; IR (KBr) ν_{max} 3446 (OH), 1735, 1638 (C=O), 1580, 1448, 1351 (s), 1237, 1173, 1034, 1019 cm^{-1}; CD (*c* 3.1 × 10^{-4} M, MeOH,) 238 ([θ] +129 529), 269 ([θ] −51 735) nm; negative FABMS *m*/*z* 871 [M − H]$^-$ (100), 872 [M]$^-$ (51), 301 (34); HRFABMS 872.1417 [M]$^-$, calcd for $C_{42}H_{32}O_{21}$, 872.1435; ^{1}H NMR (CD_3OD, 300 MHz,) δ 2.80 (1H, dd, *J* = 3.1, 17.1 Hz, H-3), 3.14 (1H, dd, *J* = 12.8, 17.1 Hz, H-3), 3.84 (1H, br t, H-2″), 3.85 (1H, br d, *J* = 13.3 Hz, H-6″), 4.23 (1H, dd, *J* = 6.2, 9.6 Hz, H-5″), 5.05 (1H, t, *J* = 9.6 Hz, H-4″), 5.18 (1H, d, *J* = 7.6 Hz, H-1″), 5.30 (1H, dd, *J* = 6.2, 13.3 Hz, H-6″), 5.40 (1H, t, *J* = 9.6 Hz, H-3″), 5.48 (1H, dd, *J* = 3.1, 12.8 Hz, H-2), 6.23 (1H, d, *J* = 2.2 Hz, H-6), 6.27 (1H, d, *J* = 2.2 Hz, H-8), 6.47 and 6.58 (each 1H, s, HHDP−H), 7.01 (2H, s, galloyl-H), 7.35−7.44 (3H, m, H-3′, 4′, 5′), 7.47−7.51 (2H, m, H-2′, 6′); ^{13}C NMR (CD_3OD, 75 MHz) δ 44.3 (C-3), 64.0 (C-6″), 71.3 (C-4″), 72.9 (C-5″), 73.3 (C-2″), 75.9 (C-3″), 80.8 (C-2), 96.8 (C-8), 98.1 (C-6), 101.4 (C-1″), 105.1 (C-10), 108.3 and 108.6 (HHDP C-3, 3′), 110.6 (galloyl C-2, 6), 116.4 and 116.7 (HHDP C-1, 1′), 121.1 (galloyl C-1), 125.9 and 126.3 (HHDP C-2, 2′), 127.4 (C-2′, 6′), 129.8 (C-3′, 4′, 5′), 137.6 (HHDP C-5, 5′), 139.9 (galloyl C-4), 140.1 (C-1′), 144.8 (HHDP C-6, 6′), 145.8 (HHDP C-4, 4′), 146.3 (galloyl C-3, 5), 164.5 (C-9), 165.0 (C-5), 166.5 (C-7), 168.0 (galloyl −COO−), 169.2, 169.6 (HHDP −COO−), 198.1 (C-4).

Acknowledgment. The study was supported by a grant from the National Science Council of the Republic of China.

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NP970428K