

· 化学成分 ·

Studies on chemical constituents from *Tetrastigma hypoglaucum*

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Abstract Object To investigate the chemical constituents in the aerial part of *Tetrastigma hypoglaucum* Planch. native to China in order to obtain a more comprehensive understanding of its effective components. **Methods** Compounds were isolated by various column chromatographies with silica gel. Their structures were elucidated by spectral analysis (IR, MS, ¹H NMR, ¹³C NMR) and chemical evidence. **Results** Ten compounds were isolated from the ethanol extracts of *T. hypoglaucum*, which were identified as β -sitosterol (I), palmitic acid (II), pentacosane (III), daucosterol (IV), resveratrol (V), gallic acid (VI), ethyl gallate (VII), catechin (VIII), 7-O-galloylcatechin (IX), and 3, 3'-dimethoxy ellagic acid-4-O β -D-glucopyranoside (X). **Conclusion** All compounds were obtained from this plant for the first time.

Key words *Tetrastigma hypoglaucum* Planch.; catechin; chemical constituents

狭叶崖爬藤化学成分的研究

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摘要: 目的 研究我国特有药用植物狭叶崖爬藤 *Tetrastigma hypoglaucum* 的化学成分,为阐明其有效成分提供依据。方法 利用各种色谱技术进行分离,根据化合物的光谱数据 (IR, MS, ¹H NMR, ¹³C NMR) 和化学方法鉴定其结构。结果 从狭叶崖爬藤地上部分分离并鉴定了 10 个化合物,分别为 β -谷甾醇 (β -sitosterol, I), 棕榈酸 (palmitic acid, II), 正二十五烷 (pentacosane, III), 胡萝卜苷 (daucosterol, IV), 白藜芦醇 (resveratrol, V), 没食子酸 (gallic acid, VI), 没食子酸乙酯 (ethyl gallate, VII), 儿茶素 (catechin, VIII), 7-氧没食子酰基儿茶素 (7-O-galloylcatechin, IX) 和 3, 3'-二甲氧基鞣花酸-4-氧葡萄糖苷 (3, 3'-dimethoxy ellagic acid-4-O β -D-glucopyranoside, X)。结论 所有化合物均系首次从该植物中分离得到。

关键词: 狭叶崖爬藤; 儿茶素; 化学成分

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Tetrastigma hypoglaucum Planch. (Vitaceae) is widely distributed in Sichuan and Yunnan Provinces of China. As a Chinese folk herb medicine, its rhizomes or the whole plants are used for the treatment of fracture, traumatic injury, and swelling pain due to pathogenic wind-dampness^[1]. In order to obtain a more comprehensive understanding of its effective constituents, ten compounds (I-X) were isolated from the 95% EtOH extracts of this plant. These ten compounds, namely, β -sitosterol (I), palmitic acid (II), pentacosane (III), daucosterol (IV), resver-

atrol (V), gallic acid (VI), ethyl gallate (VII), catechin (VIII), 7-O-galloylcatechin (IX), and 3, 3'-dimethoxy ellagic acid-4-O β -D-glucopyranoside (X) were identified by comparison with authentic samples, and on the basis of their physical constants and spectral data^[2-7].

Experimental section

General experimental procedures. Melting points were determined on a Fisher-Johns apparatus and were uncorrected. UV spectra were measured on a Philips PYE Unicam PU 8800 spectrometer. IR spectra were recorded on a Perkin-Elmer

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983 G spectrometer. NMR spectra (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) were measured on a Burkert AM-500 spectrometer, using TMS as internal standard. Coupling constants (J values) are given in Hz. VG ZAB-2F mass spectrometer was used to record the EI-MS, and an Autospec-Ultima ETOF spectrometer was used to record the FAB-MS. Si gel and Si gel GF₂₅₄ sheets (0.20–0.25 mm) (both from Qingdao Marine Chemical Group Co., Qingdao, Shandong Province, China) were used for column chromatography and TLC, respectively. D₁₀₁ microreticular resin was made by Tianjin Nankai Chemical Industry. Sephadex LH-20 (Pharmacia, 40 μm) was purchased from Sigma Chemicals.

Plant material. The whole plant of *T. hypoglaucum* was collected in Lijiang, Yunnan Province of China, in June, 1998, and identified by Professor Lian Wen-yan, from Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College. A voucher specimen (HB-98-0609) is deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College.

Extraction and isolation. The air-dried and pulverized aerial part of *T. hypoglaucum* (11.2 kg) was extracted three times with 95% EtOH for two hours under reflux. After removal of solvent, the residue (1.64 kg) was suspended in water (3 000 mL) and partitioned successively with petroleum ether (3 000 mL \times 3), and *n*-BuOH (3 000 mL \times 3). The petroleum ether-soluble fraction (150 g) was isolated by repeated column chromatography with silica gel and Sephadex LH-20 to afford compounds I (200 mg), II (40 mg), III (68 mg) and IV (40 mg). The ethyl acetate-soluble fraction (100 g) was isolated by repeated column chromatography with silica gel and Sephadex LH-20 to afford compounds V (10 mg), VI (400 mg), VII (34 mg), VIII (200 mg) and IX (25 mg). Compound X (22 mg) was obtained by repeated column chromatography with D₁₀₁ microreticular resin, silica gel and Sephadex LH-20 from the *n*-butanol-

soluble part.

β -Sitosterol (I): colorless needles, mp 137 °C–139 °C (acetone); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3 420 (OH), 2 980, 2 960, 1 650 (C=C); EI-MS m/z : 414 [M^+] (65), 396 (68), 381 (37), 329 (35), 303 (40), 273 (31), 255 (65), 233 (30), 213 (55), 159 (55), 81 (100). It was identified by comparison with an authentic sample.

Palmitic acid (II): white solid, mp 56 °C–58 °C (acetone); EI-MS m/z : 256 [M^+] (1), 99 (12), 85 (48), 71 (65), 57 (100). It was identified by comparison with an authentic sample.

Pentacosane (III): white solid; mp 56 °C–58 °C (MeOH); EI-MS m/z : 352 [M^+] (1), 99 (12), 85 (48), 71 (65), 57 (100). It was identified by comparison with an authentic sample.

Daucosterol (IV): white amorphous powder, mp 290 °C–295 °C (MeOH); EI-MS m/z : 576 [M^+] (2), 414 (28), 396 (100), 381 (45), 392 (14), 303 (14), 255 (32), 231 (12), 213 (24). It was identified by comparison with an authentic sample.

Resveratrol (V)^[3]: colorless needles, mp 258 °C–260 °C (MeOH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3 240, 1 600, 1 583, 1 510, 1 460, 1 440, 1 380, 1 260, 1 145, 1 010, 860, 805. EI-MS m/z : 228 [M^+] (100), 211 (8), 199 (6), 181 (20); ^1H NMR (CD_3OD) δ : 1.0 (1H, t, J = 2.0 Hz, H-4'); 6.39 (2H, d, J = 2.0 Hz, H-2' and H-6'), 6.70 (2H, dd, J = 7.0, 2.0 Hz, H-3 and H-5), 6.73 (1H, d, J = 16.0 Hz, H β), 6.89 (1H, d, J = 16.0 Hz, H α), 7.29 (2H, dd, J = 7.0, 2.0 Hz, H-2 and H-6); ^{13}C NMR (CD_3OD) δ : 102.6 (C-4'), 105.7 (C-2' and C-6'), 116.0 (C-3 and C-5), 127.0 (C-1), 128.8 (C-2 and C-6), 129.4 (C-1'), 130.4 (C β), 141.3 (C α), 158.3 (C-4), 159.6 (C-3' and C-5').

Gallic acid (VI)^[2]: white needles, mp 238 °C–240 °C (MeOH); EI-MS m/z : 170 [M^+] (100), 152 [$\text{M}-\text{H}_2\text{O}$] (90), 135 (22), 125 (30), 107 (16), 79 (28). ^1H NMR (CD_3OD) δ : 7.00 (2H, s, H-3 and H-7). ^{13}C NMR (CD_3OD) δ : 110.3 (C-3 and C-7), 122.0 (C-2), 139.6 (C-5), 146.4 (C-4 and C-6), 170.4 (C-1).

Ethyl gallate (VII)^[4]: white needles, mp 146

$^{\circ}\text{C}$ -148 $^{\circ}\text{C}$ (MeOH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3 400, 2 980, 1 700, 1 610, 1 530, 1 450, 1 370, 1 230, 1 100, 1 020, 870, 760. EI-MS m/z : 198 [M^+] (52), 170 (20), 153 (100), 125 (18). $^1\text{HNM R}(\text{CD}_3\text{OD})$ δ 1.27 (3H, t, $J=7.0$ Hz, H-9), 4.20 (2H, q, $J=7.0$ Hz, H-8), 6.99 (2H, s, H-3 and H-7). $^{13}\text{CNMR}(\text{CD}_3\text{OD})$ δ 14.6 (C-9), 61.7 (C-8), 109.9 (C-3 and C-7), 121.7 (C-2), 139.6 (C-5), 146.4 (C-4 and C-6), 168.5 (C-1).

Catechin (VIII) ^[5,6]: white needles, mp 154 $^{\circ}\text{C}$ - 156 $^{\circ}\text{C}$ (MeOH); UV λ_{max} (nm): 278 (MeOH); 294 (NaOMe); 278, 314 (sh), 360 (AlCl₃); 278, 314, 360 (AlCl₃ + HCl); 278 (NaOAc); 278 (H₃BO₃). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3 380, 1 623, 1 520, 1 465, 1 185, 1 146, 1 025, 820. EI-MS m/z : 290 [M^+] (45), 272 (8), 152 (60), 139 (100), 123 (63). $^1\text{HNM R}(\text{CD}_3\text{OD})$ δ 2.46 (1H, dd, $J=7.5, 16.0$ Hz, H-4a), 2.79 (1H, dd, $J=5.0, 16.0$ Hz, H-4b), 3.92 (1H, dd, $J=7.5, 8.0$ Hz, H-3), 4.50 (1H, d, $J=7.5$ Hz, H-2), 5.86 (1H, d, $J=1.5$ Hz, H-8), 5.93 (1H, d, $J=1.5$ Hz, H-6), 6.66 (1H, dd, $J=8.0$ Hz, H-6'), 6.70 (1H, d, $J=8.0$ Hz, H-5'), 6.78 (1H, s, H-2'); $^{13}\text{CNMR}(\text{CD}_3\text{OD})$ δ 28.5 (C-4), 68.8 (C-3), 82.8 (C-2), 96.2 (C-6), 95.4 (C-8), 100.8 (C-10), 115.2 (C-2'), 116.0 (C-5'), 120.0 (C-6'), 132.0 (C-1'), 146.2 (C-3' and C-4'), 156.9 (C-7), 157.6 (C-9), 157.8 (C-5).

7-O-Galloylcatechin (IX) ^[6]: white needles, mp 262 $^{\circ}\text{C}$ -264 $^{\circ}\text{C}$ (MeOH); UV λ_{max} (nm): 278 (MeOH); 294 (NaOMe); 278, 314 (sh), 360 (AlCl₃); 278, 314, 360 (AlCl₃ + HCl); 278 (NaOAc); 278 (H₃BO₃). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3 330, 1 700, 1 623, 1 530, 1 465, 1 350, 1 285, 1 203, 1 140, 1 025, 860, 760. EI-MS m/z : 290 (45), 272 (8), 152 (60), 139 (100), 123 (63); $^1\text{HNM R}(\text{CD}_3\text{OD})$ δ 2.43 (1H, dd, $J=7.5, 15.5$ Hz, H-4a), 2.69 (1H, dd, $J=5.5, 15.5$ Hz, H-4b), 3.94 (1H, dt, $J=7.5, 5.5$ Hz, H-3), 4.50 (1H, d, $J=7.0$ Hz, H-2), 6.17 (1H, d, $J=2.0$ Hz, H-8), 6.20 (1H, d, $J=2.0$ Hz, H-6), 6.66 (1H, dd, $J=7.5, 2.0$ Hz, H-

6'), 6.71 (1H, d, $J=7.5$ Hz, H-5'), 6.78 (1H, d, $J=2.0$ Hz, H-2'), 7.13 (2H, d, $J=1.0$ Hz, H-3'). $^{13}\text{CNMR}(\text{CD}_3\text{OD})$ δ 28.6 (C-4), 68.1 (C-3), 83.0 (C-2), 101.6 (C-10), 103.5 (C-8), 106.4 (C-6), 110.5 (C-3' and C-7'), 115.1 (C-2'), 116.1 (C-5'), 120.0 (C-2''), 131.7 (C-1'), 140.6 (C-4' and C-6''), 146.2 (C-4'), 146.3 (C-3'), 146.7 (C-5''), 151.7 (C-7), 157.0 (C-9), 158.1 (C-5), 166.5 (C-1'').

3, 3'-Dimethoxy ellagic acid-4-O- β -D-glucopyranoside (X) ^[7]: white amorphous powder, mp 285 $^{\circ}\text{C}$ -287 $^{\circ}\text{C}$ (MeOH); positive to Molish test; Positive-ion FAB-MS m/z 493 [$\text{M} + \text{H}^+$]; m/z : 330 [$\text{M} - 162$] (100), 315 (40); $^1\text{HNM R}(\text{DMSO}-d_6)$ δ 5.16 (1H, d, $J=8.0$ Hz, Glu-H-1), 3.22-3.71 [m, Glu-H-(2-6)], 4.09 (1H, s, 3-OCH₃), 4.05 (1H, s, 3'-OCH₃), 7.81 (1H, s, H-5), 7.53 (1H, s, H-5'). $^{13}\text{CNMR}(\text{CD}_3\text{OD})$ δ 114.8 (C-1), 142.4 (C-2), 142.4 (C-3), 153.5 (C-4), 112.6 (C-5), 112.5 (C-6), 159.1 (C-7), 111.8 (C-1'), 141.6 (C-2'), 140.8 (C-3'), 152.3 (C-4'), 113.5 (C-5'), 112.3 (C-6'), 159.1 (C-7'), 62.4 (3-OCH₃), 102.0 (C-1''), 74.0 (C-2''), 77.2 (C-3''), 70.2 (C-4''), 78.0 (C-5''), 61.2 (C-6'').

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