

Chemical Constituents of *Clematis montana*

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[ABSTRACT] **AIM:** To study the chemical constituents of *Clematis montana* Buch.-Ham. ex DC. **METHODS:** Aerial part of the plant was extracted by ethanol firstly and then the ethyl acetate part of the ethanol extract was isolated by different column chromatographic techniques including silica gel and Sephadex LH-20. Structures of these compounds were identified on the basis of spectroscopic analysis. **RESULTS:** Fourteen compounds were isolated and their structures were determined to be coniferaldehyde (1), caffeic acid (2), pluchoic acid (3), protocatechualdehyde (4), vanillin (5), hydroxytyrosol (6), 4-carbonyl-5-hydroxy methyl valerate (7), 4-hydroxydodec-2-enedioic acid (8), (+)-dihydrodehydrodiconiferyl alcohol (9), (-)-syringaresinol (10), (+)-guayanol (11), (-)-arctiin (12), (-)-lariciresinol (13), and hyperin (14). **CONCLUSION:** All the compounds were obtained from this plant for the first time.

[KEY WORDS] *Clematis montana*; Ranunculaceae; Lignans; Flavonoids

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1 Introduction

The genus of *Clematis* (Ranunculaceae) containing about 355 species is widely distributed in the world. There are 155 species in China, among 101 are endemic. *Clematis montana* Buch.-Ham. ex DC., a woody climber, usually grows in mountain slopes, valleys, and grasslands, and is distributed in Tibet, Sichuan, Guizhou, Guangxi and Yunnan^[1]. As a folk medicinal herb, the stems of this plant are used for dropsy, cystitis, proctoptosis with chronic dysentery, urethritis and galactostasis^[2] while the leaves for skin diseases. In addition, its seeds have purging properties^[3,4]. As for the chemical constituents of *C. montana*, only some triterpene saponins were reported^[3,4]. In order to investigate the bioactive constituents of this plant we have carried out a detailed chemical study on this plant and fourteen known compounds were isolated from the ethyl acetate part of the ethanol extract of the plant. These compounds were identified as coniferaldehyde (1), caffeic acid (2), pluchoic acid (3), protocatechualdehyde (4), vanillin (5), hydroxytyrosol (6), 4-carbonyl-5-hydroxy methyl valerate (7), 4-hydroxydodec-2-enedioic acid (8), (+)-dihydrode-

hydrodiconiferyl alcohol (9), (-)-syringaresinol (10), (+)-guayanol (11), (-)-arctiin (12), (-)-lariciresinol (13), and hyperin (14).

2 Experimental

2.1 General

Thin layer chromatography was performed on silica gel GF₂₅₄ plates (Qingdao Meigao Chemical Co., Ltd). TLC developing agent is 5% sulphuric acid in ethanol. Optical rotations were determined on a JASCO DIP370 digital polarimeter. Column chromatography was carried out on silica gel (200-300 mesh; Qingdao Makall Group Co., Ltd), Sephadex LH-20 (GE Healthcare Bio-Sciences AB), RP-18 gel (40-63 μm; Merck, Darmstadt, Germany), and MCI resin (75-150 μm, Mitsubishi Chemical Corporation). Preparative TLC was carried out on silica gel GF₂₅₄. MS data were measured on a VG AutoSpec 3000 mass spectrometer. All the NMR data were obtained at room temperature on AM-400 and DRX-500 spectrometers (TMS as internal reference, chemical shift in δ).

2.2 Plant Material

The aerial parts of *Clematis montana* were collected in Lijiang of Yunnan Province, China, in August 2006, and identified by Prof. PU Chun-Xia. A voucher specimen (No. 0866446) was deposited in Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2.3 Extraction and Isolation

The air-dried aerial parts of *C. montana* (4.8 kg) were ground and extracted with 90% EtOH (10 L × 3). The extracted liquid was combined and con-

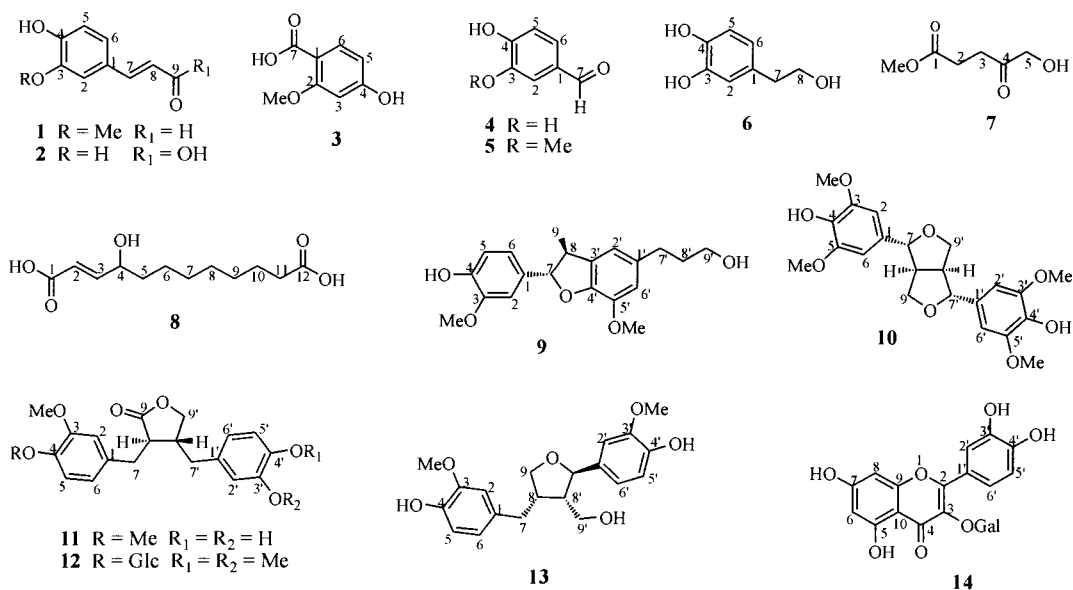
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centrated in vacuum on a rotary evaporator. The residue was suspended in water and extracted successively with petroleum ether (2 L × 3), EtOAc (2 L × 5) and *n*-BuOH (2 L × 4). The EtOAc extract (60 g) was subjected to silica gel chromatography, eluted with CHCl₃-Me₂CO (1:0 to 0:1) to provide fractions 1-12. Fraction 1 (560 mg) was subjected to repeated column chromatography on silica gel, eluted with petroleum ether-Me₂CO (20:1 to 2:1) to yield **1** (8 mg) and **5** (4 mg). Fraction 2 (790 mg) was subjected to Sephadex LH-20 column chromatography elu-

ted with MeOH to yield **10** (7 mg), **11** (15 mg) and **13** (6 mg). Fraction 7 (2.5 g) was subjected to Sephadex LH-20 CC eluted with MeOH to yield **4** (8 mg), **8** (13 mg), **9** (18 mg), **6** (11 mg), **3** (22 mg) and **2** (67 mg). Fraction 8 (3.8 g) was subjected to repeated column chromatography on silica gel, eluted with petroleum ether-EtOAc (5:1 to 0:1) to yield **7** (22 mg). Fraction 12 (12 g) was subjected to Sephadex LH-20 column chromatography eluted with MeOH to yield **12** (13 mg) and **14** (200 mg).



3 Identification

Coniferaldehyde^[5] (**1**) White powder (CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ: 7.08 (1H, d, *J* = 1.6 Hz, H-2), 6.97 (1H, d, *J* = 8.2 Hz, H-5), 7.13 (1H, dd, *J* = 8.2, 1.7 Hz, H-6), 7.41 (1H, d, *J* = 15.8 Hz, H-7), 6.61 (1H, dd, *J* = 15.8, 7.7 Hz, H-8), 9.66 (1H, d, *J* = 7.8 Hz, H-9), 3.95 (3H, s, -OMe); ¹³C NMR (CDCl₃, 100 MHz) δ: 126.6 (C-1), 109.5 (C-2), 147.0 (C-3), 149.0 (C-4), 115.0 (C-5), 124.1 (C-6), 153.2 (C-7), 126.4 (C-8), 193.7 (C-9), 56.0 (-OMe).

Caffeic acid^[6] (**2**) Yellow powder (MeOH); ESI-MS *m/z* 179[M - H]⁻; ¹H NMR (CD₃OD, 400 MHz) δ: 7.05 (1H, d, *J* = 2.0 Hz, H-2), 6.79 (1H, d, *J* = 8.2 Hz, H-5), 6.95 (1H, dd, *J* = 8.2, 2.0 Hz, H-6), 7.52 (1H, d, *J* = 15.9 Hz, H-7), 6.23 (1H, d, *J* = 15.9 Hz, H-8); ¹³C NMR (CD₃OD, 100 MHz) δ: 127.7 (C-1), 116.6 (C-2), 146.7 (C-3), 149.4 (C-4), 115.8 (C-5), 115.2 (C-6), 146.8 (C-7), 122.8 (C-8), 170.9 (C-9).

Pluchoic acid^[7] (**3**) Colorless powder (MeOH);

¹H NMR (CD₃OD, 400 MHz) δ: 6.68 (1H, d, *J* = 2.1 Hz, H-3), 6.19 (1H, d, *J* = 9.4 Hz, H-5), 7.81 (1H, d, *J* = 9.4 Hz, H-6), 3.88 (3H, s, -OMe).

Protocatechualdehyde^[8] (**4**) Colorless needles (Me₂CO); ¹H NMR (Acetone-*d*₆, 400 MHz) δ: 7.33 (1H, d, *J* = 1.9 Hz, H-2), 6.99 (1H, d, *J* = 7.9 Hz, H-5), 7.35 (1H, dd, *J* = 7.2, 1.9 Hz, H-6), 9.77 (1H, s, H-7), 8.77 (2H, br s, 3, 4-OH); ¹³C NMR (Acetone-*d*₆, 100 MHz) δ: 130.9 (C-1), 115.1 (C-2), 146.4 (C-3), 152.3 (C-4), 116.1 (C-5), 125.5 (C-6), 191.2 (C-7).

Vanillin^[9] (**5**) Colorless plate crystal (CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ: 6.37 (1H, d, *J* = 1.6 Hz, H-2), 7.06 (1H, d, *J* = 8.5 Hz, H-5), 7.44 (1H, dd, *J* = 8.0, 1.6 Hz, H-6), 9.84 (1H, s, H-7), 3.98 (3H, s, -OMe); ¹³C NMR (CDCl₃, 100 MHz) δ: 130.0 (C-1), 108.7 (C-2), 147.1 (C-3), 151.7 (C-4), 114.4 (C-5), 127.6 (C-6), 191.0 (C-7), 56.1 (-OMe).

Hydroxytyrosol^[10] (**6**) Brown oil (MeOH); ¹H NMR (CD₃OD, 500 MHz) δ: 6.64 (1H, d, *J* = 1.8 Hz, H-2), 6.66 (1H, d, *J* = 8.0 Hz, H-

5), 6.52 (1H, dd, $J = 8.0, 2.0$ Hz, H-6), 2.65 (2H, t, $J = 7.3$ Hz, H-7), 3.66 (2H, t, $J = 7.3$ Hz, H-8); ^{13}C NMR (CD_3OD , 125 MHz) δ : 131.8 (C-1), 116.3 (C-2), 146.1 (C-3), 144.6 (C-4), 117.1 (C-5), 121.2 (C-6), 39.6 (C-7), 64.6 (C-8).

4-Carbonyl-5-hydroxy methyl valerate^[11] (7)

Yellow oil (CHCl_3); ^1H NMR (CDCl_3 , 500 MHz) δ : 2.67 (2H, t, $J = 6.7$, H-2), 2.73 (2H, t, $J = 6.7$, H-3), 4.33 (2H, s, H-5), 3.69 (3H, s, -OMe); ^{13}C NMR (CDCl_3 , 125 MHz) δ : 172.7 (C-1), 27.4 (C-2), 32.7 (C-3), 208.1 (C-4), 68.1 (C-5), 51.9 (-OMe).

4-Hydroxydodec-2-enedioic acid^[12] (8)

White amorphous solid (MeOH); ^1H NMR (CD_3OD , 400 MHz) δ : 5.96 (1H, dd, $J = 15.6, 1.6$ Hz, H-2), 6.91 (1H, dd, $J = 15.6, 5.0$ Hz, H-3), 4.21 (1H, m, H-4), 1.61-1.50 (4H, m, H-5, 10), 1.45-1.34 (8H, m, H-6, 7, 8, 9), 2.27 (2H, t, $J = 7.4$ Hz, H-11); ^{13}C NMR (CD_3OD , 100 MHz) δ : 170.1 (C-1), 120.9 (C-2), 152.7 (C-3), 71.5 (C-4), 37.5 (C-5), 26.4 (C-6), 30.4 (C-7), 30.3 (C-8), 30.1 (C-9), 26.0 (C-10), 34.9 (C-11), 177.7 (C-12).

(+)-Vladinol F^[13] (9)

Colorless oil (MeOH); $[\alpha]_{\text{D}}^{25}$: +1.63° (c 2, MeOH); ESI-MS m/z 383 [$\text{M} + \text{Na}$]⁺, 743 [$2\text{M} + \text{Na}$]⁺; ^1H NMR (CD_3OD , 400 MHz) δ : 6.94 (1H, br s, H-2), 6.75 (1H, d, $J = 8.0$ Hz, H-5), 6.81 (1H, br d, $J = 6.8$ Hz, H-6), 5.48 (1H, d, $J = 6.3$ Hz, H-7), 3.46 (1H, dd, $J = 12.4, 6.2$ Hz, H-8), 3.81-3.72 (2H, m, H-9), 6.71 (2H, br s, H-2', 6'), 2.61 (2H, t, $J = 7.6$ Hz, H-7'), 1.80 (2H, dt, $J = 15.0, 6.6$ Hz, H-8'), 3.55 (2H, t, $J = 6.4$ Hz, H-9'), 3.83 (6H, s, -OMe); ^{13}C NMR (CD_3OD , 100 MHz) δ : 132.5 (C-1), 109.8 (C-2), 149.0 (C-3), 147.5 (C-4), 115.5 (C-5), 119.4 (C-6), 87.0 (C-7), 55.9 (C-8), 68.2 (C-9), 128.9 (C-1'), 114.6 (C-2'), 143.8 (C-3'), 145.1 (C-4'), 137.9 (C-5'), 114.8 (C-6'), 34.9 (C-7'), 30.9 (C-8'), 68.2 (C-9'), 55.9 (-OMe), 55.9 (-OMe).

(-)-Syringaresinol^[14] (10)

Colorless oil (CHCl_3); $[\alpha]_{\text{D}}^{25}$: -8.75° (c 2, CHCl_3); ^1H NMR (CDCl_3 , 400 MHz) δ : 6.58 (4H, br s, H-2, 2', 6, 6'), 5.55 (2H, br s, 4, 4'-OH), 4.74 (2H, d, $J = 3.6$ Hz, H-7, 7'), 3.10 (2H, m, H-8, 8'), 3.92 (2H, m, H-9 α , 9' α), 4.29 (2H, m, H-9 β , 9' β), 3.90 (12H, s, OCH_3); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 132.0 (C-1, 1'), 102.6 (C-2, 2', 6, 6'), 147.1 (C-3, 3', 5, 5'), 134.2 (C-4, 4'), 86.1 (C-7, 7'), 54.3 (C-8, 8'), 71.8 (C-9, 9'), 56.3 (-OMe).

(+)-Guayanol^[15,16] (11)

Colorless oil (CHCl_3); $[\alpha]_{\text{D}}^{25}$: +15.83° (c 2, CHCl_3); ESI-MS m/z 359

[$\text{M} + \text{H}$]⁺, 381 [$\text{M} + \text{Na}$]⁺; ^1H NMR (CDCl_3 , 500 MHz) δ : 6.59 (1H, d, $J = 1.6$ Hz, H-2), 6.80 (1H, d, $J = 8.1$ Hz, H-5), 6.61 (1H, dd, $J = 8.1, 1.6$ Hz, H-6), 2.91 (2H, m, H-7), 6.41 (1H, d, $J = 1.6$ Hz, H-2'), 6.82 (1H, d, $J = 8.3$ Hz, H-5'), 6.50 (1H, dd, $J = 8.0, 1.6$ Hz, H-6'), 2.60-2.46 (4H, m, H-7', 8', 8), 3.89 (1H, dd, $J = 9.1, 7.4$ Hz, H-9' α), 4.15 (1H, dd, $J = 9.1, 7.4$ Hz, H-9' β), 3.81 (6H, s, -OMe), 5.57 (2H, br s, 3', 4'-OH); ^{13}C NMR (CDCl_3 , 125 MHz) δ : 129.5 (C-1), 111.5 (C-2), 146.7 (C-3), 146.6 (C-4), 111.0 (C-5), 121.3 (C-6), 34.6 (C-7), 46.6 (C-8), 178.8 (C-9), 129.8 (C-1'), 114.4 (C-2'), 144.5 (C-3'), 144.4 (C-4'), 114.1 (C-5'), 122.1 (C-6'), 38.3 (C-7'), 41.0 (C-8'), 71.3 (C-9'), 55.8 (-OMe).

(-)-Arctiin^[17] (12)

Colorless oil (Me_2CO); $[\alpha]_{\text{D}}^{25}$: -7.13° (c 2, Me_2CO); ^1H NMR (Acetone- d_6 , 400 MHz) δ : 6.84 (1H, d, $J = 1.7$ Hz, H-2), 7.07 (1H, d, $J = 8.2$ Hz, H-5), 6.70 (1H, dd, $J = 7.9, 1.6$ Hz, H-6), 6.67 (1H, d, $J = 1.5$ Hz, H-2'), 6.73 (1H, d, $J = 8.0$ Hz, H-5'), 6.56 (1H, dd, $J = 8.0, 1.7$ Hz, H-6'), 4.88 (1H, d, $J = 7.3$ Hz, Glc-H-1), 3.84 (3H, s, -OMe), 3.85 (3H, s, -OMe), 3.90 (3H, s, -OMe); ^{13}C NMR (Acetone- d_6 , 100 MHz) δ : 130.9 (C-1), 115.7 (C-2), 148.3 (C-3), 146.0 (C-4), 114.6 (C-5), 122.5 (C-6), 35.0 (C-7), 46.9 (C-8), 179.0 (C-9), 133.8 (C-1'), 117.4 (C-2'), 150.4 (C-3'), 146.7 (C-4'), 113.0 (C-5'), 121.9 (C-6'), 38.4 (C-7'), 42.2 (C-8'), 71.6 (C-9'), 102.6 (C-1''), 74.7 (C-2''), 77.7 (C-3''), 71.2 (C-4''), 77.8 (C-5''), 62.6 (C-6''), 56.3 (-OMe).

(-)-Lariciresinol^[18] (13)

Colorless oil (CHCl_3); $[\alpha]_{\text{D}}^{25}$: -10.00° (c 1, CHCl_3); ^1H NMR (CDCl_3 , 400 MHz) δ : 6.70 (1H, d, $J = 1.9$ Hz, H-2), 6.81 (1H, d, $J = 8.1$ Hz, H-5), 6.67 (1H, dd, $J = 8.1, 1.9$ Hz, H-6), 2.55 (1H, dd, $J = 13.7, 10.7$ Hz, H-7 α), 2.91 (1H, dd, $J = 13.7, 4.7$ Hz, H-7 β), 2.7 (1H, m, H-8), 6.88 (1H, d, $J = 1.9$ Hz, H-2'), 6.84 (1H, d, $J = 8.1$ Hz, H-5'), 6.79 (1H, dd, $J = 8.1, 1.9$ Hz, H-6'), 4.79 (1H, d, $J = 6.6$ Hz, H-7'), 2.40 (1H, dd, $J = 13.8, 6.9$ Hz, H-8'), 3.76 (1H, dd, $J = 10.7, 6.8$ Hz, H-9'a), 3.91 (1H, dd, $J = 10.7, 7.1$ Hz, H-9'b), 3.87 (6H, br s, OCH_3); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 132.3 (C-1), 111.2 (C-2), 146.6 (C-3), 144.0 (C-4), 114.4 (C-5), 121.2 (C-6), 33.3 (C-7), 42.4 (C-8), 72.1 (C-9), 134.8 (C-1'), 108.3 (C-2'), 146.6 (C-3'), 145.0 (C-4'), 114.2 (C-5'), 118.7 (C-6'), 82.8 (C-7'), 52.6 (C-8'), 60.9 (C-9'), 55.9 (-OMe).

Hyperin^[19] (14)

Yellow needles (MeOH); ESI-MS m/z 487 [$\text{M} + \text{Na}$]⁺, 951 [$2\text{M} + \text{Na}$]⁺;

¹H NMR (DMSO-*d*₆, 400 MHz) δ: 6.18 (1H, d, *J* = 1.8 Hz, H-6), 6.39 (1H, d, *J* = 1.8 Hz, H-8), 7.51 (1H, d, *J* = 2.0 Hz, H-2'), 6.80 (1H, d, *J* = 8.4 Hz, H-5'), 7.66 (1H, dd, *J* = 8.4, 1.9 Hz, H-6'), 12.62 (1H, s, OH-5), 5.37 (1H, d, *J* = 7.7 Hz, Gal- H-1) 3.64-3.29 (6H, m, protons of galactosyl); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 156.2 (C-2), 133.5 (C-3), 177.5 (C-4), 161.2 (C-5), 98.7 (C-6), 164.3 (C-7), 93.5 (C-8), 156.2 (C-9), 103.9 (C-10), 121.1 (C-1'), 115.2 (C-2'), 144.8 (C-3'), 148.5 (C-4'), 116.0 (C-5'), 122.0 (C-6'), 101.8 (C-1''), 71.2 (C-2''), 73.2 (C-3''), 67.9 (C-4''), 75.8 (C-5''), 60.1 (C-6'').

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绣球藤的化学成分

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【摘要】 目的: 对绣球藤的化学成分进行研究。方法: 利用硅胶柱色谱、Sephadex LH-20 等方法进行分离纯化。根据理化性质和光谱分析进行结构鉴定。结果: 从绣球藤乙醇提取物的乙酸乙酯部分中分离得到 14 个化合物, 分别鉴定为: 松柏醛(1), 咖啡酸(2), pluchoic acid(3), 原儿茶醛(4), 香草醛(5), 3,4-二羟基苯乙醇(6), 4-羧基-5-羟基戊酸甲酯(7), 4-hydroxydodec-2-enedioic acid (8), (+)-川木香醇 F (9), (-)-丁香脂素(10), (+)-guayarol(11), (-)-牛蒡苷(12), (-)-落叶松树脂醇(13), 金丝桃苷(14)。结论: 这些化合物均为首次从该植物中分离得到。

【关键词】 绣球藤; 铁线莲属; 木脂素; 黄酮

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