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A New Stereoisomeric Monoterpene Glycoside from *Clematis heracleifolia* leaves

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Abstract – A new stereoisomeric monoterpene glycoside and five already-known compounds were isolated from the *n*-BuOH soluble fraction of *Clematis heracleifolia* leaves. On the basis of spectral data, the structures of the isolated compounds were identified as protocatechuic acid (**1**), ferulic acid (**2**), caffeic acid (**3**), aesculin (**4**), (6*Z*)-9-hydroxylinaloyl glucoside (**5**), and 9-hydroxylinaloyl glucoside (**6**) and these were isolated for the first time from this plant. Among these compounds, (6*Z*)-9-hydroxylinaloyl glucoside (**5**) is a newly isolated from plant source.
Keywords – *Clematis heracleifolia*, Monoterpene glycoside, (6*Z*)-9-hydroxylinaloyl glucoside

Introduction

Clematis heracleifolia DC (Ranunculaceae) is a deciduous subshrub widely distributed in Eastern Asia.[1] The root, stem, and whole plant of *C. heracleifolia* have been used as therapeutic agents for arthritis, gout and diarrhea.[2] We previously reported that chemical constituents and their acetylcholinesterase inhibitory activity of underground parts of this plant.[3] As a part to our continuous investigations to isolate and elucidate the structure, we isolated six compounds from the *n*-BuOH soluble fraction of the leaves of *C. heracleifolia*. Herein, we describe the isolation and structural elucidation of a new stereoisomeric monoterpene glycoside along with five already-known compounds.

Experimental

General experimental procedures – The HR-ESIMS was performed on QTOF/MS system equipped with a Waters Xevo G2 QTOF mass spectrometer (Waters MS Technologies, Manchester, UK). The sample was measured with the following ESI conditions: positive ion mode, capillary voltage of 3.2 kV, sampling cone voltages of 10 V, source temperature of 120 °C, desolvation temperature of 350 °C, and desolvation gas flow of 500 l/h. The instrument was calibrated using a sodium formate solution as the calibration standard suggested by the manufacturer to achieve mass accuracies of < 5 ppm. To ensure the mass accuracy and reproducibility of the optimized MS conditions, leucine enkephalin (m/z 554.2615 in negative mode) was used as a reference lock mass at a concentration of 200 *pg*/μl and a flow rate of 5 μl/min. Leucine enkephalin was sprayed into the MS instrument every 10 s. The LC-ESIMS was measured on QqQ-MS system with AB Sciex API 3200MD™ mass spectrometer (AB SCIEX, Framingham, MA, USA). In the negative mode, the sample was injected to the system with the parameters of curtain gas at 10, ion spray voltage at –4500, temperature at 0 °C, ion source gas 1 at 20 and gas 2 at 0. Declustering potential and entrance potential were –45 and –10, respectively. NMR spectra were recorded on an AVANCE 600 (Bruker, Rheinstetten, Germany). The chemical shifts were represented as parts per million (ppm) referenced to the residual solvent signal. Column chromatography was carried out using a Kieselgel 60, 63 - 200 μm and 40 - 63 μm (Merck, Darmstadt, Germany) and YMC gel ODS-A, 150 μm (YMC, Kyoto, Japan). TLC was performed on a glass backed Kieselgel 60 F254 and RP F254s plates. All other chemicals and reagents used were of analytical grade.

Plant material – The leaves of *C. heracleifolia* were collected from Mt. Samyung in Yanggu (Kangwon Province, Korea). A voucher specimen (KNUH-13-09-L-1) was deposited in the herbarium of the College of Pharmacy, Kangwon National University, Korea.

Extraction and isolation – The air dried leaves of *C. heracleifolia* were cut into the small pieces and extracted with MeOH (2.1 kg, 60 L × 2) for two weeks at room temperature. The MeOH extract (900 g) was suspended in

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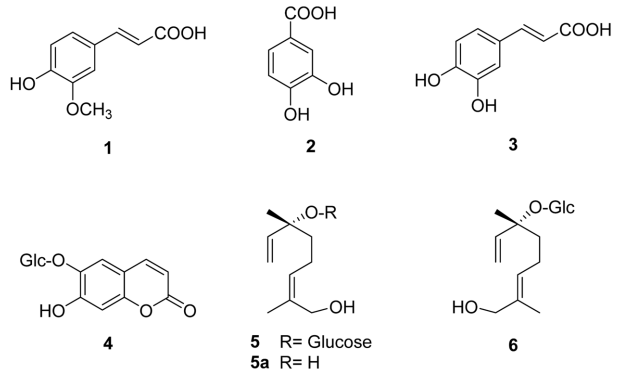


Fig. 1. Structures of the compounds isolated from *C. heracleifolia.*

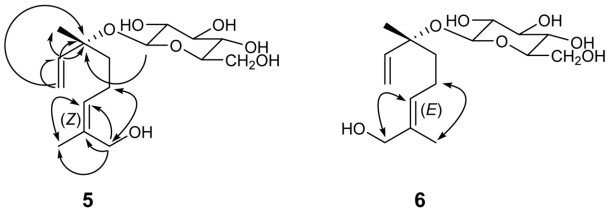


Fig. 2. Important HMBC (H → C) and NOESY (H ↔ H) correlations of compounds **5** and **6**.

water and then successively portioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, leaving a residual water soluble fraction. Each fraction was evaporated *in vacuo* to yield the residues of *n*-hexane fraction (fr.) (92 g), $CHCl_3$fr. (4.2 g), and *n*-BuOH fr. (58 g). The *n*-BuOH soluble fraction (56 g) was applied to silica gel column chromatography (1 kg, 63 - 200 μm, 15 × 50 cm) using isocratic elution with $CHCl_3$: MeOH : H_2O (3 : 1 : 0.1), in order to divide the fraction into six fractions (Fr. 1 – Fr. 6). Fr. 2 (5.1 g) underwent further chromatography on a flash column (Redisep®, silica gel, 120 g) using isocratic elution with $CHCl_3$: MeOH (9 : 1), in order to divide the seven sub-fractions (Fr. 2-1 – Fr. 2-7). Fr. 2-1 (0.9 g) was re-chromatographed on a silica gel (40 g, 40 - 63 μm, 3 × 20 cm) using isocratic elution with $CHCl_3$: MeOH (19 : 1) to give compound **1** (129.8 mg). Fr. 3 (22 g) was further chromatographed on an ODS (YMC gel, ODS-A, 150 μm, 500 g) using stepwise gradient elution with MeOH : H_2O (30 : 70 → 60 : 40) to yield five sub-fractions (Fr. 3-1 – Fr. 3-5). Fr. 3-1 (8.9 g) was re-chromatographed on silica gel (400 g, 63 - 200 μm, 6.5 × 50 cm) using isocratic elution with EtOAc : MeOH : H_2O (13 : 1.5 : 0.5) to give six sub-fractions (Fr. 3–1-1 – Fr. 3–1-6). Fr. 3–1-2 (800 mg) was further purified by flash column (Redisep®, ODS, 130 g, 25% MeOH) to give four sub-fractions (Fr. 3–1-2-1 – Fr. 3–1–2-4). Fr. 3–1–2-2 (40 mg) was re-chromatographed on a silica gel (40 g, 40 - 63 μm, 3 × 20 cm) using isocratic elution with $CHCl_3$: MeOH (6 : 1) to give compound **2** (7.8 mg). Fr. 3–1–2-3 (80 mg) was further purified by Sephadex LH - 20 (70 g, Pharmacia, 3 × 50 cm, 30% MeOH) to give compound **3** (47 mg). Fr. 3 – 1-3 (1.5 g) and Fr. 3 - 2 (2 g) were re-chromatographed on a flash column (Redisep®, silica gel, 120 g) using isocratic elution with $CHCl_3$: MeOH (7 : 1), in order to divide the four sub-fractions (Fr. 3–1–3-1 – Fr. 3–1–3-4). Fr. 3–1–3-2 (1.2 g) was re-chromatographed on a flash column (Redisep®, ODS, 40 g) using isocratic elution with MeOH : H_2O : CH_3COOH (20 : 80 : 2) to give five sub-fractions (Fr. 3–1–3–2-1 – Fr. 3–1–3–2-5). Fr. 3–1–3–2-2 (150 mg) was further purified by Sephadex LH-20 (40 g, Pharmacia, 3 × 20 cm, 30% MeOH) to give compound **4** (8 mg). Fr. 4 (3.9 g) was re-chromatographed on a flash column (Redisep®, silica gel, 80 g) using isocratic elution with $CHCl_3$: MeOH (4 : 1), in order to divide the five sub-fractions (Fr. 4-1 – Fr. 4-5). Fr. 4-3 (0.5 g) was further purified by Sephadex LH- 20 (70 g, Pharmacia, 3 × 50 cm, 30% MeOH) to give four sub-fractions (Fr. 4–3-1 – Fr. 4–3-4). Fr. 4–3-2 (187 mg) was re-chromatographed on an MPLC (μ-Bondapack C18, 19x 150 mm, Waters®) using isocratic elution with MeOH : MeOH (25 : 75) to give compounds **5** (39.2 mg) and **6** (20.3 mg).

Compound **1** – ^{1}H-NMR (600 MHz, CD_3OD) δ : 7.53 (1H, d, J = 15.8, H-8), 7.03 (1H, d, J = 1.7Hz, H-2), 6.92 (1H, dd, J = 1.7, 8.2 Hz, H-6), 6.77 (1H, d, J = 8.2Hz, H-5), 6.24 (1H, d, J = 15.8Hz, H-7), 3.74 (3H, s, O<u>C</u>H$_3$); ^{13}C-NMR (150 MHz, CD_3OD) δ : 168.37 (C-9), 148.16 (C-3), 145.54 (C-4), 145.40 (C-7), 126.31 (C-1), 121.55 (C-6), 115.12 (C-5), 113.77 (C-2), 113.48 (C-8), 50.63 (O<u>C</u>H$_3$); LC-ESI MS (negative mode) *m/z* : 193 $[M–H]^-$.

Compound **2** – ^{1}H-NMR (600 MHz, CD_3OD) δ : 7.43 (1H, br s, H-2), 7.42 (1H, dd, J = 1.9, 8.2H, H-6), 6.79 (1H, d, J = 8.2Hz, H-5); ^{13}C-NMR (150 MHz, CD_3OD) δ : 168.87 (C=O), 150.13 (C-4), 144.66 (C-3), 122.49 (C-6), 121.75 (C-1), 116.32 (C-2), 114.36 (C-5); LC-ESI MS (negative mode) *m/z* : 153 $[M–H]^-$.

Compound **3** – ^{1}H-NMR (600 MHz, CD_3OD) δ : 7.54 (1H, d, J = 15.9, H-8), 7.04 (1H, d, J = 1.4Hz, H-2), 6.93 (1H, dd, J = 1.4, 8.2 Hz, H-6), 6.78 (1H, d, J = 8.2Hz, H-5), 6.22 (1H, d, J = 15.9Hz, H-7); ^{13}C-NMR (150 MHz, CD_3OD) δ : 169.75 (C-9), 148.03 (C-7), 145.72 (C-3), 145.36 (C-4), 126.45 (C-1), 121.53 (C-6), 115.14 (C-5), 114.17 (C-2), 113.77 (C-8); LC-ESI MS (negative mode) *m/z* : 179 $[M–H]^-$.

Compound **4** – ^{1}H-NMR (600 MHz, CD_3OD) δ : 7.84 (1H, d, J = 9.4Hz, H-4), 7.43 (1H, s, H-5), 6.81 (1H, s, H-8), 6.22 (1H, d, J = 9.4Hz, H-3), 4.85 (1H, d, J = 8.0Hz,

H-1'), 3.94 (1H, dd, J = 1.9, 12.0 Hz, H-6'α), 3.72 (1H, dd, J = 5.9, 12.0 Hz, H-6'β), 3.54 – 3.39 (4H, m, H-2', H-3', H-4', H-5'); ^{13}C-NMR (150 MHz, CD_3OD) δ : 162.31 (C-2), 152.00 (C-7), 151.20 (C-9), 144.61 (C-4), 143.07 (C-6), 115.27 (C-5), 111.66 (C-3), 111.38 (C-10), 103.14 (C-8), 102.88 (C-1'), 77.11 (C-3'), 76.19 (C-5'), 73.41 (C-2'), 69.97 (C-4'), 61.13 (C-6'); LC-ESI MS (negative mode) *m/z* : 339 $[M-H]^-$.

Compound **5** – Oil; $[\alpha]_D$ −21.7° (MeOH, c 0.1); ^{1}H-NMR (600 MHz, CD_3OD) δ, 6.07 (1H, dd, J = 11.0, 17.0Hz, H-2), 5.26 (1H, br t, J = 7.0Hz, H-6), 5.19 (1H, J = 1.0, 17.0Hz, H-1a), 5.14 (1H, dd, J = 1.0, 11.0Hz, H-1b), 4.31 (1H, d, J = 7.7Hz, H-1'), 4.08 (1H, d, J = 12.0Hz, H-9a), 4.04 (1H, d, J = 12.0Hz, H-9b), 3.78 (1H, dd, J = 2.5, 12.0Hz, H-6'a), 3.62 (1H, dd, J = 5.5, 12.0Hz, H-6'b), 3.30 (2H, m, H-3', H-4'), 3.13 (2H, m, H-2', H-5'), 2.16 (1H, m, H-5), 1.74 (3H, s, 8-C$\underline{H}_3$), 1.64 (2H, m, H-4), 1.32 (3H, s, 10-C$\underline{H}_3$); ^{13}C-NMR (150 MHz, , CD_3OD) δ, see Table 1; HR-TOF MS, *m/z* 355.1736 (calcd for $C_{16}H_{28}O_7$ Na^+, 355.1733,); LC-ESI MS, *m/z* 331 $[M-H]^-$.

Compound **6** – Oil; $[\alpha]_D$ −32.4° (MeOH, c 0.1); ^{1}H-NMR (600 MHz, CD_3OD) δ, 6.09 (1H, dd, J = 11.1, 17.8 Hz, H-2), 5.39 (1H, br t, J = 6.3Hz, H-6), 5.19 (1H, dd, J = 1.1, 17.8Hz, H-1a), 5.15 (1H, dd, J = 1.1, 11.1 Hz, H-1b), 4.32 (1H, d, J = 8.0Hz, H-1'), 3.90 (2H, s, H-9), 3.79 (1H, dd, J = 2.2, 11.9 Hz, H-6'a), 3.62 (1H, dd, J = 5.5, 11.9 Hz, H-6'b), 3.30 (2H, m, H-3', H-4'), 3.13 (2H, H-2', H-5'), 2.12 (2H, m, H-5), 1.67 (2H, m, H-4), 1.63 (3H, s, 8-C$\underline{H}_3$), 1.33 (3H, s, 10-C$\underline{H}_3$); ^{13}C-NMR (150 MHz, CD_3OD) δ, see Table 1; HR-TOF MS, *m/z* 355.1736 (calcd for $C_{16}H_{28}O_7$ Na^+, 355.1733,); LC-ESI MS *m/z*, 331 $[M-H]^-$.

Acid hydrolysis of compounds 5 and 6 – Each compound (5 mg) was refluxed with 5% H_2SO_4 (5 ml) in water for 1h. The reaction mixture was diluted with water and fractionated by EtOAc. Each remaining aqueous layer was adjusted to pH 7 with $NaHCO_3$ and filtered. The filtrate was concentrated and examined by TLC with authentic sugars.

Enzymatic hydrolysis of compound 5 – A water solution of compound **5** (10 mg) was treated with cellulase (from *Aspergillus niger*) (15 mg, Sigma) at room temperature for 5 hr. The reaction mixture was diluted with H_2O and extracted with EtOAc four times. The EtOAc extract was purified by silica gel (Merck, 63 - 200 μm, 30 g) column chromatography (2.5 × 20 cm)to afford **5a** (0.3 mg); ^{1}H-NMR (600MHz, $CDCl_3$) δ, 5.94 (1H, dd, J = 10.6, 17.3 Hz, H-2), 5.34 (1H, br t, J = 7.4Hz, H-6), 5.25 (1H, J = 1.0, 17.3Hz, H-1a), 5.11 (1H, dd, J = 1.0, 10.6Hz, H-1b), 4.17 (1H, d, J = 15.3Hz, H-9a), 4.14 (1H, d, J = 15.3Hz, H-9b), 2.16 (1H, m, H-5), 1.82 (3H, s, 8-C$\underline{H}_3$), 1.61 (2H, m, H-4), 1.32 (3H, s, 10-C$\underline{H}_3$); ^{13}C-NMR (150 MHz, $CDCl_3$) δ, see Table 1.

Table 1. ^{13}C NMR data of compounds **5**, **5a** and **6**

	5[1)]	**5a**[2)]	**6**[1)]
1	113.56	111.87	113.58
2	143.18	144.93	143.09
3	79.83	73.47	79.91
4	40.11	42.05	39.84
5	21.70	22.38	21.84
6	127.62	128.49	125.58
7	134.19	134.56	134.42
8	20.08	21.43	12.33
9	60.00	61.61	67.58
10	22.40	28.14	22.14
Sugar moiety			
Glc 1	97.91		97.87
2	73.70		73.68
3	76.86		76.86
4	70.32		70.33
5	76.23		76.21
6	61.35		61.43

[1)]dissolved in CD_3OD
[2)]dissolved in $CDCl_3$

Result and Discussion

Compounds **1 - 4** were identified as ferulic acid, protocatechuic acid, caffeic acid and aesculin,[4-7] respectively. Compounds **5** and **6** have the same molecular weight, which produced a *pseudo*-molecular ion $[M]^+$ at *m/z* 355.1736 by HR-TOF-MS, consistent with a molecular formula of $C_{16}H_{28}O_7Na$ (calcd for 355.1733). The ^{1}H-NMR spectrum of compound **5** exhibited vinyl moiety signals at δ 6.07 (1H, dd, J = 11.0, 17.0Hz), 5.19 (1H, dd, J = 1.0, 17.0Hz), and 5.14 (1H, dd, J=1.0, 11.0Hz), two methyl signals (δ 1.74 and 1.32), a hydroxyl methyl signal at δ 4.08 and 4.04 (each 1H, d, J = 12.0Hz), and an anomeric proton signal at δ 4.31 (1H, d, J = 7.7Hz). These signals showed that compound **5** is a hydroxylinaloyl type glycoside.[8] In the HMBC spectrum, anomeric proton at δ_H 4.31 correlated with C-3 carbon at δ_c 79.83, which showed sugar attached at C-3 position. The attached sugar was determined as D-glucose by acid hydrolysis and TLC method. Unfortunately, we failed to obtain the rotation value and MS data of aglycone from the enzymatic hydrolysis of **5**, although we did record the ^{1}H- and ^{13}C-NMR spectra. The ^{1}H-NMR spectrum of compound **6** is very similar to that of compound **5**. The ^{13}C-NMR spectra

of these two compounds showed almost the same signals except for the chemical shift of C-8 and C-9 signals (Table 1). The C-8 and C-9 signals of compound **5** exhibited at δ 20.08 and 60.00, compared to at δ 12.33 and 67.58 for compound **6**.These signals were confirmed by COSY, DEPT, HSQC, and HMBC spectra. The structure of compound **6** was identified as 9-hydroxylinaloyl glucoside by comparison with literature values.[9] Compound **5** has a negative optical rotation value the same as that of compound **6**, which strongly suggested that these two compounds are stereoisomers. The NOESY spectra of compounds **5** and **6** provide a crucial clue for determining their structures. The NOESY spectrum of compound **5** showed correlations between H-5/H-9 and H-6/H-8, but compound **6** showed correlations between H-6/H-9 and H-5/H-8. This data confirmed the configuration of the C-6 position of **5** as (*Z*), whereas the configuration of the C-6 position of **6** is (*E*). Therefore, based on the above data, the structures of compounds **5** and **6** were identified as (*6Z*)-9-hydroxylinaloyl glucoside and 9-hydroxylinaloyl glucoside, respectively.

In conclusion, we isolated six compounds from the leaves of *C. heracleifolia*. Based on spectral data, the structures of the isolated compounds were identified asferulic acid (**1**), protocatechuic acid (**2**), caffeic acid (**3**), aesculin (**4**), 6(*Z*)-9-hydroxylinaloyl glucoside (**5**), and 9-hydroxylinaloyl glucoside (**6**). 6(*Z*)-9-Hydroxylinaloyl glucoside (**5**) was isolated for the first time from plant source.

References

(1) Lee, W. T. Standard illustrations of Korean plants; Academy Pub. Co: Seoul, **1996**, p 110.

(2) Bae, K. The medicinal plants of Korea; Kyo-Hak Pub. Co: Seoul, **2012**, p 136.

(3) Kim, M. A.; Kim, M. J.; Chun W.; Kwon Y. *Kor. J. Pharmacogn*. **2015**, *46*, 6-11.

(4) Prachayasittikul, S.; Suphapong, S.; Worachartcheewan, A.; Lawung, R.; Ruchirawat, S.; Prachayasittikul, V. *Molecules* **2009**, *14*, 850-867.

(5) Zhang, H. L.; Nagatsu, A.; Okuyama, H.; Mizukami, H.; Sakakibara, J. *Phytochemistry*, **1998**, *48*, 665-668.

(6) Lin, Z.; Fang, Y.; Huang, A.; Chen, L.; Cuo, S.; Chen, J. *Pharm. Biol*. **2014**, *52*, 1429-1434.

(7) Bayoumi, S. A.; Rowan, M. G.; Beeching, J. R.; Blagbrough, I. S. *Phytochemistry* **2010**, *71*, 598-604.

(8) Manns, D. *Phytochemistry* **1995**, *39*, 1115-1118.

(9) Uchiyama, T.; Miyase, T.; Ueno, A.; Usmanghani, K. *Phytochemistry*, **1989**, *28*, 3369-3372.

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