Chemistry of Natural Compounds, Vol. 47, No. 3, July, 2011 [Russian original No. 3, May–June, 2011]

FERULOYL, CAFFEOYL, AND FLAVONOL GLUCOSIDES FROM *Equisetum hyemale*

Byoung-Jae Park* and Matsuta Tomohiko

UDC 547.972

Feruloyl, caffeoyl, and flavonol glucosides were isolated from the stems of Equisetum hyemale *L. The structures of the compounds were elucidated as* trans-*feruloyl-4-*β-*glucoside (**1**),* cis-*feruloyl-4-*β-*glucoside (**2**),* trans-*caffeoyl-3-*β-*glucoside (**3**), kaempferol-3-sophoroside (**4**), kaempferol-3-sophoroside-7-*β-*glucoside (**5**), and herbacetin-3-sophoroside-8-*β-*glucoside (**6**) based on the spectral evidence.*

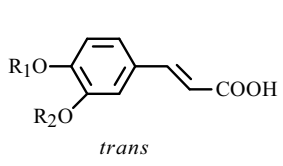
Keywords: *Equisetum hyemale* L.; feruloyl, caffeoyl, flavonol glucosides.

The stems of *Equisetum hyemale* L. have been used as a crude drug for astringent homeostasis, as a diuretic and diaphoretic, and for treating eye-diseases in traditional medicine [1, 2]. Recently, antioxidant, anti-inflammatory, and ACE inhibitory activities [3, 4] have been reported; however, the chemical constituents of *E. hyemale* have not been clarified. Therefore, we describe the isolation and structural determination of six compounds from the stem of *E. hyemale*.

The ^{1}H NMR spectrum of compounds **1** and **2** showed five protons: three aromatic protons, an olefin proton, and one methoxyl proton expected for the feruloyl moiety. Compound **3** showed five protons expected for the caffeoyl moiety. The ^{1}H NMR spectrum of compounds **1**, **2**, and **3** showed *trans* and *cis* isomers whose parameters were assigned from the coupling constant of the double bond (15.9 Hz for *trans*, 12.9 Hz for *cis*) as well as from the chemical shift [5–7]. In the HMBC spectrum, the H-1′ proton of *β*-glucose (δ 4.96, 4.94) of compounds **1** and **2** showed a correlation with C-4 (δ 149.95, 148.73) and that of compound **3** (δ 4.84) correlated with C-3 (δ 148.58). Based on the above evidence, compounds **1**, **2**, and **3** were identified as *trans*-feruloyl-4-*β*-glucoside (**1**), *cis*-feruloyl-4-*β*-glucoside (**2**), and *trans*-caffeoyl-3-*β*-glucoside (**3**).

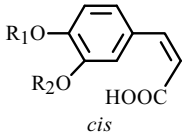
The structure of compound **4** was characterized as kaempferol-3-sophoroside on the basis of both NMR and MS spectral data and comparison with literature data [8]. In the ^{1}H NMR, and ^{13}C NMR spectra, compound **5** showed aromatic proton signals exhibited kaempferol as the aglycone moiety. The two glucose moieties were assigned to sophorose which was linked to the C-3 (δ 135.21) and H-1′′′′ proton of *β*-glucose (δ 5.05) showed a correlation with C-7 (δ 164.72) in the HMBC spectrum [8]. Based on NMR and MS spectral data, compound **5** was identified as kaempferol-3-sophoroside-7-*β*-glucoside.

In the ^{1}H NMR spectrum of compound **6**, aromatic proton signals at δ 8.31 (H-2′, 6′), δ 6.90 (H-3′, 5′), and δ 6.16 (H-6) exhibited the characteristic of herbacetin based on the aglycone moiety [9]. The two glucose moieties were assigned to sophorose, which was linked to the C-3 and H-1′′′′ proton of *β*-glucose (δ 4.61) correlation with C-8 (δ 125.46) in the HMBC spectrum [10]. Based on the above evidence, compound **6** was identified as herbacetin-3-sophoroside-8-*β*-glucoside. Although compounds **1–6** are already known [11], compounds **1–4** were isolated from *E. heymale* for the first time.



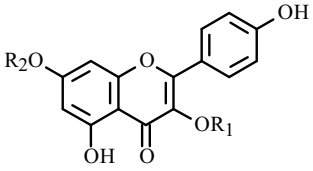
trans

1, 3

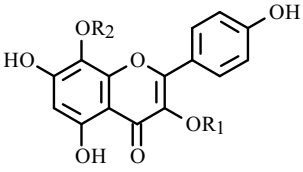


cis

2



4, 5



6

1, 2: R_1 = Glc, R_2 = CH_3; **3:** R_1 = H, R_2 = Glc
4: R_1 = sophorose, R_2 = H; **5, 6:** R_1 = sophorose, R_2 = Glc

Kitami Institute of Technology, 165 Koen-cho, Kitami 090-8507, Japan, e-mail: parkbj71@mail.goo.ne.jp. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 325–327, May–June, 2011. Original article submitted March 5, 2010.

0009-3130/11/4703-0363

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were measured with a JEOL α-500 spectrometer in methanol-d_4 and dimethyl-d_6 sulfoxide at 30°C. HR-ESI-TOF-MS spectra were recorded on a JEOL JMS-T100LC spectrometer.

Extraction and Isolation. The stems of *E. hyemale* were collected in Hokkaido of Japan in June 2009. The stems were chopped into small pieces (ca. 1 cm in length), and its moisture content was 61.4%. The stems (1 kg) were extracted with EtOH 3 times. The EtOH was removed by evaporation under reduced pressure (ca. 40°C) to give the extracts (34.1 g). It was defatted with *n*-hexane to give a hexane-insoluble fraction (18.9 g) and a hexane-soluble fraction (15.2 g). The hexane insoluble fraction (6.5 g) was applied to a Sephadex LH-20 (2.5 cm i.d × 17 cm) column using gradient elution of a H_2O–MeOH mixture to give Fraction I (H_2O), Fraction II (25% MeOH), Fraction III (50% MeOH), and Fraction IV (100% MeOH). Fraction I (5.3g) was separated by reversed phase chromatography using Wakosil 40C_{18} (2.0 cm i.d. × 20 cm) employing gradient elution of a H_2O–MeOH mixture to give eight fractions. Fraction I-1 (200.0 mg) (H_2O) was further purified by HPLC to give compound **6** (9.4 mg). Fraction I-4 (121.3 mg) (5% MeOH) was further purified by HPLC to give compound **1** (8.2 mg) and compound **2** (10.5 mg). Fraction I-6 (194.1 mg) (10% MeOH) was further purified by HPLC to give compound **5** (18.5 mg). HPLC was carried out using a Shimadzu SPD-10A system and TSK-gel ODS-80T_S (2.0 cm i.d. × 25 cm) with a H_2O–CH_3CN solvent system. Fraction II (54.5 mg) was further purified by reversed phase chromatography using Wakosil 40C_{18} (2.0 cm i.d. × 20 cm) employing gradient elution of a H_2O–MeOH mixture to give compound **3** (4.4 mg) (12.5% MeOH) and compound **4** (6.2 mg) (27.5% MeOH).

***trans*-Feruloyl-4-β-glucoside (1),** white powder. HR-ESI-TOF-MS *m/z*: 357.1195 $[M + H]^+$ (calcd for $C_{16}H_{21}O_9$, 357.1186), 195.0550 $[(M + H) – Glc]^+$. ^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.59 (1H, d, J = 15.9, H-7), 7.23 (1H, d, J = 1.8, H-2), 7.17 (1H, d, J = 8.2, H-5), 7.14 (1H, dd, J = 1.8, 8.2, H-6), 6.39 (1H, d, J = 15.9, H-8), 4.96 (1H, d, J = 7.6, H-1′), 3.89 (3H, s, OCH_3), 3.87 (1H, dd, J = 2.1, 11.9, H-6a′), 3.69 (1H, dd, J = 5.2, 11.9, H-6b′), 3.51 (1H, dd, J = 8.9, 7.6, H-2′), 3.47 (1H, m, H-3′), 3.42 (1H, br.dd, J = 2.1, 5.2, H-5′), 3.39 (1H, dd, J = 8.2, 9.5, H-4′). ^{13}C NMR spectrum (125 MHz, CD_3OD, δ): 170.89 (C-9), 151.06 (C-3), 149.95 (C-4), 145.76 (C-7), 130.78 (C-1), 123.33 (C-6), 118.38 (C-8), 117.52 (C-5), 112.51 (C-2), 102.29 (C-1′), 78.29 (C-5′), 77.88 (C-3′), 74.84 (C-2′), 71.31 (C-4′), 62.49 (C-6′), 56.80 (OCH_3) [5, 6].

***cis*-Feruloyl-4-β-glucoside (2)**, white powder. HR-ESI-TOF-MS *m/z*: 357.1198 $[M + H]^+$ (calcd for $C_{16}H_{21}O_9$, 357.1186), 195.0656 $[(M + H) – Glc]^+$. ^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.64 (1H, d, J = 1.8, H-2), 7.13 (1H, dd, J = 1.8, 8.2, H-6), 7.12 (1H, d, J = 8.2, H-5), 6.81 (1H, d, J = 12.9, H-7), 5.89 (1H, d, J = 12.9, H-8), 4.94 (1H, d, J = 7.6, H-1′), 3.87 (1H, dd, J = 2.1, 11.9, H-6a′), 3.85 (3H, s, OCH_3), 3.68 (1H, dd, J = 5.2, 11.9, H-6b′), 3.51 (1H, dd, J = 9.2, 7.6, H-2′), 3.45–3.37 (3H, m, H-3′, 4′, 5′). ^{13}C NMR spectrum (125 MHz, CD_3OD, δ): 170.43 (C-9), 150.03 (C-3), 148.73 (C-4), 142.37 (C-7), 131.23 (C-1), 125.36 (C-6), 119.88 (C-8), 116.82 (C-5), 115.31 (C-2), 102.38 (C-1′), 78.40 (C-5′), 77.59 (C-3′), 74.83 (C-2′), 71.31 (C-4′), 62.49 (C-6′), 56.67 (OCH_3) [5, 6].

***trans*-Caffeoyl-3-β-glucoside (3)**, white powder. HR-ESI-TOF-MS *m/z* 343.1070 $[M + H]^+$ (calcd for $C_{15}H_{19}O_9$, 343.1029). ^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.51 (1H, d, J = 15.9, H-7), 7.19 (1H, d, J = 8.2, H-5), 7.08 (1H, d, J = 1.8, H-2), 7.02 (1H, dd, J = 1.8, 8.2, H-6), 6.31 (1H, d, J = 15.9, H-8), 4.84 (1H, d, J = 7.6, H-1′), 3.90 (1H, dd, J = 2.1, 11.9, H-6′a), 3.72 (1H, dd, J = 5.2, 11.9, H-6′b), 3.41–3.33 (4H, m, H-2′, 3′, 4′, 5′). ^{13}C NMR spectrum (125 MHz, CD_3OD, δ): 171.41 (C-9), 148.58 (C-3), 148.57 (C-4), 145.19 (C-7), 131.55 (C-1), 121.94 (C-6), 119.07 (C-8), 118.30 (C-5), 115.83 (C-2), 103.65 (C-1′), 78.40 (C-5′), 77.59 (C-3′), 74.83 (C-2′), 71.31 (C-4′), 62.43 (C-6′) [7].

Kaempferol-3-sophoroside (4), yellow powder. HR-ESI-TOF-MS *m/z*: 611.1577 $[M + H]^+$ (calcd for $C_{27}H_{31}O_{16}$, 611.1612), 449.1052 $[(M + H) – Glc]^+$, 287.0582 $[(M + H) – 2Glc]^+$. ^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 8.02 (2H, d, J = 8.8, H-2′, 6′), 6.89 (2H, d, J = 8.8, H-3′, 5′), 6.37 (1H, d, J = 2.1, H-8), 6.18 (1H, d, J = 2.1, H-6), 5.41 (1H, d, J = 7.3, H-1″), 4.74 (1H, d, J = 7.6, H-1‴), 3.78 (1H, dd, J = 2.4, 11.9, H-6‴a), 3.73 (1H, dd, J = 7.6, 8.8, H-2″), 3.68 (1H, dd, J = 2.4, 11.9, H-6″a), 3.68 (1H, dd, J = 5.2, 11.9, H-6‴b), 3.59 (1H, t, J = 8.8, H-3″), 3.48 (1H, dd, J = 5.5, 12.2, H-6″b), 3.41–3.37 (2H, m, H-3‴, 4‴), 3.36 (1H, m, H-2‴), 3.35 (1H, m, H-4″), 3.28 (1H, m, H-5‴), 3.19 (1H, m, H-5″). ^{13}C NMR spectrum (125 MHz, CD_3OD, δ): 179.64 (C-4), 166.59 (C-7), 163.11 (C-5), 161.54 (C-4′), 158.85 (C-9), 158.58 (C-2), 134.91 (C-3), 132.31 (C-2′, 6′), 122.86 (C-1′), 116.29 (C-3′, 5′), 105.65 (C-10), 104.71 (C-1‴), 101.09 (C-1″), 100.09 (C-6), 94.87 (C-8), 82.52 (C-2″), 78.26 (C-5″), 78.22 (C-5‴), 77.93 (C-3″), 77.89 (C-3‴), 75.55 (C-2‴), 71.35 (C-4‴), 71.14 (C-4″), 62.64 (C-6″, C-6‴) [8].

Kaempferol-3-sophoroside-7-β-glucoside (5), yellow powder. HR-ESI-TOF-MS *m/z*: 773.2064 $[M + H]^+$ (calcd for $C_{33}H_{41}O_{21}$, 773.2140), 611.1650 $[(M + H) – Glc]^+$, 449.1061 $[(M + H) – 2Glc]^+$, 287.0699 $[(M + H) – 3Glc]^+$. ^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 8.07 (2H, d, J = 8.6, H-2′,6′), 6.90 (2H, d, J = 8.6, H-3′, 5′), 6.76 (1H, d, J = 2.4,

H-8), 6.48 (1H, d, J = 2.4, H-6), 5.48 (1H, d, J = 7.3, H-1″), 5.05 (1H, d, J = 7.3, H-1‴″), 4.74 (1H, d, J = 7.3, H-1‴), 3.91 (1H, dd, J = 2.4, 12.2, H-6‴″a), 3.78 (1H, dd, J = 2.4, 11.9, H-6‴a), 3.73 (1H, dd, J = 7.6, 8.8, H-2″), 3.69 (1H, dd, J = 2.4, 12.2, H-6″a), 3.68 (1H, dd, J = 5.2, 11.9, H-6‴b), 3.68 (1H, m, H-6‴″b), 3.60 (1H, t, J = 8.8, H-3″), 3.53 (1H, m, H-5‴″), 3.49 (1H, dd, J = 4.9, 12.1, H-6″b), 3.48 (1H, m, H-2‴″), 3.36 (1H, m, H-2‴), 3.34 (1H, m, H-4″), 3.18 (1H, m, H-5″), 3.50–3.27 (5H, m, H-3‴, 4‴, 5‴, H-3‴″, 4‴″). ^{13}C NMR spectrum (125 MHz, CD_3OD, δ): 179.89 (C-4), 164.72 (C-7), 162.83 (C-5), 161.71 (C-4′), 159.46 (C-2), 158.07 (C-9), 135.21 (C-3), 132.48 (C-2′, 6′), 122.70 (C-1′), 116.28 (C-3′, 5′), 107.62 (C-10), 104.81 (C-1‴), 101.64 (C-1‴″), 100.87 (C-6, 1″), 95.79 (C-8), 82.74 (C-2″), 78.34 (C-5″, 5‴″), 78.21 (C-5‴), 77.93 (C-3″, 3‴″), 77.86 (C-3‴), 75.62 (C-2‴), 74.75 (C-2‴″), 71.34 (C-4‴), 71.29 (C-4‴″), 71.20 (C-4″), 62.64 (C-6‴″), 62.49 (C-6″, 6‴) [8].

Herbacetin-3-sophoroside-8-β-glucoside (6), yellow powder. HR-ESI-TOF-MS *m/z*: 789.2075 $[M + H]^+$ (calcd for $C_{33}H_{41}O_{22}$, 789.2090), 627.1539 $[(M + H) – Glc]^+$, 465.1022 $[(M + H) – 2Glc]^+$, 303.0973 $[(M + H) – 3Glc]^+$. ^{1}H NMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.31 (2H, d, J = 8.8, H-2′, 6′), 6.90 (2H, d, J = 8.8, H-3′, 5′), 6.16 (1H, s, H-6), 5.64 (1H, d, J = 7.5, H-1″), 4.61 (1H, d, J = 7.7, H-1‴″), 4.60 (1H, d, J = 7.1, H-1‴), 3.69–3.03 (18H, m, sugar-H). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ): 177.25 (C-4), 159.91 (C-5, 4′), 156.90 (C-7), 155.22 (C-2), 148.30 (C-9), 132.76 (C-3), 131.47 (C-2′, 6′), 125.46 (C-8), 120.83 (C-1′), 115.14 (C-3′, 5′), 106.58 (C-1‴″), 103.76 (C-1‴), 102.76 (C-10), 99.38 (C-6), 98.43 (C-1″), 82.03 (C-2″), 77.38 (C-5″), 77.23 (C-5‴″), 76.80 (C-5‴), 76.47 (C-1, 3″, C-3‴), 76.08 (C-3‴″), 74.29 (C-2‴), 74.03 (C-2‴″), 69.70 (C-4‴), 69.53 (C-4‴″), 69.19 (C-4″), 60.79 (C-6‴″), 60.65 (C-6‴), 60.63 (C-6″) [9, 10].

REFERENCE

1. A. Nitta, S. Yoshida, and T. Tagaeto, *Chem. Pharm. Bull*., **25**, 1135 (1977).
2. T. Namba, T. Tani, and M. Kubo, *J. Jap. Bot*., **49**, 138 (1974).
3. E. Y. Park and H. Jeon, *Nat. Prod. Sci.*, **14**, 239 (2008).
4. G. P. Choi, B. H. Chung, D. I. Lee, H. Y. Lee, J. H. Lee, and J. D. Kim, *Kor. J. Med. Crop Sci.*, **10**, 399 (2002).
5. P. Johnsson, N. Peerlkamp, A. Kamal-Eldin, R. E. Andersson, R. Andersson, L. N. Lundgren, and P. Aman, *Food Chem.*, **76**, 211 (2002).
6. M. Rahouti, F. Seigle-Murandi, R. Steiman, and K. E. Eriksson, *Appl. Environ. Microbiol*., **55**, 2394 (1989).
7. H. Meyermans, K. Morreel, C. Lapierre, B. Pollet, A. D. Bruyn, R. Busson, P. Herdewijn, B. Devreese, J. V. Beeumen, J. M. Marita, J. Ralph, C. Chen, B. Burggraeve, M. V. Montagu, E. Messens, and W. Boerjan, *J. Biol. Chem.*, **275**, 36903 (2000).
8. W. Schlieman, B. Schneider, V. Wray, J. Schmidt, M. Nimtz, A. Porzel, and H. Bohm, *Phytochemistry*, **67**, 200 (2006).
9. G. G. Zapesochnaya and V. A. Kurkin, *Chem. Nat. Comp.*, **19**, 24 (1982).
10. K. Struijsa, J. Vinckena, R. Verhoefa, W. H. M. O. Casterena, A. G. J. Voragena, and H. Gruppen, *Phytochemistry*, **68**, 1230 (2007).
11. H. Geiger, S. Reichert, and K. R. Markham, *Z. Naturforsch.,* **37B**, 504 (1982).