ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2014, Vol. 40, No. 7, pp. 777–780. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © V.M. Bonacheva, E.Kh. Botirov, 2013, published in Khimiya Rastitel'nogo Syr'ya, 2013, No. 4, pp. 109–113.

LOW-MOLECULAR-WEIGHT COMPOUNDS

Kaempferol and Its Glycosides from *Equisetum silvaticum* L. from the Khanty-Mansi Autonomous Area

V. M. Bonacheva[1] and E. Kh. Botirov

Surgut State University, ul. Lenina 1, Surgut, 628412 Russia

Received February 13, 2012

Abstract—Three flavonoids were isolated from the aerial part of the wood horsetail (*Equisetum silvaticum* L.); two of them were found for the first time. The compounds were identified as kaempferol, kaempferol 3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranoside and kaempferol 3-*O*-rutinosyl-7-*O*-L-rhamnopyranoside on the basis of the chemical transformations and IR, UV, ^{1}H-NMR and mass spectra.

Keywords: *Equisetum silvaticum L., flavonoids, kaempferol, glycosides, aglycone, β-D-glucopyranoside, α-L-rhamnopyranoside, rutinoside, β-D-galactopyranoside, hydrolysis*

DOI: 10.1134/S1068162014070048

INTRODUCTION

In recent decades plants containing flavonoids have been of special interest due to their importance for medicine and pharmacology as the sources of broad-spectrum drugs. They possess antioxidant, anti-inflammatory, capillary restorative, choleretic, antitumor, immunomodulating and other activities, and the preparations created on their basis are low-toxic [1, 2].

From this point of view *Equisetum silvaticum* L. is promising but understudied. In this paper, the phytochemical study of the wood horsetail from the territory of the Khanty-Mansi autonomous area is reported for the first time.

Equisetum silvaticum L. (wood horsetail) is a perennial plant with a height of up to 60 cm. It is spread in forest areas of taiga, forest tundra, forest steppe, and mountain forests of Russia, South-East Asia and North America. The wood horsetail is commonly found in forest edges, near water basins, in wet shady places and marshy and peat meadows [3]. This horsetail species is poisonous for cattle and horses.

From the earliest times, the wood horsetail was used in folk medicine as a medicinal plant with diuretic, anti-inflammatory, antifungal and other properties [4].

The wood horsetail was also used to dye wool gray-yellow [5–6].

Due to its occurrence, rich raw material resources and the presence of phenol components the wood horsetail is the most promising for chemicopharmaceutical studies. This fact indicates that studies of the wood horsetail flavonoids are important.

RESULTS AND DISCUSSION

The isolated individual compounds belong to the flavonoid class. Identification was performed according to the results of chemical conversion and spectral data. The obtained results were compared with the literature data.

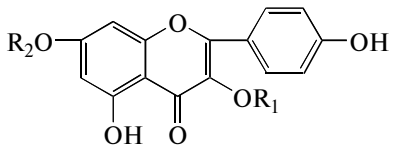
3,5,7,4'-tetrahydroxyflavon (kaempferol) (1) is a light-yellow crystalline substance of the $C_{15}H_{10}O_6$ composition with the melting point of 268–270°C and the mass spectrum (m/z): M^+286. The UV spectrum: λ_{max} (ethanol) 266 and 369 nm, which is characteristic of flavonols; + NaOH: 281 and 415 nm; +CH_3COOH: 274 and 387 nm; it means that the hydroxyl group at the position 7 is free because there are bathochromic shifts of band II for 8 nm and band I for 18 nm.

The IR spectrum of the substance 1 contains the absorption bands of hydroxyl groups (3323–3277 cm^{-1}), γ-pyrone carbonyl (1662 cm^{-1}), and aromatic C=C bonds (1591 cm^{-1}).

The ^{1}H NMR spectrum of the substance 1 registered in DMSO-d_6 has the signals of protons of 3,5,7,4'-tetrasubstituted flavonol: 6.19 (H, d, 2.0 Hz, H-6), 6.44 (H, d, 2.0 Hz, H-8), 6.93 (2H, d, 8.9 Hz, H-3', 5'), 8.05 (2H, d, 8.9 Hz, H-2', 6'), 12.49 (1H, bs, 5-OH).

[1] Corresponding author; phone: (34624) 76-28-00; e-mail: bwmbeml@mail.ru.

Based on comparison with authentic kaempferol by TCL analysis in the chloroform–ethyl acetate solvent system (6 : 3 : 1), the spectral data and physicochemical properties compound 1 was determined as 3,5,7,4'-tetrahydroxyflavon (kaempferol) [12, 13]. Kaempferol from *Equisetum silvaticum* L. was isolated for the first time.



1. $R_1 = H$, $R_2 = H$
2. $R_1 = \beta$-D-Galp, $R_2 = \alpha$-L-Rhap
3. $R_1 = \beta$-D-Glcp)6←1)-α-L-Rhap, $R_2 = \alpha$-L-Rhap.

Kaempferol 3-O-β-D-galactopyranosyl-7-O-α-L-rhamnopyranoside (2) is a yellow crystalline compound with the molecular formula of $C_{27}H_{30}O_{15}$, the melting point of 188–190°C, and the mass spectrum (m/z): 286 (M^+ of kaempferol aglycone). The UV spectrum: λ_{max} (ethanol): 272 and 359 nm; + NaOH: 277 and 399 nm with the decreased intensity of band I; +$AlCl_3$: 276, 348, and 400 nm; +CH_3COOH: 267 and 354 nm. On the basis of UV spectra we concluded that the substance belongs to 3,7-di-O-substituted flavonols.

The IR spectrum of substance 2 contains the absorption bands of hydroxyl groups (3392–3219 cm^{-1}), γ-pyrone carbonyl (1647 cm^{-1}), glycoside C-O (1006–1141 cm^{-1}) and aromatic bonds.

^{1}H NMR (β, DMSO-d_6): 12.6 (1H, bs, 5-OH), 8.06 (2H, d, 8.8 Hz, H-2',6'), 6.95 (2H, d, 8.8 Hz, H-3',5'), 6.80 (1H, d, 1.9 Hz, H-8), 6.43 (1H, d, 1.9 Hz, H-6), 5.53 (s, H-1" anomer of H-Rha), 5.47 (d, 7.3 Hz, H-1'" anomer of H-Gal), 3.05–3.62 (m, protons of the sugar part), 1.05 (d, 6 Hz, CH_3-Rha).

Substance 2 (14 g) underwent complete acid hydrolysis by a mixture of 5% HCl–C_2H_5OH (1 : 1) in a water bath for 2 h. The aglycone precipitate formed during ethanol distillation under vacuum was separated by filtration and recrystallized. It resulted in kaempferol of the $C_{15}H_{10}O_6$ composition (λ_{max} 266, 369 nm; melting point of 270–272°C). The filtrate was evaporated to dryness; the precipitate was dissolved in ethanol. Its TLC analysis in the n-butanol-acetic acid-water solvent system (6: 1.5 : 2.5) in the presence of authentic samples revealed D-galactose and L-rhamnose.

We also performed alkaline hydrolysis of 10 mg of substance 2 by 0.5% KOH (10 mL) in a water bath for 2 h. It resulted in kaempferol 3-*O*-β-D-galactopyranoside and L-rhamnose (TLC). Consequently, we can conclude the hydroxyl group at the position 7 is substituted for L-rhamnose while 3-OH is glycosylated by D-galactose.

The signal of anomeric proton of D-galactose in the ^{1}H NMR spectrum (DMSO-d_6) was observed as a doublet with the spin-spin coupling constant of 7.3 Hz that indicates that D-galactose is in β-configuration.

On the basis of the chemical conversion and comparison of the spectra with the literature data we identified compound 2 as kaempferol 3-*O*-β-D-galactosyl-7-*O*-rhamnopyranoside [14]. Kaempferol 3-*O*-β-D-galactosyl-7-*O*-rhamnopyranoside was isolated from *Equisetum* L. plants for the first time.

Kaempferol 3-*O*-β-rutinosyl-7-*O*-rhamnopyranoside (3) is a light-yellow crystalline substance with the molecular formula of $C_{33}H_{40}O_{19}$, the melting point of 147–148°C, and the mass spectrum (m/z): 286 (M^+ of aglycone). The UV spectrum: λ_{max} (ethanol) 272 and 361 nm; + NaOH: 277 and 400 nm; +$AlCl_3$: 276, 350, and 402 nm; +CH_3COONa: 267 and 356 nm.

The IR spectrum: OH-groups (3373–3277 cm^{-1}), γ-pyrone C=O (1654 cm^{-1}), aromatic C=C (1591 cm^{-1}) and glycoside C-O (1100–1000 cm^{-1}) bonds, etc.

^{1}H NMR spectrum (δ, DMSO-d_6): 0.98 (d, 6.95 Hz, CH_3 of rhamnose biose), 1.13 (d, 6 Hz, CH_3-7-Rha), 3.05–3.87 (m, protons of the sugar part), 5.16 (d, 2.1 Hz, H-1'" anomer of H-Rha biose), 5.32 (d, 7.5 Hz, H-1'" anomer of H-Glc), 5.53 (d, 2 Hz, H-1" of H-Rha), 6.42 (H, d, 2.0 Hz, H-6), 6.76 (H, d, 2.0 Hz, H-8), 6.87 (2H, d, 8.8 Hz, H-3', H-5'), 8.00 (2H, d, 8.8 Hz, H-2', H-6'), 12.6 (bs, 5-OH).

The complete acid hydrolysis of glycoside 3 by the mixture of 5% HCl and ethanol (1 : 1) resulted in kaempferol (4 mg), L-rhamnose, and D-glucose.

In order to reveal the positions of carbohydrate residues we performed alkaline and mild stepwise acid hydrolysis of substance 3. A diglycoside which was identified as kaempferol 3-*O*-glucosyl-7-*O*-rhamnoside (melting point of 151–153°C; UV spectrum: λ_{max} 272 and 359 nm) and a monosaccharide L-rhamnose were formed at the first stage (after 15 min) of mild acid hydrolysis [4, 8]. A glycoside (melting point of 177–179°C; UV spectrum: λ_{max} 260 and 366 nm) and D-glucose are formed at the second stage (after 30 min) (TLC, IR spectrum). We identified the glycoside as kaempferol 7-*O*-rhamnoside by comparison with the literature data [15–16].

The alkaline hydrolysis of compound 3 resulted in a monosaccharide L-rhamnose and the substance with the melting point of 182–185°C and the UV spectrum: λ_{max} 266 and 352 nm [4]. The comparison with the literature data allowed us to identify the substance as kaempferol 3-*O*-rutinoside which had been isolated from the wood horsetail earlier. The sugar unit was

determined by TLC comparison with the authentic L-rhamnose sample.

Consequently, integration of the experimental data and comparison of the spectra and physicochemical properties with the literature data allowed us to identify the substance 3 as kaempferol 3-*O*-rutinosyl-7-*O*-rhamnoside [4, 15–17].

CONCLUSIONS

Three flavonoids—3,5,7,4'-tetrahydroxyflavon (kaempferol), kaempferol 3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranoside, and kaempferol 3-*O*-rutinosyl-7-*O*-L-rhamnopyranoside—were isolated from the aerial part of the wood horsetail. The two first compounds are reported for the first time. The structures were elucidated on the basis of chemical transformations and UV, ^{1}H-NMR, IR and mass spectra.

EXPERIMENTAL

Flavonoids were isolated by five-fold extraction of the air-dried milled aerial part of the wood horsetail (700 g) by 85% ethanol at room temperature. The joined extract was concentrated under vacuum, diluted with water in the ratio of 1 : 1 and then sequentially treated with petroleum ether, chloroform, ethyl acetate, and n-butanol in a separating funnel. It yielded 1.8 g of chloroform, 9.5 g of ethyl acetate, and 19.5 g of butanol fractions after solvent removal. Compound 1 was isolated by the chromatography of the ethyl acetate fraction in a column (120 × 3 cm) packed with silicagel (220 g) in the ethyl acetate-ethanol gradient system. Substance 1 was eluted by a mixture of ethyl acetate and ethanol (98 : 2); the yield was 0.2 g. Substance 2 (yield of 0.45 g) was obtained by the chromatography of a part of the butanol fraction (12 g) in a column packed with silicagel (240 g) in the ethyl acetate-ethanol solvent system (94 : 6). Substance 3 was eluted by the ethyl acetate-ethanol solvent system (86 : 14); the yield was 0.8 g.

The substances obtained were purified by the Woelm polyamide (Germany) column chromatography in the chloroform-ethanol gradient system.

In order to reveal the positions of the carbohydrate residues we performed alkaline and acid hydrolysis (mild stepwise and complete) of substances 2 and 3.

Alkaline hydrolysis was performed as follows: a glycoside sample was dissolved in a 0.5% water solution of potassium hydroxide and hydrolyzed in boiling water bath under reflux for 2 h. The solution was neutralized with a 2% water solution of sulfuric acid and evaporated to dryness. The products were identified by thin layer chromatography (TLC) comparison with authentic samples [7–8]. In order to perform stepwise mild acid hydrolysis a glycoside was heated up to 50–60°C in a 0.16% water solution of hydrochloric acid. Samples were taken every 15 min, and the products were analyzed by TLC using the standard samples [9].

Complete acid hydrolysis of flavonoid glycosides was performed as follows: a glycoside sample (10 mg) was dissolved in 10 mL of a mixture of 5% hydrochloric acid and ethanol (1 : 1) and hydrolyzed in boiling water bath under reflux for 2 h [10]. The aglycone precipitate formed during ethanol vacuum distillation was separated by filtration. The filtrate was evaporated to dryness; the precipitate was dissolved in ethanol. Carbohydrates were analyzed by thin layer chromatography in the presence of authentic monosaccharide samples in the solvent mixture of n-butanol–acetic acid–water (6 : 1.5 : 2.5). The plates were visualized by the mixture of n-butanol–water–acetic acid–phosphoric acid–aniline–diphenylamine (60 mL–25 mL–15 mL–10 mL–1 mL–2 g) and dried at 120°C for 5 min [11].

UV spectra were recorded in ethanol using a Specord M 400 spectrophotometer. IR spectra were recorded using an IR Prestige-21 Fourier spectrometer. Mass spectrometry was performed using a Thermo Finnigan MAT 95 XP instrument with an ionization energy of 70 eV. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker Avance III instrument at an operating frequency of 500 MHz. Chemical shifts are shown in parts per million (ppm) in the δ-scale.

Melting points were determined using a Kofler bench.

TLC was performed using Sorbfil PTSKh-P-A-UF (polyethylene terephthalate film analytic UV-fluorescent) plates. Flavonoid spots were visualized by the 1% spirit solution of $AlCl_3$. Column chromatography was performed using the KSK 100/160 μm silicagel.

REFERENCES

1. Hollman, P.C.H., Feskens, E.I.M., and Katan, M.B., *Proc. Soc. Exp. Biol. Med.*, 1999, vol. 220, no. 4, pp. 198–202.
2. Hernandez, N.E., Tereschur, M.L., and Abdala, L.R., *J. Ethnopharmacol.,* 2000, vol. 73, nos. 1–2, pp. 317–322.
3. *Flavonoids in Health and Disease*, Rice-Evans, C.A. and Packer, L., Eds., New-York: Marcel Decker, Inc., 2003.
4. Kolomiets, N.E. and Kalinkina, G.I., *Khim. Rastit. Syr'ya*, 2010, no. 1, pp. 149–154.
5. Nosov, A., *Lekarstvennye rasteniya* (Medicinal Plants), Moscow, 2001.

6. Shamruk, S.G., *Lekarstvennye rasteniya* (Medicinal Plants), Moscow, 1989.

7. Makarov, V.A., Shinkarenko, A.L., Litvinenko, V.I., and Kovalev, I.P., *Khim. Prir. Soedin.*, 1969, no. 5, pp. 366–369.

8. Litvinenko, V.I. and Makarov, V.A., *Khim. Prir. Soedin.*, 1969, no. 5, pp. 366–369.

9. Maksyutina, N.P., Litvinenko, V.I., and Kovalev, I.P., *Khim. Prir. Soedin.*, 1966, no. 6, pp. 367–454.

10. Chander, R.F. and Harpek, K.A., *Aust. J. Chem.*, 1961, vol. 14, no. 4, pp. 586–595.

11. *Metody issledovaniya uglevodov* (Methods of Carbohydrate Research), Khorlin, A.Ya., Ed., Moscow, 1975.

12. Markham, K.R., *Techniques of Flavonoid Identification*, London: Academic Press, 1982.

13. Yuldashev, M.P., Muminova, B.A., Drenin, A.A., and Botirov, E.Kh., *Chem. Nat. Comp*, 2007, vol. 43, no. 1, pp. 34–36.

14. Perez-Jimenez, J., Neveu, V., Vos, F., and Scalbert, A., *Eur. J. Clin. Nutr.*, 2010, vol. 64, pp. 112–120.

15. Samokina, O.V. and Shinkarenko, A.P., *Khim. Prir. Soedin.*, 1969, no. 5, p. 441.

16. Yonekura-Sakakibara, K., Tohge, T., Niida, R., and Saito, K., *Biol. Chem.,* 2007, no. 2, p. 282.

17. Zapesochnaya, G.G., *Khim. Prir. Soedin.*, 1982, no. 5, pp. 695–709.

Translated by O. Maloletkina