Chemistry of Natural Compounds, Vol. 48, No. 2, May, 2012 [Russian original No. 2, March–April, 2012]

FLAVONOL GLYCOSIDES OF *Pseudodrynaria coronans* AND THEIR ANTIOXIDANT ACTIVITY

Zhigang Tai, Fengmei Zhang, Le Cai, Jie Shi, Qiue Cao, and Zhongtao Ding*

UDC 547.972

A new flavonol glycoside and four known flavonol glycosides were isolated from the whole plant of Pseudodrynaria coronans*. By means of spectroscopic analysis, their structures were established as kaempferol-3-*O*-(6″-*O*-feruloyl-4″-*O*-acetyl)-β-D-glucopyranoside (**1**), kaempferol-3-*O*-(6″-*O*-feruloyl)-β-D-glucopyranoside (**2**), kaempferol-3-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (**3**), astragalin (**4**), and isoquercitrin (**5**). The DPPH radical scavenging activities of these compounds were also assayed.*

Keywords: *Pseudodrynaria coronans*, flavonol glycoside, antioxidant activity.

Pseudodrynaria coronans Wall.ex.Mett. (Drynariaceae) is mainly distributed over southern China and southern of Asia. As a folk medicinal plant, it has been used to treat traumatic injury, rheumatic arthritis, tinnitus, and toothache in China [1]. Its antioxidant activity and polyphenol content have been reported [2]. However, in a series of chemical studies on fern plants [3, 4], we carried out an investigation of the chemical constituents of *Pseudodrynaria coronans*. A new flavonol glycoside, kaempferol-3-*O*-(6″-*O*-feruloyl-4″-*O*-acetyl)-*β*-D-glucopyranoside (**1**), was isolated, together with four known flavonoids: kaempferol-3-*O*-(6″-*O*-feruloyl)-*β*-D-glucopyranoside (**2**), kaempferol-3-*O*-(6″-*O*-acetyl)-*β*-D-glucopyranoside (**3**), astragalin (**4**), and isoquercitrin (**5**). The antioxidant activities of these compounds were herein investigated by the DPPH method.

Compound **1** was obtained as a yellow powder. HR-ESI-MS showed molecule ion peaks at *m/z* 667.1668 $[M + H]^+$, corresponding to the molecular formula $C_{33}H_{30}O_{15}$. Its ^1H NMR spectrum revealed one typical kaempferol core according to the signals at δ 6.26 (1H, s, H-6), 6.48 (1H, s, H-8), 8.12 (2H, d, J = 8.7 Hz, H-2′, 6′), and 6.97 (2H, d, J = 8.7 Hz, H-3′, 5′) [5]. In addition, a glucopyranose unit at δ 5.43 (1H, d, J = 7.7 Hz, H-1″), 3.79 (1H, m, H-2″), 3.61 (1H, m, H-3″), 4.92 (1H, m, H-4″), 3.81 (1H, m, H-5″), and 4.11 (2H, m, H-6″), a feruloyl moiety at 7.47 (1H, d, J = 15.9 Hz, H-7‴), 6.24 (1H, d, J = 15.9 Hz, H-8‴), 7.24 (1H, s, H-2‴), 6.87 (1H, d, J = 8.2, H-5‴), 7.03 (1H, d, J = 8.2 Hz, H-6‴), and 3.93 (3H, s, 3‴-OCH_3), and an acetyl at δ 2.06 (3H, s) were observed. These data were also supported by the ^{13}C NMR spectrum (Table 1). The linkage of the feruloyl moiety, glucose unit, acetyl group, and kaempferol was confirmed by the HMBC spectrum (Fig. 1). The carbonyl group (δ 168.0) of the feruloyl moiety correlated not only with the double bond protons at δ 6.24 but also with the 6″-methylene protons (4.11) of the glucose unit. This implies that the feruloyl moiety is connected to the C-6″ of the glucose unit. Moreover, the correlation between C-3 (δ 136.1) and the anomeric proton (5.43) showed that the site of glycosidation was at the C-3 position of kaempferol, and the coupling constant of the anomeric proton at 7.7 Hz suggested that the glucose moiety was a *β*-type sugar. This was also confirmed by the C-6″ of the sugar portion at a relative downfield of δ 64.2 [6]. The acetyl group was confirmed to attach at C-4″ (δ 72.7) of the glucose unit because of the HMBC correlation between H-4″ (δ 4.92) of the glucose unit and the carbonyl group (δ 171.5) of the acetyl. Therefore, the structure of compound **1** was determined to be kaempferol-3-*O*-(6″-*O*-feruloyl-4″-*O*-acetyl)-*β*-D-glucopyranoside.

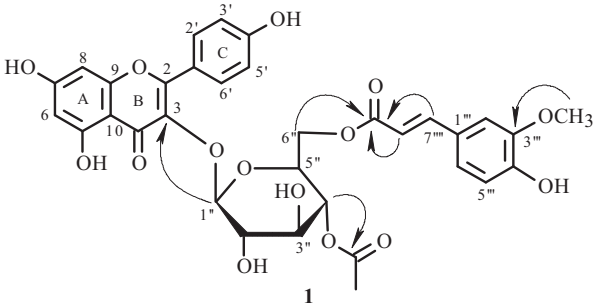
Compounds **2**–**5** were also isolated from this plant for the first time. Their spectral data were in agreement with the literature data.

Key Laboratory of Medicinal Chemistry for Nature Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, P. R. China, fax: +86 871 5033726, e-mail: ztding@ynu.edu.cn. Published in *Khimiya Prirodnykh Soedinenii*, No. 2, March–April, 2012, pp. 202–204. Original article submitted June 7, 2010.

0009-3130/12/4802-0221

TABLE 1. ^{13}C NMR Spectral Data of Compound **1** (125 MHz, acetone-d_6)

C atom	δ_C	C atom	δ_C
2	159.7	4″	72.7
3	136.1	5″	76.4
4	179.9	6″	64.2
5	163.8	1‴	128.3
6	100.8	2‴	112.3
7	166.2	3‴	151.0
8	95.7	4‴	150.0
9	158.9	5‴	117.0
10	106.4	6‴	125.0
1′	123.3	7‴	147.0
2′, 6′	133.0	8‴	116.1
3′, 5′	117.0	9‴	168.0
4′	162.0	MeO	57.4
1″	105.1	Me-C=O	21.9
2″	74.2	O=C-Me	171.5
3″	76.6		



1

Fig. 1. Structure and key HMBC (H→C) correlations of compound **1.**

The DPPH assay was performed as reported by P. V. Hung, T. Maeda, and K. Miyatake with some modification [7]. Rutin, a well-known antioxidant compound, was used as the reference control in this study; its IC_{50} value (the inhibitory concentration at which the DPPH radicals were scavenged by 50%) was 6.8 ± 1.5 μmol L^{-1}. The IC_{50} values of compounds **1**–**5** were determined to be 47.3 ± 1.8, 84.4 ± 2.1, 86.4 ± 2.3, 115.4 ± 1.8, and 4.57 ± 0.8 μmol L^{-1}, respectively. Of these, compound **5** exhibited the strongest activity.

The antioxidant capacity of flavonoids was based on the chemical structure characteristics, which mainly depended on the substitution pattern of hydroxyl groups and the extent of structural conjugations [8]. In this study, compound **5** and rutin bearing 3′, 4′-OH groups were more efficient than the other compounds in DPPH scavenging ability. In addition, it is clearly seen that the antioxidant capacities of compounds **1** and **3** with one additional acetyl in their glucose moiety were much stronger than that of compounds **2** and **4**. This discovery might provide an insight into the structure–activity relationships of flavonoid compounds.

EXPERIMENTAL

General Experimental Procedures. Melting point was measured on a Dresden HMK micromelting point apparatus and was uncorrected. MS spectra were obtained on an Agilent G3250AALC/MSD TOF instrument. 1D and 2D NMR spectra were recorded on a Bruker AV-500 spectrometer with TMS as an internal reference. UV spectra were recorded on a Hitachi UV-2550 spectrometer. Specific rotation was taken on a JASCOP-1020 digital polarimeter. Silica gel (200–300 mesh) was a product of the Qingdao Marine Chemical Ltd., Qingdao, P. R. China. Sephadex LH-20 (Amersham Biosciences) was used for column chromatography. Reversed-phase chromatography was with RP-18 (LiChroprep, 40–63 μm, Merck, Darmstadt, Germany). DPPH (2,2′-diphenyl-1-picrylhydrazyl) was purchased from Sigma (St. Louis, MO, USA).

Plant Material. The plant of *Pseudodrynaria coronans* was collected in Pingbian County, Yunnan Province, China, in February 2008 and identified by Prof. Shugang Lu (School of Life Science, Yunnan University, China). A voucher specimen (2008-Ding-Zhang-1) was deposited in the Key Laboratory of Medicinal Chemistry for Nature Resource of Yunnan University.

Extraction and Isolation. The air-dried and powered whole plant of *Pseudodrynaria coronans* (3.0 kg) was extracted three times with 85% CH_3OH at room temperature. The extract was partitioned with petroleum ether, AcOEt and *n*-BuOH to yield the petroleum ether extract (20 g), the AcOEt extract (37 g), and the *n*-BuOH extract (75 g), respectively. The AcOEt extract was subjected to silica gel chromatography eluted with $CHCl_3$–MeOH–H_2O (20:3:0.5) to give five fractions (Fr.1-5). Fraction 4 was purified by column chromatography on silica gel with AcOEt–MeOH (9:1), then on Sephadex LH-20 with MeOH–H_2O (9:1) to afford compounds **1** (13 mg), **2** (8 mg), and **5** (9 mg). Fraction 3 was purified by column chromatography on silica gel with $CHCl_3$–CH_3OH (8:1), then on RP-18 with CH_3OH–H_2O (9:1) to afford compounds **3** (12 mg) and **4** (10 mg).

Kaempferol-3-*O*-(6″-*O*-feruloyl-4″-*O*-acetyl)-*β*-D-glucopyranoside (**1**): yellow powder, mp 168–169°C; $[\alpha]_D^{20}$ –49.3° (*c* 0.014; MeOH). UV/Vis (MeOH, λ_{max}, nm): 330, 296, 282, 205. HR-ESI-MS *m/z* 667.1668 $[M + H]^+$, $C_{33}H_{30}O_{15}$ (calcd 667.1663). ^{13}C NMR spectra, see Table 1.

Kaempferol-3-*O*-(6″-*O*-feruloyl)-*β*-D-glucopyranoside (2) [9]: yellow powder, mp 190–192°C. ESI-MS *m/z* 647 $[M + Na]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 8.14 (2H, d, J = 8.7, H-2′, 6′), 7.46 (1H, d, J = 15.8), 7.24 (1H, s, H-2‴), 7.03 (1H, d, J = 8.2), 6.97 (2H, d, J = 8.7, H-3′, 5′), 6.87 (1H, d, J = 8.2), 6.48 (1H, s, H-8), 6.26 (1H, s, H-6), 6.24 (1H, d, J = 15.8), 5.37 (1H, d, J = 7.7, H-1″), 4.12 (2H, m, H-6″), 3.81–4.64 (4H, m, H-2″, 3″, 4″, 5″), 3.92 (3H, s, MeO). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 179.4 (C-4), 167.8 (C-9‴), 165.7 (C-7), 163.3 (C-5), 159.2 (C-4′), 161.5 (C-2), 158.4 (C-9), 150.0 (C-4‴), 149.1 (C-3‴), 146.3 (C-7‴), 135.8 (C-3), 132.5 (C-2′, 6′), 127.8 (C-1‴), 124.4 (C-6‴), 122.8 (C-1′), 111.7 (C-2‴), 116.5 (C-3′, 5′), 115.9 (C-5‴), 115.9 (C-8‴), 105.8 (C-10), 105.1 (C-1″), 100.2 (C-6), 95.2 (C-8), 75.9 (C-3″), 75.6 (C-2″), 78.4 (C-5″), 71.3 (C-4″), 64.4 (C-6″), 56.8 (MeO).

Kaempferol-3-*O*-(6″-*O*-acetyl)-*β*-D-glucopyranoside (**3**) [10]: yellow powder, mp 185–187°C. ESI-MS *m/z* 491 $[M + H]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 8.14 (2H, d, J = 8.7, H-2′, 6′), 6.97 (2H, d, J = 8.7, H-3′, 5′), 6.48 (1H, s, H-8), 6.26 (1H, s, H-6), 5.22 (1H, d, J = 7.7, H-1″), 4.21 (1H, m, H-6a″), 4.12 (1H, m, H-6b″), 3.41–3.58 (4H, m, H-2″, 3″, 4″, 5″), 1.92 (3H, s, H-Me (Ac)). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 179.4 (C-4), 165.7 (C-7), 163.0 (C-5), 162.2 (C-4′), 160.0 (C-2), 158.4 (C-9), 135.8 (C-3), 132.5 (C-2′, 6′), 122.8 (C-1′), 116.5 (C-3′, 5′), 105.3 (C-10), 104.9 (C-1″), 100.2 (C-6), 95.2 (C-8), 78.1 (C-3″), 75.9 (C-2″), 75.9 (C-5″), 71.5 (C-4″), 64.6 (C-6″), 21.0 (C-Me(Ac)), 172.3 (C-(C=O)(Ac)).

Astragalin (**4**) [11]: yellow powder, mp 176–179°C. ESI-MS *m/z* 449 $[M + H]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 8.11 (2H, d, J = 8.5, H-2′, 6′), 6.96 (2H, d, J = 8.5, H-3′, 5′), 6.27 (1H, s, H-6), 6.44 (1H, s, H-8), 5.28 (1H, d, J = 7.5, H-1″), 3.74 (1H, m, H-6b″), 3.60 (1H, m, H-6a″), 3.26–3.50 (4H, m, H-2″, 3″, 4″, 5″). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 179.1 (C-4), 165.8 (C-7), 163.0 (C-5), 161.7 (C-4′), 159.6 (C-2), 158.9 (C-9), 135.8 (C-3), 132.7 (C-2′, 5), 122.9 (C-1′), 116.3 (C-3′, 6), 105.0 (C-10), 104.7 (C-1″), 100.6 (C-6), 95.7 (C-8), 78.9 (C-5″), 78.7 (C-3″), 76.3 (C-2″), 71.6 (C-4″), 63.2 (C-6″).

Isoquercitrin (**5**) [11]: yellow powder, mp 239–242°C. ESI-MS *m/z* 465 $[M + H]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.76 (1H, s, H-2′), 7.55 (1H, d, J = 8.5, H-6′), 6.87 (1H, d, J = 8.5, H-5′), 6.33 (1H, s, H-8), 6.16 (1H, s, H-6), 5.22 (1H, d, J = 7.7, H-1″), 3.73 (1H, m, H-6a″), 3.60 (1H, m, H-6b″), 3.20–3.51 (4H, m, H-2″, 3″, 4″, 5″). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 179.8 (C-4), 166.4 (C-7), 163.3 (C-5), 158.5 (C-2), 158.8 (C-9), 150.3 (C-4′), 146.2 (C-3′), 136.1 (C-3), 123.7 (C-6′), 123.5 (C-1′), 118.2 (C-2′), 116.5 (C-5′), 106.0 (C-10), 105.1 (C-1″), 100.5 (C-6), 94.3 (C-8), 78.7 (C-5″), 78.5 (C-3″), 76.2 (C-2″), 71.6 (C-4″), 63.0 (C-6″).

ACKNOWLEDGMENT

This work was supported by a program from the National Natural Science Foundation of China (No. 30560178) and a grant from the Natural Science Foundation of Yunnan Province (No. 2008CD066).

REFERENCES

1. Editorial Committee of Flora of China, *Flora of China*, Vol. **6**, Science Press, Beijing (2000), p. 274.
2. H. Changi, G. Huang, D. C. Agrawal, C. Kuo, C. Wu, and H. Tsay, *Bot. Stud.*, **48**, 397 (2007).

3. Z. T. Ding, Y. S. Fang, Z. G. Tai, M. H. Yang, Y. Q. Xu, F. Li, and Q. E. Cao, *Fitoterapia*, **79**, 581 (2008).
4. Y. S. Fang, Y. B. Yang, M. H. Yang, X. Q. Yang, L. H. Dong, and Z. T. Ding, *Acta Bot. Yunnan,* **30**, 725 (2008).
5. M. M. Marzouk, S. A. Kawashty, N. Y. M. Saleh, and A. S. M. Al-Nowaihi, *Chem. Nat. Comp.*, **45**, 483 (2009).
6. I. Hideji, S. Keiichi, and T. Koichi, *Chem. Pharm. Bull*., **29**, 1777 (1981).
7. P. V. Hung, T. Maeda, and K. Miyatake, *Food Res. Int*., **42**, 185 (2009).
8. C. A. Rice-Evans, N. J. Miller, and G. Paganga, *Free Radical Biol. Med.,* **20**, 933 (1996).
9. I. Merfort, *Phytochemistry*, **27**, 3281 (1988).
10. S. Catalano, A. R. Bilia, and M. Martinozzi, *Phytochemistry,* **37**, 1777 (1994).
11. H. Y. Kim, B. H. Moon, H. J. Lee, and D. H. Choi, *J. Ethnopharmacol.*, **93**, 227 (2004).