Phenolic Constituents from the Rhizomes of *Dryopteris crassirhizoma*

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A new phenolic glycoside, dryopteroside (1), was isolated from the rhizomes of *Dryopteris crassirhizoma* (Dryopteridaceae), together with five known compounds, 4β-carboxymethyl-(−)-epicatechin (2), isobiflorin (3), biflorin (4), 1-β-D-glucopyranosyloxy-3-methoxy-5-hydroxybenzene (5) and (+)-catechin-6-*C*-β-D-glucopyranoside (6). The new compound was elucidated to be 1-butanoyl-3-*C*-β-D-glucopyranosyl-5-methyl-phloroglucinyl-6-*O*-β-D-glucopyranoside (1) by chemical and various spectroscopic analyses. The known compounds 2—6 were first reported from the genus *Dryopteris*.

Key words *Dryopteris crassirhizoma*; Dryopteridaceae; dryopteroside

Dryopteris crassirhizoma NAKAI (Dryopteridaceae) is distributed mainly in the northeast of China, which rhizomes (common name: Dong-Bei-Guan-Zhong) are widely used as a traditional Chinese medicine for the treatment of intestinal worms, fever caused by influenza and vomiting of blood.[1] Phloroglucinol derivatives and flavonoid glycosides have been reported from the rhizomes of *D. crassirhizoma*,[2,3] and have demonstrated antibacterial, antitumor-promoting, antioxidant and HIV-1 reverse transcriptase inhibitory activities.[4—7] As a part of our ongoing investigation on medicinal plants in the northeast of China, we carried out a phytochemical investigation on the rhizomes of *D. crassirhizoma*, which resulted in the isolation of a new phenolic glycoside, dryopteroside (**1**), together with five known compounds. In this paper, we report the isolation and structural determination of the new compound on the basis of chemical and various spectroscopic analyses.

The air-dried rhizomes of *D. crassirhizoma* were extracted with MeOH and the extract was partitioned with EtOAc and H_2O. The H_2O layer was passed through a Diaion HP-20 column, and washed with H_2O, 40% MeOH and MeOH. The 40% MeOH eluate fraction was separated by normal-phase and reversed-phase (RP) silica gel column chromatography (CC), and purified by repeated RP-HPLC to afford compounds **1**—**6**. The known compounds **2**—**6** were identified as 4β-carboxymethyl-(−)-epicatechin (**2**),[8] isobiflorin (**3**),[9] biflorin (**4**),[9] 1-β-D-glucopyranosyloxy-3-methoxy-5-hydroxybenzene (**5**)[10] and (+)-catechin 6-*C*-β-D-glucopyranoside (**6**)[11] by comparison of their spectral data with the reported values. The known compounds **2**—**6** were first reported from the genus *Dryopteris*.

Dryopteroside (**1**) was obtained as a pale yellow amorphous powder with a molecular formula of $C_{23}H_{34}O_{14}$, as determined by the HR-FAB-MS data, implying the presence of seven degrees of unsaturation in the molecule. In the ^{1}H- and ^{13}C-NMR spectra of **1**, the signals assignable to the *C*-β-glucopyranosyl and the *O*-β-glucopyranosyl moieties were observed with the aromeric proton signals at δ_H 5.79 (1H, d, J=9.8 Hz, H-1′) and 5.22 (1H, d, J=7.4 Hz, H-1″), correlated in the HMQC spectrum with two anomeric carbon signals at δ_C 76.8 (C-1′) and 105.9 (C-1″), respectively. The presence of the butanoyl moiety was indicated by the proton signals of one methyl at δ_H 0.81 (3H, t, J=7.5 Hz, H_3-10), two methylene at δ_H 1.66 (1H, m, H_a-9), 1.73 (1H, m, H_b-9) and 3.24 (1H, ddd, J=17.0, 8.7, 6.1 Hz, H_a-8), δ_H 3.61 (1H, ddd, J=17.0, 8.7, 6.1 Hz, H_b-8) in the ^{1}H-NMR spectrum, and the carbon signals at δ_C 18.1 (C-10), 14.1 (C-9), 46.4 (C-8) and 208.9 (C-7) in the ^{13}C-NMR spectrum,[12] and further supported by the DQF-COSY and HMBC correlations as shown in Fig. 1. Besides the signals due to sugars and the bu-

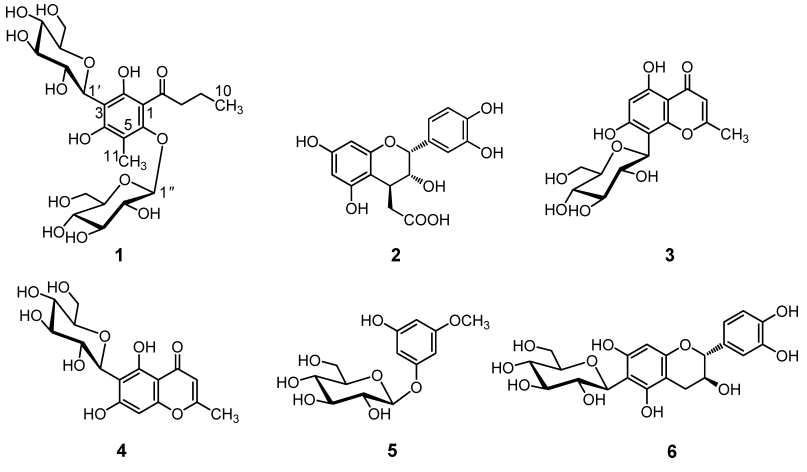

Chart 1

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Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Spectral Data of **1** in C_5D_5N

Position	**1**		Position	**1**	
	δ_H (mult, J in Hz)	δ_C		δ_H (mult, J in Hz)	δ_C
1		112.9	Glc-1′	5.79 (d, 9.8)	76.8
2		159.6	2′	4.51 (t, 9.8)	74.3
3		109.8	3′	4.36 (t, 9.2)	79.7
4		161.6	4′	4.49 (t, 9.4)	70.8
5		111.4	5′	4.06 (dt, 9.7, 2.9)	82.7
6		156.6	6′	4.45 (dd, 11.8, 2.2)	61.5
7		208.9		4.48 (dd, 11.8, 3.2)	
8	3.24 (ddd, 17.0, 8.7, 6.1)	46.4	Glc-1″	5.22 (d, 7.4)	105.9
	3.61 (ddd, 17.0, 8.7, 6.1)		2″	4.31 (dd, 8.0, 7.4)	75.8
9	1.66 (m)	14.1	3″	4.30 (t, 8.0)	78.3
	1.73 (m)		4″	4.25 (t, 8.3)	71.8
10	0.81 (t, 7.5)	18.5	5″	3.88 (ddd, 9.3, 5.7, 2.8)	78.5
11	2.54 (s)	9.6	6″	4.26 (dd, 11.4, 5.3)	62.7
				4.37 (dd, 11.4, 2.8)	

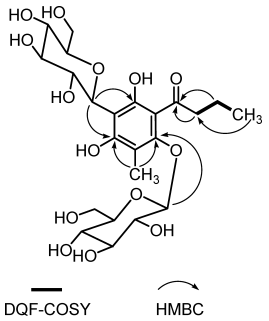

Fig. 1. Selected DQF-COSY and HMBC Correlations for **1**

tanoyl moiety, the ^{1}H-NMR spectrum showed a methyl singlet at δ_H 2.54 and the ^{13}C-NMR spectrum showed six additional quaternary carbons. Taking the remaining three degrees of unsaturation into consideration, the presence of one hexasubstituted aromatic ring in **1** was suggested. In the HMBC spectrum, the correlations between δ_H 5.79 (H-1′) and δ_C 159.6 (C-2) and 161.6 (C-4) suggested that the *C*-β-glucosyl moiety was located at C-3. The HMBC correlations between δ_H 2.54 (H-11) and δ_C 161.6 (C-4), 156.6 (C-6), and δ_H 5.22 (H-1″) and δ_C 156.6 (C-6) established the attachment of the methyl group at C-5 and the *O*-β-glucosyl moiety at C-6. On acid hydrolysis, **1** afforded D-glucose as a component sugar, which was identified by gas-liquid chromatography (GLC) analysis of its trimethylsilyl thiazolidine derivative.[13] The absolute configuration of the *C*-β-glucosyl moiety was not chemically corrected. However, it was considered as D-form in keeping with those mostly encountered among plant *C*-glucosides.[14—16] Thus, the structure of dryopteroside (**1**) was determined to be 1-butanoyl-3-*C*-β-D-glucopyranosyl-5-methyl-phloroglucinyl-6-*O*-β-D-glucopyranoside.

Experimental

General Experimental Procedures The UV spectra were obtained with a Shimadzu UV-160 spectrophotometer, whereas the IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5-dm cell. The FAB-MS was taken on a JEOL JMS-700 MStation spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL ECP-500 spectrometer or a JEOL AL-400 spectrometer in the solution with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). RP-HPLC separation was carried out with a JASCO PU-2080 HPLC system, equipped with a Shodex RI-101 Differential Refractometer detector and a Senshu Pak RP-C_{18} column (20×150 mm i.d.). RP CC was accomplished with RP-C_{18} silica gel (100—200 mesh, Chromatorex DM1020T ODS, Fuji Silysia Chemical Ltd.). Silica gel CC was carried out with Kieselgel 60 (E. Merck). TLC was conducted in Kieselgel 60 F_{254} plates (E. Merck). GLC was carried out on a PerkinElmer Clarus 500 GC-MS instrument.

Extraction and Isolation The rhizomes of *D. crassirhizoma* used in this study were purchased in Shenyang "Bo Kang" pharmacy, Liaoning Province, P. R. China, and identified by Professor Qishi Sun, Shenyang Pharmaceutical University. The air-dried rhizomes (1.5 kg) were extracted with MeOH by ultrasonic treatment three times for 1 h each at room temperature. Evaporation of the solvent under reduced pressure provided a MeOH extract (171.8 g). The MeOH extract was suspended in H_2O and then partitioned with EtOAc. The H_2O layer was subjected to a Diaion HP-20 column, and washed with H_2O, 40% MeOH, and MeOH. The 40% MeOH fraction (8.4 g) was chromatographed over a silical gel column eluted with $CHCl_3$–MeOH–H_2O (60 : 29 : 6) to give two fractions, A (1.1 g) and B (7.0 g). Further purification of fraction A by repeated RP-HPLC with 50% MeOH or 25% CH_3CN afforded six compounds, **1** (16 mg), **2** (17 mg), **3** (27 mg), **4** (6 mg), **5** (6 mg) and **6** (55 mg).

Dryopteroside (**1**): Pale yellow amorphous powder; $[\alpha]_D^{25}$ +103.4° (c=1.0, MeOH); UV (MeOH) λ_{max} (log ε): 222 (4.29), 280 (4.05), 324 (3.79); IR ν_{max} (KBr) cm^{-1}: 3402, 2927, 1614, 1074; ^{1}H-NMR (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz): see Table 1; FAB-MS (negative) m/z 533 $[M-H]^-$; HR-FAB-MS (negative) m/z 533.1879 $[M-H]^-$ (Calcd for $C_{23}H_{33}O_{14}$, 533.1870).

Acid Hydrolysis of 1 Compound **1** (1 mg) in 1 M HCl (dioxane–H_2O, 1 : 1 v/v, 200 μl) was heated at 100 °C for 1 h under an Ar atmosphere. After dioxane was removed, the solution was extracted with EtOAc (1 ml×3) to remove the aglycon. The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column, concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (1 ml), to which 0.1 M L-cysteine methyl ester hydrochloride in pyridine (2 ml) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsily limidazole (0.2 ml) for 2 h. The mixture was partitioned between hexane and H_2O (0.3 ml each) and the hexane extract was analyzed by GLC under the following conditions: capillary column, EQUITY^{TM}-1 (30 m×0.25 mm×0.25 μm, Supelco), column temperature, 230 °C; injection temperature, 250 °C; carrier N_2 gas. In the acid hydrolysate of **1**, D-glucose was confirmed by comparison of the retention times of their derivatives with those of D-glucose and L-glucose derivatives prepared in a similar way, which showed retention times of 10.80 and 11.20 min, respectively.

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