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Flavonoids from *Goodyera schlechtendaliana*

Xiao-Ming Du[a], Ning-Yi Sun[b], Yukihiro Shoyama[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[b]*Seiwa Pharmaceutical Ltd., 1-12-15 Shiba-Daimon, Minato-ku, Tokyo 105-8585, Japan*

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Abstract

A flavonol glycoside, 3-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxi]-5,7-dihydroxy-8-[(4-hydroxy-3,5-dimethoxyphenyl)methyl]-2-(3,4-dihydroxypheny)-4*H*-1-benzopyran-4-one, trivially named goodyerin, was isolated from the whole plant of *Goodyera schlechtendaliana*, along with three known flavonoids, rutin, kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside. The structures were established by spectroscopic analysis. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Goodyera schlechtendaliana*; Orchidaceae; Flavonoids; Goodyerin

1. Introduction

G. schlechtendaliana Reichb (Orchidaceae) is a herbal drug used in Chinese folk medicine for fever, pain, snake-bites and lung disease since ancient time (Jiangsu New Medicinal College, 1986). Recently, it has also been used as a substitute for the precious crude drug, *Anoectochilus formosanus* Hayata (Kan, 1986; Lin & Namba, 1981a, 1981b; Du, Yoshizawa & Shoyama, 1998a). There have been no previous reports on the chemical constituents of *G. schlechtendaliana*. The present paper describes the isolation and structure elucidation of a novel flavonol glycoside, goodyerin (**1**), together with three known flavonol glycosides (**2–4**) from *G. schlechtendaliana*.

2. Results and discussion

Compound **1**, a yellow amorphous solid, gave positive results in Molish and Mg-HCl tests. Its UV spectrum in methanol showed characteristic absorption at 271 (band II), 320 (sh) and 360 nm (band I), indicating a 3-*O*-substituted flavonol skeleton (Markham, 1982), and analysis with the usual flavonoid shift reagents suggesting the presence of free hydroxyl groups at positions C-5, C-7, C-3′ and C-4′ (Markham, 1982). The monosaccharides obtained after complete acid hydrolysis were identified as D-glucose and L-rhamnose following GC analysis using standard samples (Hara, Okabe & Mihasi, 1987). Thus, **1** was deduced to be a 3-*O*-substituted flavonol glycoside.

The positive ion FABMS of **1** showed two quasimolecular ion peaks at m/z 777 $[M + H]^+$ and 799 $[M + Na]^+$, and high resolution MS analysis of quasimolecular ion peak $[M + Na]^+$ revealed the molecular formula of **1** to be $C_{36}H_{40}O_{19}$. Fragment ion peaks at m/z 653 $[M + Na - 146$ (deoxyhexose unit)$]^+$, and 491 $[653 - 162$ (hexose unit)$]^+$, confirmed the presence of rhamnosyl and glucosyl moieties in the molecule.

All the ^{1}H- and ^{13}C-NMR signals of **1** in CD_3OD were assigned using ^{1}H–^{1}H COSY, NOESY, DEPT, HMQC, and HMBC experiments (Table 1). The ^{1}H- and ^{13}C-NMR spectra of **1** showed the presence of a 3,8-disubstituted quercetin moiety, a glucose moiety, and a rhamnose moiety, in addition to a symmetric

* Corresponding author.: Tel.: +81-92-642-6545; fax: +81-92-642-6580.
E-mail address: shoyama@shoyaku.pharm.kyushu-u.ac.jp (Y. Shoyama).

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1,3,4,5-tetrasubstituted benzene ring system. In the ^{1}H-NMR spectrum, a set of aromatic signals due to the 3′,4′-disubstituted ring B were evident at δ 7.75 (1H, *dd*, J = 1.5, 0.7 Hz, H-2′), 6.87 (1H, *dd*, J = 8.4, 0.7 Hz, H-5′), and 7.58 (1H, *dd*, J = 8.4, 1.5 Hz, H-6′). A signal at δ 6.31 (1H, *s*), which correlated to carbon at δ 99.5 in the HMQC spectrum, was characteristic of proton H-6 of ring A in the flavonoid skeleton (Markham, 1982). The H-6 signal was a singlet, suggesting that ring A of the flavonol has an 8-substituted skeleton. In the HMBC spectrum, the H-6 signal showed correlations with four quaternary carbons at δ 160.8 (C-5), 163.2 (C-7), 108.5 (C-8) and 105.8 (C-10), confirming the 8-substituted flavonol skeleton. Furthermore, a signal due to the methylene group at δ 4.04 (2H, *s*, H-7″) in ^{1}H-NMR spectrum, correlated with the ^{13}C-NMR signal at δ 29.1 (C-7″) in the HMQC spectrum. In addition, a chemically equivalent aromatic two-proton singlet at δ 6.58 (2H, *s*, H-2″ and H-6″) correlated with the ^{13}C-NMR signal at δ 106.7 (C-2″and C-6″), and two chemically equivalent methoxyl groups at δ 3.58 (3H × 2, *s*, 3″- and 5″-OMe) correlated with the ^{13}C-NMR signal at δ 6.5 in the HMQC spectrum (Fig. 1).

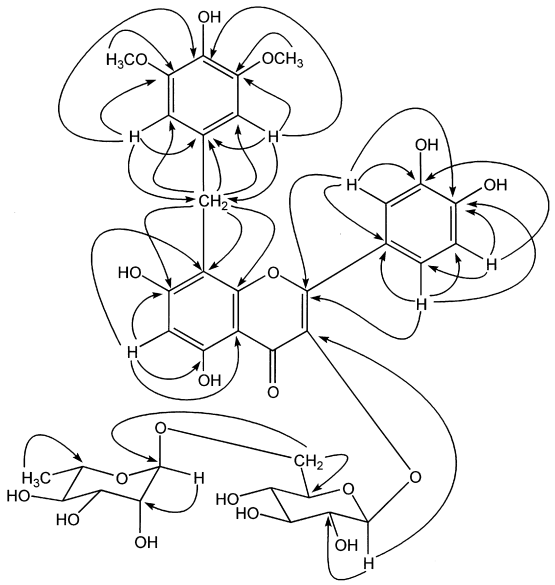


Fig. 1. Significant HMBC correlations observed for **1**.

Table 1
^{1}H- and ^{13}C-NMR spectral data of **1** in CD_3OD[a]

Attribution	δ_C (mult.)	δ_H (mult. J_{H-H})
2	159.3 (*s*)	
3	135.6 (*s*)	
4	179.7 (*s*)	
5	160.8 (*s*)	
6	99.5 (*d*)	6.31 (1H, *s*)
7	163.2 (*s*)	
8	108.5 (*s*)	
9	155.8 (*s*)	
10	105.8 (*s*)	
1′	123.5 (*s*)	
2′	117.9 (*d*)	7.75 (1H, *dd*, 1.5, 0.7)
3′	146.0 (*s*)	
4′	149.8 (*s*)	
5′	116.1 (*d*)	6.87 (1H, *dd*, 8.4, 0.7)
6′	123.7 (*d*)	7.58 (1H, *dd*, 8.4, 1.5)
1″	133.2 (*s*)	
2″, 6″	106.7 (*d*)	6.58 (2H, *s*)
3″, 5″	149.0 (*s*)	
4″	134.5 (*s*)	
7″	29.1 (t)	4.04 (2H, *s*)
3″, 5″ OMe	56.5 × 2 (*q*)	3.58 (3H × 2, *s*)
Glc-1	104.9 (*d*)	5.06 (1H, d, 7.3)
2	75.7 (*d*)	3.44 (1H, *dd*, 9.2, 7.3)
3	78.2 (*d*)	3.39 (1H, *dd*, 9.2, 9.2)
4	71.3 (*d*)	3.25 (1H, *dd*, 9.2, 9.2)
5	77.2 (*d*)	3.29 (1H, *m*)
6	68.5 (*t*)	3.37 (1H, *dd*, 11.0, 5.5) 3.77 (1H, *dd*, 11.0, 1.2)
Rha-1	102.4 (*d*)	4.51 (1H, *d*, 1.0)
2	72.1 (*d*)	3.63 (1H, *dd*, 3.3, 1.0)
3	72.3 (*d*)	3.54 (1H, *dd*, 9.5, 3.3)
4	73.9 (*d*)	3.27 (1H, *dd*, 9.5, 9.5)
5	69.7 (*d*)	3.43 (1H, *m*)
6	17.9 (*q*)	1.09 (3H, *d*, 6.2)

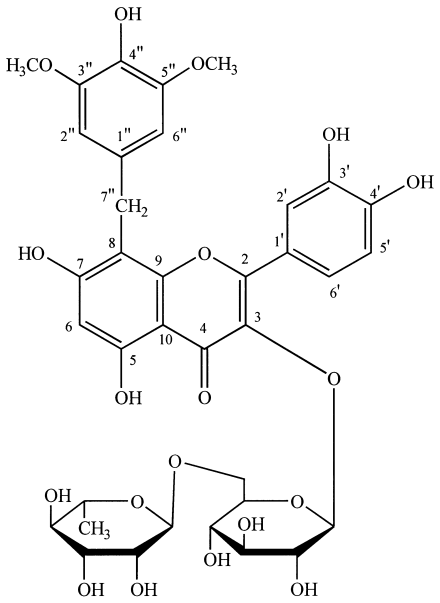
[a] ^{13}C multiplicities from DEPT experiments.

In the HMBC spectrum, long range correlations were observed from H-7″ to C-7, C-8, C-9, C-1″, C-2″ and C-6″. The same correlation of H-2″ and H-6″ was seen in C-1″, C-3″, C-4″, C-5″ and C-7″. The methoxyl group signals at δ 3.58 correlated to the carbons at δ 149.0 (C-3″ and C-5″) in the HMBC, and cross peaks between the methoxyl groups and H-2″ and H-6″ signals, and between H-7″ and H-2′ signals were also observed in the NOESY spectra. On the basis of the above data, the symmetric 1,3,4,5-tetra-substituted benzene ring system was identified as a (4-hydroxy-3,5-dimethoxyphenyl)methyl group, attached to C-8 of the flavonol skeleton.

Two anomeric protons in the ^{1}H-NMR spectrum of **1** were observed, and a signal at δ 5.06 (1H, *d*, J = 7.3 Hz) was assigned as a glucosyl anomeric proton, suggesting that the glycosidic bond had a β linkage (Du, Kohinata, Kawasaki, Guo & Miyahara, 1998b). Another signal at δ 4.51 (1H, *d*, J = 1.0 Hz) was assigned the rhamnosyl anomeric proton with an α linkage (Du et al., 1998b). The sequence of sugar moieties was determined by NOESY spectroscopy, supported by a correlation between H_2-6 of the glucosyl group and the anomeric proton of rhamnosyl group. In the HMBC spectrum, the anomeric proton of the glucosyl group was correlated with C-3 (δ 135.6) of the aglycone. From these results, the structure of **1** was concluded to be 3-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxi]-5,7-dihydroxy-8-[(4-

hydroxy-3,5-dimethoxyphenyl)methyl]-2-(3,4-dihydroxypheny)-4H-1-benzopyran-4-one, and was trivially named goodyerin

The known flavonol glycosides were identified by UV, MS, ^{1}H- and ^{13}C-NMR spectra data in agreement with literature data (Markham, 1982; Agrawal, 1989) as rutin (**2**), kaempferol 3-*O*-rutinoside (**3**) and isorhamnetin 3-*O*-rutinoside (**4**).



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3. Experimental

3.1. General

Melting points were determined with a YAZAWA BY-2 apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1010 digital polarimeter in MeOH at 26°C. UV spectra were recorded on a BECKMAN DU-70 spectrophotometer. The MS were recorded on a JEOL AX-500 instrument. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 instrument (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, respectively) using TMS as an internal standard.

3.2. Plant material

The whole plants of *G. schlechtendaliana* were collected in Taiwan, in August 1997. A voucher specimen has been deposited at the Herbarium of Medicinal Plant Garden, Graduate School of Pharmaceutical Sciences, Kyushu University.

3.3. Extraction and isolation

The air-dried whole plants of *G. schlechtendaliana* (1.5 kg) were powdered and percolated with MeOH at room temperature. Evaporation of the solvent from the extract under reduced pressure furnished the MeOH extract (667 g). The extract was suspended in H_2O and partitioned successively with *n*-hexane, $CHCl_3$, and *n*-BuOH. The *n*-BuOH soluble portion (58.2 g) was applied to a Diaion HP-20 column and eluted with H_2O and 20, 40, 50, 80 and 100% MeOH, successively. The 40% MeOH eluate (2.51 g) was chromatographed over silica gel and eluted with stepwise gradients of $CHCl_3$–MeOH–H_2O (8:2:0.2 to 7:3:0.5) solvent system, then rechromatographed over a column of Polyamide C-200 (80% EtOH), and finally purified by silica gel column ($CHCl_3$–MeOH–H_2O, 7:3:0.3) to give **2** (249 mg). The 50% MeOH eluate (5.49 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH–H_2O (8:2:0.2 to 7:3:0.5) to give three fractions (frs. 1, 2 and 3). Fr. 3 (2.82 g) was further fractionated on a Sephadex LH-20 column eluted with MeOH, rechromatographed over silica gel eluted with $CHCl_3$–MeOH–H_2O (8:2:0.2 to 7:3:0.5), and finally purified by reversed-phase HPLC (YMC-parck ODS-A) with 50% MeOH to yield of **3** (5 mg) and **4** (9 mg). The 60% MeOH eluate (545 mg) was chromatographed using Sephadex LH-20 column eluted with 80% MeOH, silica gel eluted with $CHCl_3$–MeOH–H_2O (7:3:0.5), Polyamide C-200 (95% EtOH), then a silica gel column, and was finally crystallized from 80% EtOH to give **1** (282 mg).

3.4. Goodyerin (*1*)

Yellow amorphous solid, mp: 180°C (80% EtOH), $[\alpha]_D^{26}$ + 56.8° (*c* 0.61, MeOH); UV λ_{max} (MeOH) nm: 271, 320 (*sh*), 360; +NaOMe: 281, 336 (*sh*), 415; +$AlCl_3$: 279, 370 (*sh*), 440; +$AlCl_3$/HCl: 279, 360 (*sh*), 408; +NaOAc: 278, 329 (*sh*), 398; +NaOAc/H_3BO_3: 268, 309 (*sh*), 381; positive ion HR-FABMS *m/z* 799.2025 $[M + Na]^+$ (calculated for $C_{36}H_{40}O_{19}Na$: 799.2022); positive ion FABMS *m/z*: 815 $[M + K]^+$, 799 $[M + Na]^+$, 777 $[M + H]^+$, 653 $[M + Na - 146$ (deoxyhexose unit)$]^+$, 491 $[653 - 162$ (hexose unit)$]^+$; ^{1}H- and ^{13}C-NMR spectral data (CD_3OD): Table 1.

3.5. Acid hydrolysis of *1*

A solution of **1** (2 mg) in 2N H_2SO_4 (0.5 ml) was heated at 95°C for 0.5 h. After cooling, the reaction mixture was neutralized with $Ba(OH)_2$ and the insoluble portion was removed by filtration. The filtrate was evaporated under reduced pressure and the residue was separated on a Sep-Pack C18 cartridge column

eluted with H_2O. The eluate was concentrated to give a syrup which exhibited two spots identical with those of authentic samples of D-glucose and L-rhamnose on Avicel SF TLC [BuOH–pyridine–H_2O (6:2:3) upper layer + pyridine(1)] R_f: 0.38 (D-glucose), 0.63 (L-rhamnose). The syrup was converted to the trimethylsilyl ether of the thiazolidine derivative according to Hara et al., and was examined by GC (GL Sciences, OV-17, capillary columu, 0.25 mm i.d. × 50 m; column temperature 220°C; carrier gas: He, 1.5 kg/cm^2); R_t (min): 25.8 (D-glucose), 19.3 (L-rhamnose).

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