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Flavonoid and benzophenone glycosides from *Coleogyne ramosissima*

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Abstract

A benzophenone glucoside and two flavonol glycosides were isolated together with 27 known polyphenols from the aerial parts of *Coleogyne ramosissima*, and their structures were elucidated by spectroscopic and chemical methods as iriflophenone 2-*O*-β-glucopyranoside, isorhamnetin 3-*O*-2^{G}-rhamnopyranosylrutinoside-7-*O*-α-rhamnopyranoside and limocitrin 3-*O*-rutinoside-7-*O*-β-glucopyranoside, respectively. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Coleogyne ramosissima*; Rosaceae; Iriflophenone 2-*O*-glucopyranoside; Isorhamnetin 3-*O*-2^{G}-rhamnopyranosylrutinoside-7-*O*-rhamnopyranoside; Limocitrin 3-*O*-rutinoside-7-*O*-glucopyranoside

1. Introduction

Coleogyne ramosissima Torr. (Rosaceae) is a densely branched shrub widely distributed in western North America. As for the chemical constituents of this plant, the presence of condensed tannins (Provenza and Malechek, 1983) and cyanogenic glycosides (Fikenscher et al., 1981) in the leaves was reported, and lignans (Tachimoto et al., 1993) and phenylpropanoid glycosides (Tachimoto et al., 1994) were isolated from the stem extract as inhibitors of Epstein–Barr virus early antigen activation induced by 12-*O*-tertadecanoylphorbol-13-acetate. The polar polyphenolic constituents in the plant have, however, been investigated little, although reversed-phase HPLC of the aqueous acetone extract with diode array detection at 280 nm suggested the occurrence of unidentified UV-sensitive compounds in large amount. In the present study, three new polyphenols, a benzophenone and two flavonol glycosides along with 27 known polyphenols were isolated from the aerial parts of the plant. We describe herein the structure elucidation of these new polyphenols.

2. Results and discussion

A concentrated solution of an aq. acetone homogenate of the dried aerial parts of *C. ramosissima* was extracted with Et_2O, AcOEt and *n*-BuOH, successively. A new benzophenone glucoside, iriflophenone 2-*O*-β-glucopyranoside (**1**), and two new flavonoid glycosides, isorhamnetin 3-*O*-2^{G}-rhamnopyranosylrutinoside-7-*O*-α-rhamnopyranoside (**2**) and limocitrin 3-*O*-rutinoside-7-*O*-β-glucopyranoside (**3**) were obtained by repeated column chromatography of the AcOEt and the *n*-BuOH extracts, respectively. Twenty-seven known polyphenols were also isolated from the AcOEt and *n*-BuOH extracts as described in Section 3, and were identified as (+)-catechin, (−)-epicatechin, procyani-

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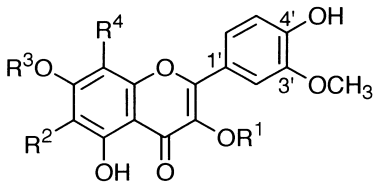

	R^1	R^2	R^3	R^4
2	Rha-Rut	H	Rha	H
3	Rut	H	Glc	OCH_3
4	Rha-Rut	H	H	H
5	Glc	H	H	H
6	Rut	H	H	H
7	Glc	H	H	OCH_3
8	Rut	H	H	OCH_3
9	GlcA	H	H	H
10	Rut	H	Glc	H
11	Glc	OCH_3	H	H

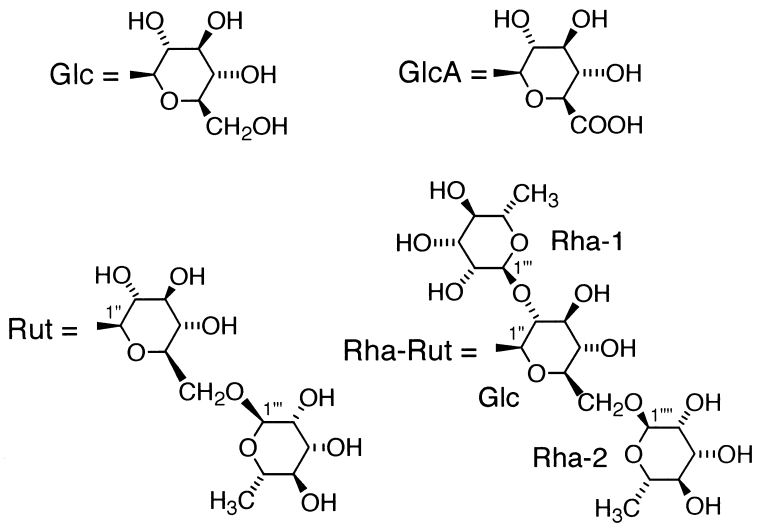

Chart 1.

dins B-1, B-2, B-3, B-4, B-5, C-1, isoquercitrin (Markham et al., 1978), quercetin 3-*O*-β-xylopyranoside (Kadota et al., 1990), 8-methoxykaempferol 3-*O*-β-sophoroside[1]) (Ferreres et al., 1989), (±)-eriodictyol 7-*O*-β-glucopyranoside (Cui et al., 1990), (+)-taxifolin 7-*O*-β-glucopyranoside (Foo and Karchesy, 1989), (±)-naringenin 8-*C*-β-glucopyranoside (Ge et al., 1991), 1-*O*-(*E*)-cinnamyl-β-glucopyranose (Tachimoto et al., 1994), (*E*)-coniferyl alcohol 9-*O*-β-glucopyranoside (Andersson and Lundgren, 1988), picein (Perry et al., 1996), liriodendrin (Deyama, 1983) and N^1-, N^5-, N^{10}-tri-*p*-(*E*)-coumaroylspermidine (Strack et al., 1990; Bokern et al., 1995; Werner et al., 1995), and eight isorhamnetin glycosides, isorhamnetin 3-*O*-2^G-rhamnopyranosylrutinoside (**4**) (Masterova et al., 1991), isorhamnetin 3-*O*-β-glucopyranoside (**5**) (Ishida et al., 1988), narcissin (**6**) (Markham et al., 1978), limocitrin 3-*O*-β-glucopyranoside (**7**) (Ulubelin et al., 1984), limo-

[1] This compound was erroneously reported to be 6-methoxykaempferol 3-*O*-β-sophoroside in our study on the anti-tumor promoting activity of the rosaceous plants (Ito et al., 1999).

citrin 3-*O*-rutinoside (**8**) (Harborne, 1981), isorhamnetin 3-*O*-β-glucuronopyranoside (**9**) (Merfort and Wendisch, 1988), isorhamnetin 3-*O*-rutinoside-7-*O*-β-glucopyranoside (**10**) (Dembinska-Migas et al., 1985) and spinacetin 3-*O*-β-glucopyranoside (**11**) (Merfort and Wendisch, 1987), by comparison of the physicochemical data with those reported (see Chart 1).

Compound **1** was obtained as a pale yellow amorphous powder. The molecular formula was established as $C_{19}H_{20}O_{10}$ by HRESIMS (*m/z* 409.1186 $[M + H]^+$, calculated for $C_{19}H_{20}O_{10}$ + H, 409.1135). The ^1H NMR spectrum of **1** indicated the presence of a 1-, 4-disubstituted [δ 7.64 (2H, *d*, $J = 8$ Hz) and δ 6.84 (2H, *d*, $J = 8$ Hz)] and 1-, 2-, 4-, 6-tetrasubstituted benzene nucleus [δ 6.33 (1H, *d*, $J = 2$ Hz) and δ 6.12 (1H, *d*, $J = 2$ Hz)]. A seven-spin system characteristic of a $^4C_1$ glucopyranose residue was observed in the aliphatic region. The ^{13}C NMR spectrum showed a ketonic carbon resonance besides 12 sp^2 and glucose carbon signals, suggesting that **1** is a glucoside of iriflophenone. The HMBC spectrum of **1** revealed that the anomeric proton at δ 4.80 (H-1″) was correlated through three-bond coupling with the signal at δ 158.7 (C-2) which also showed two-bond coupling with one (δ 6.33, H-3) of the two *meta*-coupled proton signals in the phloroglucinol unit (Fig. 1). The location of the glucose residue was thus established to be C-2 of the aglycone. A β-glucosidic linkage was suggested from the coupling constant ($J = 8$ Hz) of the anomeric proton signal. Based on these data, compound **1** was iriflophenone 2-*O*-β-glucopyranoside.

Compound **2**, a yellow powder, showed an $[M + H]^+$ ion peak at *m/z* 917 in ESIMS. The ^1H NMR spectrum of **2** exhibited 1,3,4-trisubstituted benzene signals [δ 7.85 (*d*, $J = 2$ Hz), 7.54 (*dd*, $J = 2$, 8 Hz) and 6.90 (*d*, $J = 8$ Hz)], *meta*-coupled signals [δ 6.78 and 6.43 (each *d*, $J = 2$ Hz)] in the aromatic proton region, and a methoxyl signal (δ 3.85), which were similar to the aglycone signals of isorhamnetin glycosides (**4**–**6**). Further, the signals due to four anomeric proton signals [δ 5.63 (*d*, $J = 7$ Hz), 5.54, 5.02, 4.37 (each *br s*)] and three methyl groups [δ 1.11, 0.93, 0.70 (each 3H, *d*, $J = 6$ Hz)] revealed the presence of three rhamnosyl residues in **2**. In fact, the ^{13}C NMR spectrum of **2** (Table 1) was very similar to that of isorhamnetin 3-*O*-2^G-rhamnopyranosylrutinoside (**4**), except for the presence of signals ascribable to an extra rhamnose core and a remarkable upfield shift ($\Delta\delta$ 2.7 ppm) of the aglycone C-7. The linkage pos-

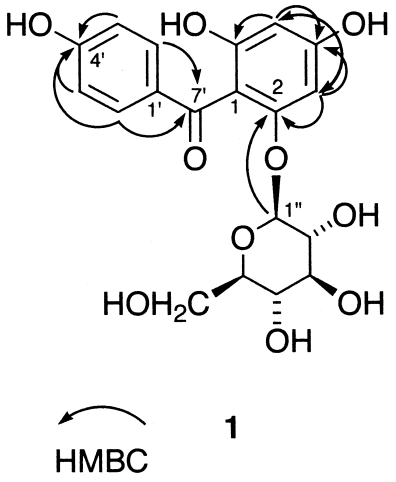

Fig. 1. HMBC correlations of **1**.

Table 1
^{13}C NMR spectral data for compounds **2**, **3**, **4** and **8** (126 MHz in DMSO-d_6)

	2	**4**		**3**	**8**
Aglycone moiety					
C-2	157.1	157.0	C-2	155.9	156.1
C-3	132.9	133.0	C-3	133.4	133.3
C-4	177.5	177.7	C-4	177.9	177.7
C-5	161.1	161.4	C-5	157.0	156.5
C-6	99.5	99.2	C-6	98.8	99.2
C-7	161.8	164.5	C-7	156.2	157.2
C-8	95.0	94.4	C-8	129.2	127.7
C-9	156.2	157.0	C-9	149.9	149.7
C-10	105.8	104.6	C-10	105.5	104.1
C-1′	120.9	121.7	C-1′	121.2	121.4
C-2′	113.4	113.7	C-2′	113.3	113.3
C-3′	149.9	149.6	C-3′	147.1	147.1
C-4′	147.1	147.3	C-4′	148.3	148.9
C-5′	115.4	115.6	C-5′	115.6	115.6
C-6′	122.5	122.6	C-6′	122.5	122.4
OMe	55.8	56.2	8-OMe	60.8	61.2
			3′-OMe	55.7	55.8
Sugar moieties			Rutinose (Rut)		
Glucose (Glc)			Glucose		
C-1″	98.8	99.1	C-1″	101.4	101.5[a]
C-2″	77.8	78.2	C-2″	74.5	74.5
C-3″	77.2	77.3	C-3″	76.8	76.6
C-4″	70.5	70.8	C-4″	70.3	70.3
C-5″	76.0	76.3	C-5″	76.1	76.2
C-6″	66.5	67.4	C-6″	67.2	67.1
Rhamnose (Rha-1, 2, 3)			Rhamnose		
C-1‴,1⁗,1⁗′	101.04	101.4	C-1‴	101.1	101.1[a]
	100.98	101.3	C-2‴	70.5	70.6
	98.6		C-3‴	70.8	70.8
C-2‴,2⁗,2⁗′	70.7 (3C)	70.7 (2C)	C-4‴	71.9	72.0
C-3‴,3⁗,3⁗′	70.5 (3C)	70.9 (2C)	C-5‴	68.4	68.5
C-4‴,4⁗,4⁗′	71.9 (2C)	72.1 (2C)	C-6‴	17.9	17.9
	71.8				
C-5‴,5⁗,5⁗′	68.5	68.8 (2C)	Glucose (Glc)		
	68.4 (2C)		C-1⁗	100.4	
C-6‴,6⁗,6⁗′	18.1	18.1	C-2⁗	73.4	
	17.8	17.5	C-3⁗	77.4	
	17.3		C-4⁗	69.8	
			C-5⁗	76.5	
			C-6⁗	61.5	

[a] These values may be interchanged.

itions of sugar units with the aglycone in **2** were substantiated by HMBC spectrum ($J_{CH} = 6$ Hz), in which H-1″ (δ 5.63) of glucose and H-1‴′ (δ 5.54) of rhamnose (Rha-3) were correlated with C-3 (δ 132.9) and C-7 (δ 161.8) of the aglycone through three-bond couplings, respectively. Similarly, anomeric proton signals of the other rhamnoses (Rha-1 H-1‴ at δ 5.02 and Rha-2 H-1⁗ at δ 4.37) exhibited three-bond cross peaks with C-2″ at δ 77.8 and C-6″ at δ 66.5 carbon resonances of glucose, respectively (Fig. 2). These spectral data indicated the presence of the 2^G-rhamnopyranosylrutinosyl and a rhamnosyl residue, at positions 3 and 7 of the aglycone, respectively. Enzymatic hydrolysis of **2** with hesperidinase (Ishida et al., 1988) afforded isorhamnetin 3-*O*-β-glucopyranoside (**5**), narcissin (**6**) and **4** to establish that the 2^G-rhamnopyranosylrutinose unit is located at O-3. On the basis of these data, the structure of **2** was determined as isorhamnetin 3-*O*-2^G-rhamnopyranosylrutinoside-7-*O*-α-L-rhamnopyranoside.

Compound **3** was obtained as a yellow powder. The UV and ESIMS (*m/z* 817 $[M + H]^+$) suggested that **3** is also a flavonoid glycoside. The ^{1}H NMR spectrum of **3** showed signals attributable to an aglycone at δ 7.88 (*d*, $J = 2$ Hz), 7.56 (*dd*, $J = 2$, 8 Hz) and 6.94 (*d*, $J = 9$ Hz) (B-ring), δ 6.65 (*s*) and two methoxyl signals (δ 3.84 and 3.83), which were similar to those of limocitrin 3-*O*-rutinoside (**8**) except for a downfield shift of H-6 (Δ 0.37 ppm). The spectrum also exhibited three anomeric proton signals at δ 5.43 (*d*, $J = 8$ Hz), δ 5.06 (*d*, $J = 7.5$ Hz) and δ 4.39 (*br s*) and a methyl signal at δ 0.95 (*d*, $J = 6$ Hz), indicating the presence of a rhamnose and two hexose units in **3**. The sugar moiety carbon resonances in the ^{13}C NMR spectrum of **3** corresponded to those of **8** (Harborne, 1981) and quercetin 7-*O*-glucoside (Markham et al., 1978). A significant difference in the ^{13}C NMR spectrum is in the chemical shifts of C-7, C-8 and C-10 (Δ δ −1.0, +1.5 and +1.4 ppm). This suggests that **3** is a 7-*O*-glucoside of **8**. The proposed structure **3** was substantiated by the HMBC spectrum which showed correlation of H-1‴ (δ 5.43) with C-7 (δ 156.2) of the aglycone through three-bond coupling. Further, the anomeric proton of the glucose core exhibited an NOE correlation with H-6 at δ 6.65, and 8-methoxy signal at δ 3.84 displayed NOE with H-6′ signal at δ 7.56. Upon enzymatic hydrolysis with β-glucosidase, **3** yielded **8** as judged by reversed-phase HPLC. Consequently, compound **3** was established as limocitrin 3-*O*-rutinoside-7-*O*-β-glucopyranoside.

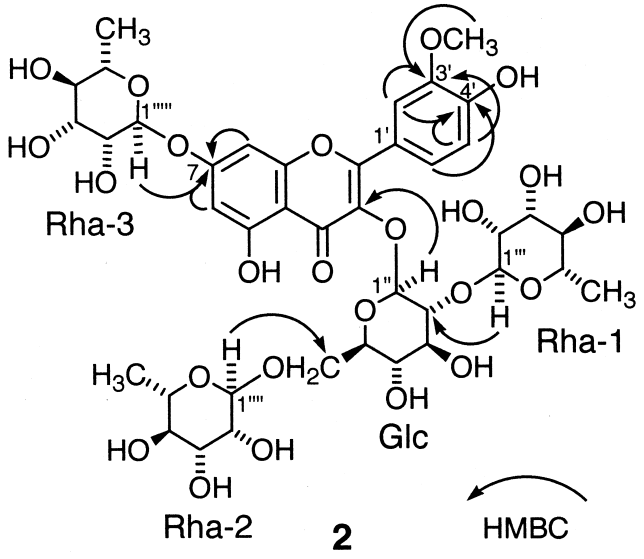

Fig. 2. HMBC Correlations Of **2**.

3. Experimental

3.1. General

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. ^{1}H (500 MHz) and ^{13}C NMR (126 MHz) spectra were measured on a Varian VXR-500 instrument, and chemical shifts are given in δ-values from tetramethylsilane. Average *J*-values for one-bond coupling and two- or three-bond coupling in HMQC and HMBC were set at 140 Hz and 6 or 8 Hz. ESI-MS were measured with a Micromass Auto Spec OA-Tof mass spectrometer (solvent: 50% MeOH containing 0.1% $AcONH_4$, flow rate: 0.02 ml min^{-1}). Normal phase HPLC was conducted on a YMC-Pack SIL A-003 (YMC Co., Ltd.), column (4.6 × 250 mm) developed with *n*-hexane–MeOH–THF–HCO_2H (55:33:11:1) containing oxalic acid (450 mg l^{-1}) (flow rate, 1.5 ml min^{-1}; detection 280 nm) at room temperature. Reversed-phase HPLC was performed on a YMC-Pack AQ-302 (ODS) (YMC Co., Ltd.) column (4.6 × 150 mm) developed with 10 mM H_3PO_4–10 mM KH_2PO_4–EtOH–AcOEt (42.5:42.5:10:5) (flow rate, 1.0 ml min^{-1}; detection 280 nm) at 40°C.

3.2. Plant material

The aerial parts of *C. ramosissima* Torr. were collected at Beaver Dam Mts, Utah, USA in May 1992, and the plant was identified by Dr. R.J. Kass, Brigham Young University, Utah. A voucher specimen is deposited at the Herbal Garden of Kyoto Pharmaceutical University.

3.3. Extraction and isolation

The dried aerial parts (1 kg) were homogenized (3×) in Me_2CO–H_2O (7:3) (3 l × 3) and the homogenate was filtered. The filtrate was concentrated and

extracted with Et_2O (1 l × 4), EtOAc (1 l × 6) and *n*-BuOH saturated with H_2O (1 l × 6). The EtOAc extract (16.5 g) was subjected to chromatography over Toyopearl HW-40 (coarse) (2.2 cm i.d. × 66 cm) with MeOH–H_2O (4:6 → 5:5 → 6:4 → 7:3) → Me_2CO–H_2O (7:3) in a stepwise gradient mode. Fractions showing similar HPLC patterns were combined and further purified by a combination of CC over Toyopearl HW-40 (fine), MCI-gel CHP-20P and/or YMC GEL ODS-AQ 120-S50 with aq. MeOH to afford (+)-catechin (35 mg), (−)-epicatechin (270 mg), procyanidins B-2 (203 mg), B-3 (47 mg), B-4 (383 mg), B-5 (9 mg), C-1 (16 mg), isoquercitrin (47 mg), quercetin 3-*O*-β-xylopyranoside (9 mg), (±)-eriodictyol 7-*O*-β-glucopyranoside (119 mg), (±)-naringenin 8-*C*-β-glucopyranoside (11 mg), 1-*O*-cinnamyl-β-glucopyranose (125 mg), (*E*)-coniferyl alcohol 9-*O*-β-glucopyranoside (5 mg), picein (308 mg), N^1-, N^5-, N^{10}-tri-*p*-(*E*)-coumaroylspermidine (59 mg), iriflophenone 2-*O*-β-glucopyranoside (**1**) (6 mg), isorhamnetin 3-*O*-β-glucopyranoside (**5**) (36 mg), narcissin (**6**) (85 mg), limocitrin 3-*O*-β-glucopyranoside (**7**) (10 mg), limocitrin 3-*O*-rutinoside (**8**) (22 mg) and spinacetin 3-*O*-β-glucopyranoside (**11**) (12 mg).

The *n*-BuOH extract (56.3 g) was subjected to column chromatography over Diaion HP-20 (6.0 cm i.d. × 40 cm) and developed with H_2O → MeOH–H_2O (1:9 → 2:8 → 3:7 → 4:6 → 6:4) → MeOH in a stepwise gradient mode. The eluate with MeOH–H_2O (2:8) was further subjected to chromatography over MCI-gel CHP-20P to give picein (145 mg). The eluate with MeOH–H_2O (3:7) was similarly subjected to repeated column chromatographies to give (−)-epicatechin (66 mg), procyanidins B-1 (40 mg), B-2 (94 mg), C-1 (29 mg), isorhamnetin 3-*O*-rutinoside-7-*O*-β-glucopyranoside (**10**) (23 mg), (+)-taxifolin 7-*O*-β-glucopyranoside (8 mg), isorhamnetin 3-*O*-2^G-rhamnopyranosylrutinoside-7-*O*-α-rhamnopyranoside (**2**) (39 mg) and limocitrin 3-*O*-rutinoside-7-*O*-β-glucopyranoside (**3**) (12 mg). From the eluate with MeOH–H_2O (4:6), procyanidins B-2 (52 mg), C-1 (10 mg), liriodendrin (9 mg), 8-methoxykaempferol 3-*O*-sophoroside (63 mg) and isorhamnetin 3-*O*-2^G-rhamnopyranosylrutinoside (**5**) (120 mg) were obtained after repeated column chromatography. The eluate with MeOH–H_2O (6:4) was similarly purified by a combination of column chromatographies to yield **6** (63 mg) and **8** (17 mg). The known polyphenols were identified by comparison of their physical data with the reported values.

3.3.1. Iriflophenone 2-O-β-glucopyranoside (*1*)

A pale yellow amorphous powder, $[\alpha]_D$ −24° (MeOH; *c* 1.0). UV λ_{max} MeOH nm (log ε): 233 sh (4.08), 289 (4.12). ESIMS *m/z*: 409 $[M + H]^+$. HR-ESIMS *m/z*: 409.1186 $[M + H]^+$, calculated for $C_{19}H_{20}O_{10}$ + H, 409.1135. ^{1}H NMR (Me_2CO-d_6 + D_2O): δ 7.64 (2H, *d*, *J* = 8 Hz, H-2′,6′), 6.84 (2H, *d*, *J* = 8 Hz, H-3′, 5′), 6.33 (1H, *d*, *J* = 2 Hz, H-3), 6.12 (1H, *d*, *J* = 2 Hz, H-5), 4.80 (1H, *d*, *J* = 8 Hz, H-1″), 3.86 (1H, *dd*, *J* = 2.5, 12 Hz, H-6″), 3.64 (1H, *dd*, *J* = 6, 12 Hz, H-6″), 3.44 (1H, *ddd*, *J* = 2.5, 6, 9 Hz, H-5″), 3.41 (1H, *t*, *J* = 9 Hz, H-3″), 3.2–3.4 (overlapped with HDO, H-4″), 3.02 (1H, *dd*, *J* = 8, 9 Hz, H-2″). ^{13}C NMR (MeOH-d_4): δ 197.5 (C-7′), 163.6 (C-4′), 162.4 (C-4), 159.7 (C-6), 158.7 (C-2), 133.5 (2C) (C-2′,6′), 124.4 (C-1′), 115.8 (2C) (C-3′,5′), 102.4 (C-1″), 99.8 (C-1), 98.1 (C-5), 95.9 (C-3), 78.2 (C-5″), 77.8 (C-3″), 74.7 (C-2″), 71.1 (C-4″), 62.5 (C-6″).

3.3.2. Isorhamnetin 3-O-2^G-rhamnosylrutinoside-7-O-α-L-rhamnopyranoside (*2*)

A yellow powder, $[\alpha]_D$ −35° (MeOH; *c* 1.0). UV λ_{max} MeOH nm (log ε): 248 sh (4.01), 254 (4.17), 265 sh (4.07), 354 (4.07). ESIMS *m/z*: 917 $[M + H]^+$, 939 $[M + Na]^+$. HRESIMS *m/z*: 917.2965 $[M + H]^+$, calculated for $C_{40}H_{52}O_{24}$ + H, 917.2927. ^{1}H NMR (DMSO-d_6): δ 7.85 (1H, *d*, *J* = 2 Hz, H-2′), 7.54 (1H, *dd*, *J* = 2, 8 Hz, H-6′), 6.90 (1H, *d*, *J* = 8 Hz, H-5′), 6.78 (1H, *br s*, H-8), 6.43 (1H, *br s*, H-6), 5.63 [1H, *d*, *J* = 7 Hz, glucose (Glc) H-1″], 5.54 (1H, *br s*, Rha-3 H-1‴′), 5.02, 4.37 (each 1H, *br s*, Rha-1, 2 H-1‴,1⁗), 3.85 (3H, *s*, 3′-OCH_3), 1.11 (3H, *d*, *J* = 6 Hz, Rha-3 H-6‴′), 0.93, 0.70 (each 3H, *d*, *J* = 6 Hz, Rha-1, 2 H-6‴,6⁗). ^{13}C NMR: Table 1.

3.3.3. Enzymatic hydrolysis of *2* with hesperidinase

A solution of **2** (1 mg) in 0.05 M McIlvaine buffer (pH 3.8) (1 ml) and 4% glucose-DMSO (0.1 ml) was incubated with hesperidinase (1 mg) at 37°C for 21 h. The reaction mixture was analyzed by reversed-phase HPLC to show peaks identical with those of the authentic **4–6**.

3.3.4. Limocitrin 3-O-rutinoside-7-O-β-glucopyranoside (*3*)

A yellow powder, $[\alpha]_D$ −23° (MeOH; *c* 1.0). UV λ_{max} MeOH nm (log ε): 257 (4.13), 270 sh (4.05), 360 (4.01); + $AlCl_3$ 276, 305 sh, 377, 418. ESIMS *m/z*: 817 $[M + H]^+$. HRESIMS *m/z*: 817.2423 $[M + H]^+$, calculated for $C_{35}H_{44}O_{22}$ + H, 817.2402. ^{1}H NMR (DMSO-d_6 + D_2O): δ 7.88 (1H, *d*, *J* = 2 Hz, H-2′), 7.56 (1H, *dd*, *J* = 2, 8 Hz, H-6′), 6.94 (1H, *d*, *J* = 8 Hz, H-5′), 6.65 (1H, *s*, H-6), 5.43 (1H, *d*, *J* = 8 Hz, Glc H-1″), 5.06 (1H, *d*, *J* = 7.5 Hz, Glc H-1‴), 4.39 (1H, *br s*, Rha H-1⁗), 3.84 (3H, *s*, 8-OCH_3), 3.83 (3H, *s*, 3′-OCH_3), 0.95 (3H, *d*, *J* = 6 Hz, Rha H-6⁗) ^{13}C NMR: Table 1.

3.3.5. Enzymatic hydrolysis of *3* with β-glucosidase

A solution of **3** (1 mg) in 0.1 M acetate buffer (pH 5) (1 ml) was incubated with β-glucosidase (1 mg) at 37°C for 1 h. Reversed-phase HPLC of the reaction mixture showed a peak identical with that of **8**.

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