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THREE FLAVONOL GLYCOSIDES FROM LEAVES OF *MYRSINE SEGUINII*

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Key Word Index—*Myrsine seguinii*; *Rapanea neriifolia*; Myrsinaceae; flavonol glycosides; quercitrin; myricitrin.

Abstract—From the leaves of *Myrsine seguinii*, five flavonol glycosides were isolated. Two were identified as quercitrin and myricitrin, and the structures of the remaining three are quercetin 3-rhamnoside-3′-glucoside, myricetin 3-rhamnoside-3′-glucoside, and myricetin 3,4′-dirhamnoside. © 1997 Elsevier Science Ltd

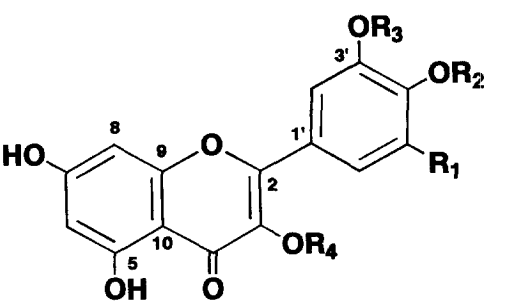
INTRODUCTION

Myrsine seguinii Lev. (syn. *Rapanea neriifolia*) is a perennial tree, and grows in moderate and subtropical areas. In China, a plant of this family, *Ardisia japonica*, is used as an antitussive, expectorant, antidote and diuretic [1]. Phytochemical investigation of *M. seguinii* afforded five flavonol glycosides, of which two (**1** and **3**) were known, and three (**2**, **4** and **5**) are new. This paper deals with structural elucidation of these compounds.

RESULTS AND DISCUSSION

Flavonol glycosides were isolated from the *n*-BuOH soluble fraction of a methanol extract of *M. seguinii* by a combination of highly porous synthetic resin (Diaion HP-20), normal and reversed-phase silica gel column chromatographies, and droplet counter-current chromatography (DCCC). Compounds **1** and **3** were identified as quercitrin and myricitrin, respectively, on comparison of the spectroscopic data with reported values [2].

Quercetin 3-rhamnoside-3′-glucoside (**2**), $[\alpha]_D$ −135.5°, was isolated as yellow crystals with an elemental composition of $C_{27}H_{30}O_{16}$, as judged from the results of HR-FAB mass spectral analysis. The IR spectrum showed the presence of hydroxyl (3300 cm^{-1}) and ketone (1655 cm^{-1}) groups, and aromatic rings(s) (1600 and 1505 cm^{-1}). The ^1H and ^{13}C NMR spectra were similar to those of quercitrin, except for the presence of an additional β-glucopyranosyl moiety. Spectral shift data (see Experimental) showed that this glucose was linked to the hydroxyl group on C-3′ [3]. This was confirmed by comparison of the ^{13}C NMR data with those reported for quercetin 4′-glucoside (**6**) (see Table 1) and by the HMBC spectrum, in which a cross peak between δ_H 4.76 (H-1‴) and δ_C 145.2 (C-3′) was observed (see Fig. 1). Therefore, **2** has the structure shown.



	R_1	R_2	R_3	R_4
1	H	H	H	Rha
2	H	H	Glc	Rha
3	OH	H	H	Rha
4	OH	H	Glc	Rha
5	OH	Rha	H	Rha
6	H	Glc	H	H

Myricetin 3-rhamnoside-3′-glycoside (**4**), $[\alpha]_D$ −152.1°, was isolated as a yellow amorphous powder whose elemental composition was determined to be $C_{27}H_{30}O_{17}$. The IR and UV spectra also showed the

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Table 1. ^{13}C NMR data for quercitrin (**1**) and myricitrin (**3**), and **2**, **4** and **5** [100 MHz, CD_3OD (DMSO-d_6 in parentheses)]

Carbon Number	1	2	6*	3	4		5
2	158.6	(156.8)	(147.0)	159.5	158.6		158.6
3	136.3	(134.1)	(136.5)	136.4	136.2		136.4
4	179.7	(177.6)	(176.3)	179.7	179.7		179.7
5	163.3	(161.1)	(161.0)	163.3	163.2		163.2
6	99.8	(98.6)	(98.7)	99.8	99.9		100.0
7	165.9	(164.1)	(164.3)	165.9	165.9		166.0
8	94.7	(93.9)	(93.9)	94.7	94.9		94.8
9	159.3	(156.4)	(156.7)	158.6	158.9		158.9
10	105.9	(104.0)	(103.5)	105.9	106.0		106.0
1′	122.9	(124.8)	(125.8)	122.0	122.3		127.3
2′	116.9	(115.6)	(115.7)	109.6	113.1		110.0
3′	146.4	(145.2)	(147.0)	146.9	147.3		152.3
4′	149.8	(149.5)	(146.4)	137.9	140.3		136.9
5′	116.4	(116.9)	(117.0)	146.9	147.3		152.3
6′	123.0	(120.9)	(120.0)	109.6	112.3		110.0
1″	103.6	(101.5)	( )	103.7	103.3	1″,1‴	103.2, 103.9
2″	72.1	(70.2)	( )	72.1	72.1	2″,2‴	72.1, 72.1
3″	72.1	(70.5)	( )	72.2	72.2	3″,3‴	72.2, 72.3
4″	73.3	(70.9)	( )	73.4	73.2	4″,4‴	73.3, 73.8
5″	71.9	(69.9)	( )	71.9	71.9	5″,5‴	71.4, 71.9
6″	17.7	(17.4)	( )	17.7	17.8	6″,6‴	17.8, 18.0
1‴		(102.5)	(102.2)		105.1		
2‴		(73.2)	(73.4)		75.0		
3‴		(75.7)	(76.4)		78.3		
4‴		(69.6)	(70.5)		71.1		
5‴		(77.0)	(77.5)		77.5		
6‴		(60.6)	(61.4)		62.3		

* Data taken from ref. [2].

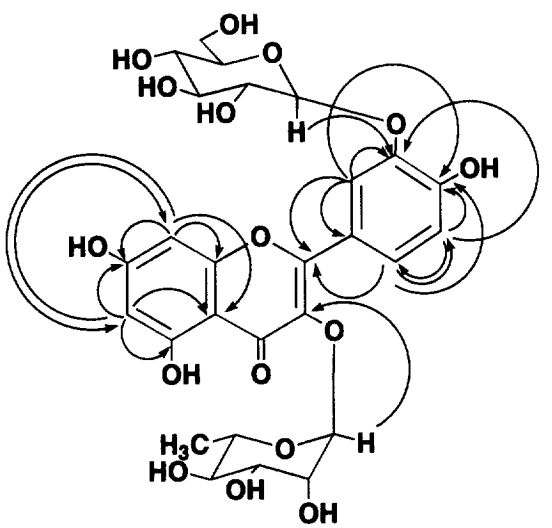

Fig. 1. The HMBC correlations of **2**. The arrowheads indicate carbon atoms and the tails indicate protons.

features of a flavonol glycoside and the NMR spectra indicated that **4** was a derivative of myricitrin with an extra glucose unit. Spectral shift data indicated that the glucose was located at the 3′-hydroxyl, as in **2**. This was confirmed on observation of a cross peak between δ_H 4.86 (H-1‴) and δ_C 147.3 (C-3′) in the HMBC spectrum. Carbon signals of C-3′ and 5′ (δ_C 147.3) accidentally appeared in the ^{13}C spectrum at the same positions in CD_3OD, since in DMSO-d_6, these carbons resonated at different frequencies (δ_C 145.56 and 145.81).

Myricetin 3,4′-dirhamnoside (**5**), $[\alpha]_D - 154.9°$, was isolated as a yellow amorphous powder whose elemental composition was determined to be $C_{27}H_{30}O_{16}$. The NMR data showed that this compound was a derivative of myricitrin with an extra sugar, α-rhamnopyranose, on the B ring. The UV spectral shift data indicated that the hydroxy group on the 4′-position was substituted by the sugar. The NMR spectra also supported that the B-ring have a symmetric substitution and thus **5** is the 4′-*O*-α-rhamnopyranoside of myricitrin.

EXPERIMENTAL

General. Mp. uncorr.; ^1H and ^{13}C NMR: 400 MHz and 100 MHz, respectively, and TMS as an int. standard.

Plant material. Leaves of *Myrsine seguinii* were collected in Okinawa Prefecture in 1992 and the plant

was identified by one of the authors (A. T.). A voucher specimen was deposited in the Herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine (MS-92-Okinawa).

Extraction and isolation. Dried leaves (5.95 kg) were extracted with MeOH (40 l × 3) and then the MeOH extract was concd to 2 l. The concd MeOH extract was washed with *n*-hexane (1 l × 2, *n*-hexane soluble fr., 61.1 g), and the MeOH layer was concd to yield a viscous gummy material. The latter was suspended in H_2O (3 l), and then extracted with EtOAc (3 l) and *n*-BuOH (3 l) successively, to give EtOAc- and *n*-BuOH-soluble frs (195.0 g and 200.0 g, respectively). The remaining H_2O layer was concd to furnish a H_2O-soluble fr. (380 g).

A portion (50.0 g) of the *n*-BuOH soluble fr. was sepd first by CC on a highly porous synthetic resin, Diaion HP-20 (Mitsubishi Kasei, Tokyo), with MeOH–H_2O [(1:4, 3.5 l), (2:3, 3 l), (3:2, 3 l) and (4:1, 3 l), and MeOH (3 l)], 500 ml frs being collected. The residue (14.8 g in frs 10–16) of the 40% MeOH eluate was sepd by silica gel (450 g) CC with $CHCl_3$ (3 l) and $CHCl_3$–MeOH [(99:1, 6 l), (97:3, 6 l), (19:1, 6 l), (37:3, 6 l), (9:1, 6 l), (17:3, 6 l), (4:1, 6 l), (3:1, 6 l), and (7:3, 6 l)], 500 ml frs being collected. The residue (1.18 g in frs 70–80) of the 15% MeOH eluate was subjected to reversed-phase silica gel [ODS: Cosmosil, 75C_{18}-OPN; Nakarai Tesque Co., Ltd., Kyoto], CC (RPCC) with a linear gradient solvent system of H_2O–MeOH (9:1, 1.5 l) → (3:7, 1.5 l), 10 g frs being collected. The residue (99 mg) of frs 148–155 was finally purified by DCCC [500 columns (2 mm id × 40 cm), $CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2), ascending method]. Frs were numbered according to elution with the mobile phase, 5 g frs being collected. Compound **2** was obtained in frs 34–43 as yellow crystals (20 mg). From the residue (89 mg in frs 166–180) on RPCC, compound **1** was isolated by DCCC in frs 51–58 as yellow crystals (41 mg).

Compounds **3**, **4** and **5** were isolated from the residue (805 mg) of the 20% MeOH eluate on silica gel CC in a similar manner in the following amounts, 77 mg, 146 mg and 31 mg, respectively.

Known compounds isolated. Quercitrin (**1**), yellow crystals (MeOH–H_2O), mp 179–182° [4], $[\alpha]_D^{21}$ −156.7° (MeOH, *c* 0.67), ^{13}C NMR (CD_3OD): see Table 1. Myricitrin (**3**), yellow crystals (MeOH–H_2O), mp 197–201° [4], $[\alpha]_D^{21}$ −152.8° (MeOH, *c* 0.89), ^{13}C NMR (CD_3OD): see Table 1. Identification of compounds **1** and **3** was performed by comparison of the ^{13}C NMR spectral data in DMSO-d_6 with reported values [2].

Quercetin 3-rhamnoside-3′-glucoside (**2**). Yellow crystals (MeOH), mp 195–197°, $[\alpha]_D^{21}$ −135.5° (pyridine, *c* 0.43), IR υ_{max}^{KBr} cm^{-1}: 3300, 1655, 1600, 1505, 1355, 1285, 1195, 1060; UV λ_{max}^{MeOH} nm (log ε): 251 sh (4.18), 267 (4.26), 340 (4.11), $\lambda_{max}^{MeOH+AcONa}$ nm (log ε): 270 (4.30), 305 (3.96), 376 (4.12), $\lambda_{max}^{MeOH+CH_3ONa}$ nm (log ε): 272 (4.35), 328 (4.05), 392 (4.33), $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 274 (4.35), 303 (4.04), 349 (4.16), 400 (4.15), $\lambda_{max}^{MeOH+AcONa+H_3BO_3}$ nm (log ε): 266 (4.28), 341 (4.15), $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 274 (4.10), 300 sh (4.00), 347 (4.16), 397 (4.16); ^1H NMR (DMSO-d_6): δ 0.79 (3H, *d*, *J* = 6 Hz, H_3-6″), 4.76 (1H, *d*, *J* = 7 Hz, H-1‴), 5.33 (1H, *d*, *J* = 2 Hz, H-1″), 6.21 (1H, *d*, *J* = 2 Hz, H-6), 6.47 (1H, *d*, *J* = 2 Hz, H-8), 6.95 (1H, *d*, *J* = 8 Hz, H-5′), 7.51 (1H, *dd*, *J* = 2 and 8 Hz, H-6′), 7.64 (1H, *d*, *J* = 2 Hz, H-2′), 9.39, 10.87 (each 1H, each *br s*, OH-7 and −4′), 12.60 (1H, *s*, *ex.* with D_2O, OH-5); ^{13}C NMR (DMSO-d_6): see Table 1; HR-FAB-MS (negative centroid) *m/z* (rel. int.): 609.1435 (100) $[M-H]^-$ ($C_{27}H_{29}O_{16}$ requires 609.1456), 463.0896 (41) [M-rhamnose]$^-$ ($C_{21}H_{19}O_{12}$ requires 463.0876), 447.0908 (53) [M-glucose]$^-$ ($C_{21}H_{19}O_{11}$ requires 447.0927).

Myricetin 3-rhamnoside-3′-glucoside (**4**). Yellow amorphous powder, $[\alpha]_D^{21}$ −152.1° (MeOH, *c* 1.21), IR υ_{max}^{KBr} cm^{-1}: 3300, 1650, 1600, 1500, 1355, 1200, 1090 ~ 1000; UV λ_{max}^{MeOH} nm (log ε): 214 (4.36), 255 (4.29), 260 sh (4.28), 308 sh (4.03), 347 (4.22), $\lambda_{max}^{MeOH+AcONa}$ nm (log ε): 217 (4.40), 267 (4.32), 375 (4.14), $\lambda_{max}^{MeOH+CH_3ONa}$ nm (log ε): 219 (4.43), 267 (4.40), 328 (4.04), 401 (4.32), $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 217 (4.41), 272 (4.42), 306 (3.92), 429 (4.33), $\lambda_{max}^{MeOH+AcONa+H_3BO_3}$ nm (log ε): 259 (4.33), 304 (3.82), 369 (4.24), $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 274 (4.33), 305 (3.96), 359 (4.12), 401 (4.15); ^1H NMR (CD_3OD): δ 0.94 (3H, *d*, *J* = 6 Hz, H_3-6‴), 3.82 (1H, *dd*, *J* = 4 and 12 Hz, H-6‴a), 3.94 (1H, *br d*, *J* = 12 Hz, H-6‴b), 4.23 (1H, *dd*, *J* = 2 and 3 Hz, H-2″), 4.86 (1H, *d*, *J* = 8 Hz, H-1‴), 5.44 (1H, *d*, *J* = 2 Hz, H-1″), 6.20 (1H, *d*, *J* = 2 Hz, H-6), 6.40 (1H, *d*, *J* = 2 Hz, H-8), 7.15 (1H, *d*, *J* = 2 Hz, H-2′), 7.35 (1H, *d*, *J* = 2 Hz, H-6′); ^{13}C NMR (CD_3OD): see Table 1; HR-FAB-MS (negative centroid) *m/z* (rel. int.): 625.1376 (100) $[M-H]^-$ ($C_{27}H_{29}O_{17}$ requires 625.1405), 479.0819 (41) [M-rhamnose]$^-$ ($C_{21}H_{19}O_{13}$ requires 479.0826), 463.0855 (38) [M-glucose]$^-$ ($C_{21}H_{19}O_{12}$ requires 463.0876), 301 (59) [M-rhamnose-glucose]$^-$.

Myricetin 3,4′-dirhamnoside (**5**). Yellow amorphous powder, $[\alpha]_D^{21}$ −154.9° (MeOH, *c* 2.04), IR υ_{max}^{KBr} cm^{-1}: 3300, 1650, 1600, 1495, 1360, 1290, 1195, 1055, 950; UV λ_{max}^{MeOH} nm (log ε): 214 (4.25), 263 (4.17), 346 (3.98), $\lambda_{max}^{MeOH+AcONa}$ nm (log ε): 217 (4.29), 269 (4.24), 305 (3.95), 347 (3.96), $\lambda_{max}^{MeOH+CH_3ONa}$ nm (log ε): 221 (4.33), 267 (4.30), 328 sh (3.93), 372 (4.07), $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 217 (4.30), 273 (4.24), 304 (3.91), 347 (4.00), 396 (3.99), $\lambda_{max}^{MeOH+AcONa+H_3BO_3}$ nm (log ε): 265 (4.24), 305 sh (4.05), 346 (4.08), $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 274 (4.30), 305 (4.01), 346 (4.10), 394 (4.02); ^1H NMR (CD_3OD): δ 0.97 (3H, *d*, *J* = 6 Hz, H_3-6″), 1.25 (3H, *d*, *J* = 6 Hz, H_3-6‴), 3.77 (1H, *dd*, *J* = 3 and 10 Hz, H-3″), 3.48 (1H, *t*, *J* = 10 Hz, H-4‴), 4.00 (1H, *dd*, *J* = 3 and 10 Hz, H-3‴), 4.24, 4.25 (each 1H, each *dd*, *J* = 2 and 3 Hz, H-2″ and 2‴), 4.35 (1H, *qd*, *J* = 6 and 10 Hz, H-5‴), 5.30 (1H, *d*, *J* = 2 Hz, H-1‴), 5.55 (1H, *d*, *J* = 2 Hz, H-1″), 6.21 (1H, *d*, *J* = 2 Hz, H-6), 6.37 (1H, *d*, *J* = 2 Hz, H-8), 6.93 (2H, *s*, H-2′ and 6′); ^{13}C NMR (CD_3OD): see Table 1; NR-FAB-MS (negative centroid) *m/z* (rel. int.): 609.1461 (74) $[M-H]^-$

($C_{27}H_{29}O_{16}$ requires 609.1455), 463.0877 (100) [M-rhamnose]$^-$ ($C_{21}H_{19}O_{12}$ requires 463.0876).

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