Flavonoid Glycosides from *Rhazya orientalis*

Atsuko Itoh,[†] Tomoko Kumashiro,[†] Takao Tanahashi,*[,†] Naotaka Nagakura,[†] and Toyoyuki Nishi[‡]

Kobe Pharmaceutical University, Higashinada-ku, Kobe 658-8558, Japan, and The Nippon Shinyaku Institute for Botanical Research, Yamashina-ku, Kyoto 607-8182, Japan

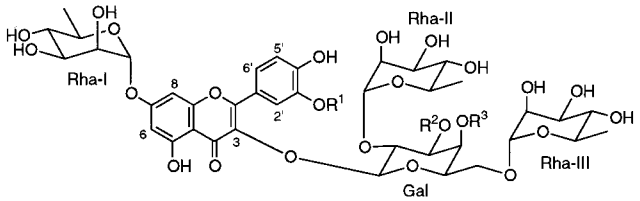
Received October 19, 2001

Six new flavonoid glycosides, quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**1**), quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**2**), isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**3**), isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-[α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**4**), isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*cis*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**5**), and isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-feruloyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**6**), were isolated from the dried aerial parts of *Rhazya orientalis*. The structures of **1**−**6** were determined by spectroscopic and chemical means.

The genus *Rhazya* (Apocynaceae) comprises only two species, *Rhazya orientalis* (Decne.) A. DC. (=*Amsonia orientalis* Decne.), indigenous to Greece and Turkey, and *R. stricta* Decne., which is widely distributed in Western Asia and abundantly found in Pakistan and has long been used in the indigenous system of medicine for treatment of various diseases.[1,2] Both plants are known to be rich sources of indole alkaloids.[1] On the other hand, no detailed study on glycosidic constituents of *R. orientalis* has been reported. We have examined the constituents of the glycosidic fraction and have isolated six new flavonoid glycosides. The structure elucidation of these new compounds is reported here.

Results and Discussion

The dried aerial parts of *R. orientalis* were extracted with hot MeOH. The extract was suspended in H_2O and successively partitioned with $CHCl_3$ and *n*-BuOH. The *n*-BuOH fraction was separated by a combination of chromatographic procedures to afford six new compounds, **1**−**6**, along with 11 known glycosides, (5*S*)-5-carboxystrictosidine,[3] strictosamide,[4] loganic acid,[5] secologanin,[5] sweroside,[5] 1-*O*-sinapoyl-*β*-D-glucose,[6] kaempferol 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside,[7] quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside,[7] quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-glucopyranoside (rutin),[7] isorhamnetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside,[7] and isorhamnetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-glucopyranoside.[7] The latter 10 glycosides were isolated for the first time from this plant species. The structures of six new glycosides, **1**−**6**, were determined as follows.



1: R^1=R^2=H, R^3=*trans*-*p*-coumaroyl
2: R^1=R^3=H, R^2=*trans*-*p*-coumaroyl
3: R^1=Me, R^2=H, R^3=*trans*-*p*-coumaroyl
4: R^1=Me, R^2=*trans*-*p*-coumaroyl, R^3=H
5: R^1=Me, R^2=H, R^3=*cis*-*p*-coumaroyl
6: R^1=Me, R^2=H, R^3=*trans*-feruloyl
7: R^1=R^2=R^3=H

Compound **1** was obtained as a yellow amorphous powder, $[\alpha]_D$ −193°. Its HR-SI mass spectrum showed a pseudomolecular ion $[M - H]^-$ at *m*/*z* 1047.2951, indicating a molecular formula $C_{48}H_{56}O_{26}$. It showed UV maxima at 227sh, 256.5, 270sh, 299sh, 315.5, and 364sh nm and IR bands at 3394, 1655, 1516, and 1495 cm^{-1}. Its ^1H NMR spectrum exhibited a pair of *meta*-coupled aromatic protons at *δ* 6.45 (d, *J* = 2.0 Hz) and 6.79 (d, *J* = 2.0 Hz) and an AMX spin system at *δ* 6.92 (d, *J* = 8.5 Hz), 7.64 (d, *J* = 2.0 Hz), and 7.75 (dd, *J* = 8.5 and 2.0 Hz). These spectral data indicated that **1** possessed a quercetin moiety as a basic skeleton. The ^1H and ^{13}C NMR spectra showed, besides the signals due to a quercetin moiety, the signals assignable to three α-rhamnopyranosyl units, a *β*-galactopyranosyl unit, and a *trans*-*p*-coumaroyl unit (Tables 1 and 2). This suggestion was further supported by chemical means. An alkaline methanolysis of **1** afforded a flavonoid glycoside, **7**, and methyl *trans*-*p*-coumarate, and subsequent acid hydrolysis of **7** liberated D-galactose and L-rhamnose, which were identified by GLC analysis of their thiazolidine derivatives.[8] Detailed NMR studies determined the glycosidic linkage in **7**. When ^{13}C NMR data of **7** were compared with those of quercetin, the effects of glycosylation of the 7-OH, i.e., the upfield shift of C-7 and the downfield shifts of C-6, 8, 10, and the effect of glycosylation of 3-OH, i.e., the downfield shift of C-2, were observed.[9] An attachment of one rhamnosyl group at C-7 of the quercetin moiety was shown by the anomeric proton signal at *δ* 5.55.[10] Its HMBC spectrum showed correlations between H-1 of rhamnose-I [*δ* 5.55 (d, *J* = 1.5 Hz)] and C-7 (*δ* 163.5); H-1 of rhamnose-II [*δ* 5.22 (d, *J* = 1.5 Hz)] and C-2 of galactose (*δ* 77.5); and H-1 of rhamnose-III [*δ* 4.54 (d, *J* = 1.5 Hz) and C-6 of galactose (*δ* 67.2), indicating the attachment of a rhamnosyl group at C-7 and a α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-*β*-D-galactopyranosyl group at C-3. Acy-

* To whom correspondence should be addressed. Fax: +81-78-441-7546. E-mail: tanahash@kobepharma-u.ac.jp.
[†] Kobe Pharmaceutical University.
[‡] The Nippon Shinyaku Institute for Botanical Research.

10.1021/np010518w CCC: $22.00 © 2002 American Chemical Society and American Society of Pharmacognosy
Published on Web 02/19/2002

Table 1. ^{1}H NMR Spectral Data of **1**−**7** at 500 MHz

H	**1**[a]			**1**[b]			**2**[b]			**3**[b]		
flavonol												
6	6.45	d	(2.0)	6.46	d	(2.0)	6.45	d	(2.0)	6.45	d	(2.0)
8	6.79	d	(2.0)	6.73	d	(2.0)	6.71	d	(2.0)	6.71	d	(2.0)
2′	7.64	d	(2.0)	7.74	m		7.76	d	(2.0)	8.08	d	(2.0)
5′	6.92	d	(8.5)	6.93	d	(8.5)	6.91	d	(8.5)	6.95	d	(8.5)
6′	7.75	dd	(8.5, 2.0)	7.74	dd	(8.5, 2.0)	7.62	dd	(8.5, 2.0)	7.64	dd	(8.5, 2.0)
OMe-3′										4.03	s	
galactose												
1	5.71	d	(7.5)	5.73	d	(7.0)	5.91	d	(8.0)	5.84	d	(7.5)
2	3.84	dd	(9.0, 7.5)	3.96−4.03	m		4.29	dd	(10.0, 8.0)	3.99	dd	(9.5, 7.5)
3	3.95	brd	(9.0)	3.96−4.03	m		5.11	dd	(10.0, 3.0)	4.00−4.05	m	
4	5.22	brd	(3.0)	5.36	brd	(3.0)	4.13	brd	(3.0)	5.40	dd	(3.0, 0.5)
5	3.90	brt	(6.5)	3.87	brt	(6.5)	3.87	brt	(6.5)	3.94	brt	(6.5)
6	3.15	dd	(10.0, 6.5)	3.25	dd	(10.5, 6.5)	3.47	dd	(10.0, 6.0)	3.31	dd	(10.0, 6.5)
6	3.33	dd	(10.0, 6.5)	3.49	dd	(10.5, 6.5)	3.78	dd	(10.0, 6.5)	3.53	dd	(10.0, 6.5)
rhamnose-I												
1	5.57	brs		5.56	brs		5.56	brs		5.57	d	(1.0)
2	3.87	brs		3.96−4.03	m		4.03	brs		4.00−4.05	m	
3	3.66	dd	(9.5, 3.0)	3.83[c]	dd	(9.5, 3.5)	3.83	dd	(9.5, 3.5)	3.84[g]	dd	(9.5, 3.5)
4	3.32	t	(9.5)	3.48[d]	t	(9.5)	3.48	t	(9.5)	3.49[h]	t	(9.5)
5	3.45	dq	(9.5, 6.0)	3.61[e]	dq	(9.5, 6.0)	3.61	dq	(9.5, 6.0)	3.62[i]	dq	(9.5, 6.0)
6	1.14	d	(6.0)	1.26[f]	d	(6.0)	1.26	d	(6.0)	1.27[j]	d	(6.0)
rhamnose-II												
1	5.04	brs		5.21	d	(1.5)	5.07	brs		5.16	d	(1.0)
2	3.76	dd	(3.0, 1.5)	3.96−4.03	m		3.83	brs		4.02	dd	(3.5, 1.0)
3	3.52	dd	(9.5, 3.0)	3.81[c]	dd	(9.5, 3.5)	3.74	dd	(9.5, 3.5)	3.81[g]	dd	(9.5, 3.5)
4	3.16	t	(9.5)	3.37[d]	t	(9.5)	3.32	t	(9.5)	3.36[h]	t	(9.5)
5	3.82	dq	(9.5, 6.0)	4.15[e]	dq	(9.5, 6.0)	4.04	dq	(9.5, 6.0)	4.12[i]	dq	(9.5, 6.0)
6	0.85	d	(6.0)	1.05[f]	d	(6.0)	0.95	d	(6.0)	1.00[j]	d	(6.0)
rhamnose-III												
1	4.37	brs		4.46	d	(1.5)	4.57	d	(1.5)	4.49	d	(1.5)
2	3.38	dd	(3.0, 1.5)	3.56	dd	(3.5, 1.5)	3.63	dd	(3.5, 1.5)	3.58	dd	(3.5, 1.5)
3	3.29	dd	(9.5, 3.5)	3.50	dd	(9.5, 3.5)	3.52	dd	(9.5, 3.5)	3.50	dd	(9.5, 3.5)
4	3.06	t	(9.5)	3.21	t	(9.5)	3.28	t	(9.5)	3.22	t	(9.5)
5	3.21	dq	(9.5, 6.0)	3.36	dq	(9.5, 6.0)	3.53	dq	(9.5, 6.0)	3.37	dq	(9.5, 6.0)
6	0.92	d	(6.0)	1.04	d	(6.0)	1.18	d	(6.0)	1.04	d	(6.0)
acyl												
2	7.55	d	(8.5)	7.52	d	(8.5)	7.50	d	(8.5)	7.51	d	(8.5)
3	6.85	d	(8.5)	6.66	d	(8.5)	6.81	d	(8.5)	6.86	d	(8.5)
5	6.85	d	(8.5)	6.66	d	(8.5)	6.81	d	(8.5)	6.86	d	(8.5)
6	7.55	d	(8.5)	7.52	d	(8.5)	7.50	d	(8.5)	7.51	d	(8.5)
α	6.41	d	(16.0)	6.43	d	(16.0)	6.46	d	(16.0)	6.35	d	(16.0)
β	7.54	d	(16.0)	7.64	d	(16.0)	7.72	d	(16.0)	7.65	d	(16.0)
OMe-3												

H	**4**[b]			**5**[b]			**6**[b]			**7**[b]		
flavonol												
6	6.46	d	(2.0)	6.45	brs		6.47	d	(2.0)	6.46	d	(2.0)
8	6.74	d	(2.0)	6.72	brs		6.75	d	(2.0)	6.72	d	(2.0)
2′	8.09	d	(2.0)	8.03	d	(2.0)	8.06	d	(2.0)	7.72	d	(2.0)
5′	6.94	d	(8.5)	6.92	d	(8.5)	6.96	d	(8.5)	6.88	d	(8.5)
6′	7.58	dd	(8.5, 2.0)	7.60	brd	(8.5)	7.67	dd	(8.5, 2.0)	7.60	dd	(8.5, 2.0)
OMe-3′	4.03	s		4.00	s		4.04	s				
galactose												
1	6.00	d	(7.5)	5.82	d	(7.5)	5.81	d	(7.5)	5.69	d	(8.0)
2	4.28	dd	(10.0, 7.5)	3.95	dd	(9.5, 7.5)	4.00	m		3.96	dd	(9.5, 8.0)
3	5.11	dd	(10.0, 3.0)	3.99−4.03	m		4.00	m		3.72	dd	(9.5, 3.5)
4	4.09	dd	(3.0, 1.0)	5.34	brd	(3.5)	5.38	brd	(2.0)	3.81	d	(3.5)
5	3.86	td	(6.5, 1.0)	3.91	brt	(6.0)	3.91	brt	(6.5)	3.67	brt	(6.5)
6	3.50	dd	(10.0, 6.5)	3.32	dd	(10.5, 6.5)	3.29	dd	(10.5, 6.5)	3.48	dd	(10.5, 6.5)
6	3.78	dd	(10.0, 6.5)	3.55	dd	(10.5, 5.5)	3.52	dd	(10.5, 6.5)	3.75	dd	(10.5, 6.0)
rhamnose-I												
1	5.56	d	(1.5)	5.56	brs		5.57	d	(1.5)	5.55	d	(1.5)
2	4.02	dd	(3.5, 1.5)	3.99−4.03	m		4.02	dd	(3.5, 1.5)	4.00	dd	(3.5, 1.5)
3	3.83	dd	(9.5, 3.5)	3.83	dd	(9.5, 3.5)	3.83[k]	dd	(9.5, 3.5)	3.83	dd	(9.5, 3.5)
4	3.48	t	(9.5)	3.48	t	(9.5)	3.48[l]	t	(9.5)	3.48	t	(9.5)
5	3.61	dq	(9.5, 6.0)	3.61	dq	(9.5, 6.0)	3.61[m]	dq	(9.5, 6.5)	3.61	dq	(9.5, 6.0)
6	1.26	d	(6.0)	1.26	d	(6.0)	1.26[n]	d	(6.5)	1.26	d	(6.0)
rhamnose-II												
1	5.01	d	(1.5)	5.17	d	(1.5)	5.16	d	(1.5)	5.22	d	(1.5)
2	3.80	dd	(3.5, 1.5)	3.99−4.03	m		4.00	m		4.01	dd	(3.5, 1.5)
3	3.71	dd	(9.5, 3.5)	3.79	dd	(9.5, 3.5)	3.79[k]	dd	(9.5, 3.5)	3.78	dd	(9.5, 3.5)
4	3.29	t	(9.5)	3.34	t	(9.5)	3.34[l]	t	(9.5)	3.33	t	(9.5)
5	4.02	dq	(9.5, 6.0)	4.08	dq	(9.5, 6.0)	4.10[m]	dq	(9.5, 6.5)	4.03	dq	(9.5, 6.0)
6	0.88	d	(6.0)	0.94	d	(6.0)	0.97[n]	d	(6.5)	0.95	d	(6.0)

Table 1 (Continued)

H	**4**[b]			**5**[b]			**6**[b]			**7**[b]		
rhamnose-III												
1	4.57	d	(1.5)	4.49	d	(1.5)	4.49	d	(1.5)	4.54	d	(1.5)
2	3.61	dd	(3.5, 1.5)	3.55	dd	(3.0, 1.5)	3.56	dd	(3.5, 1.5)	3.55	dd	(3.5, 1.5)
3	3.51	dd	(9.5, 3.5)	3.47	dd	(9.5, 3.5)	3.50	dd	(9.5, 3.5)	3.49	dd	(9.5, 3.5)
4	3.27	t	(9.5)	3.21	t	(9.5)	3.21	t	(9.5)	3.26	t	(9.5)
5	3.54	dq	(9.5, 6.0)	3.39	dq	(9.5, 6.0)	3.37	dq	(9.5, 6.5)	3.53	dq	(9.5, 6.0)
6	1.18	d	(6.0)	1.08	d	(6.0)	1.05	d	(6.5)	1.18	d	(6.0)
acyl												
2	7.51	d	(8.5)	7.71	d	(9.0)	7.28	d	(2.0)			
3	6.81	d	(8.5)	6.75	d	(9.0)						
5	6.81	d	(8.5)	6.75	d	(9.0)	6.85	d	(8.5)			
6	7.51	d	(8.5)	7.71	d	(9.0)	7.10	dd	(8.5, 2.0)			
α	6.45	d	(16.0)	5.81	d	(13.0)	6.40	d	(16.0)			
β	7.72	d	(16.0)	6.95	d	(13.0)	7.65	d	(16.0)			
OMe-3							3.96	s				

[a] Measured in DMSO-d_6. [b] Measured in CD_3OD. [c−n] Values with the same superscript are interchangeable.

lation of the hydroxyl group at C-4 of the galactose moiety in **1** was determined by comparison of the ^{13}C NMR data of **1** with those of **7** and by HMBC correlation between galactose H-4 [*δ* 5.22 (br d, *J* = 3.0 Hz)] and the *trans*-*p*-coumaroyl carbonyl carbon (*δ* 165.9). The linkages of sugar and acyl moieties were further supported by the ROESY spectrum of **1**. Thus, the structure of **1** was established as quercetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside.

Compound **2** was assigned the same molecular formula as **1** by its HR-SIMS. Its ^1H and ^{13}C NMR spectral features were closely similar to those of **1**. But there were remarkable differences between **1** and **2** in the chemical shifts of the proton and carbon signals due to a galactose moiety. Whereas a downfield-shifted proton at *δ* 5.36 (br d, *J* = 3.0 Hz) was assigned to H-4 of galactose in **1**, a downfield shifted proton signal at *δ* 5.11 (dd, *J* = 10.0 and 3.0 Hz) was assigned to H-3 of galactose in **2** from 2D NMR, suggesting that the acyl group was linked to the hydroxyl group at C-3 of galactose in **2** instead of that at C-4 of galactose in **1**. Further evidence for acylation of the hydroxyl group at C-3 of the galactose moiety was provided by the HMBC correlation between H-3 of the galactose moiety and a carbonyl carbon signal (*δ* 168.3), which was correlated with a pair of doublets (*J* = 16.0 Hz) at *δ* 6.46 and 7.72. Thus, the structure of **2** was determined to be quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside.

The HR-SIMS of **3** and **4** showed the same molecular formula, $C_{49}H_{58}O_{26}$. The spectral features of **3** and **4** were similar to those of **1** and **2**, respectively, except for the presence of an additional methoxyl signal in their ^1H and ^{13}C NMR spectra and the chemical shifts of the signals arising from the B-ring in their aglycone portions. These results suggested that the linkages of sugar and acyl units of **3** and **4** were the same as in **1** and **2**. The methoxyl signal (**3** and **4**, *δ* 4.03) was correlated to H-2′ of the aglycone moiety [**3**, *δ* 8.08 (d, *J* = 2.0 Hz); **4**, *δ* 8.09 (d, *J* = 2.0 Hz)] in each ROESY spectrum, indicating a methoxyl group at C-3′. Accordingly, compounds **3** and **4** were deduced to be isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside and isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside, respectively.

Compound **5**, $C_{49}H_{58}O_{26}$, and compound **6**, $C_{50}H_{60}O_{27}$, were also obtained as yellow powders. The spectral features of **5** and **6** were quite similar to those of **3**. The differences could be ascribed to their acyl units. The ^1H NMR spectrum of **5** exhibited *cis*-olefinic proton signals at *δ* 6.95 and 5.81 (each d, *J* = 13.0 Hz) instead of *trans*-olefinic proton signals in **3**, suggesting a *cis*-*p*-coumaroyl unit in **5**. The ^1H and ^{13}C NMR spectra of **6** exhibited an additional methoxyl signal at *δ* 3.96 and an AMX spin system at *δ* 7.28 (d, *J* = 2.0 Hz), 7.10 (dd, *J* = 8.5, 2.0 Hz), and 6.85 (d, *J* = 8.5 Hz) instead of an AA′BB′ spin system in **1**−**5**. In the ROESY spectrum the methoxyl signal at *δ* 3.96 was correlated to a doublet at *δ* 7.28, which was correlated to an olefinic proton at *δ* 7.65 (d, *J* = 16.0 Hz), suggesting a *trans*-feruloyl group in **6**. Thus, the structures of **5** and **6** were determined to be isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*cis*-*p*-coumaroyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside and isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl-(1→2)]-(4-*O*-*trans*-feruloyl)-*β*-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside, respectively.

Although the isolation of flavonoid glycosides, robonin, isorhamnetin 3-(6-α-L-rhamnopyranosyl-*β*-D-galactopyranoside)-7-α-L-rhamnopyranoside, and isorhamnetin 3-(2,6-di-α-L-rhamnopyranosyl-*β*-D-galactopyranoside)-7-α-L-rhamnopyranoside, was reported from *R. stricta*,[11] this is the first isolation of flavonoid glycosides from *R. orientalis*.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra on a Shimadzu FTIR-8200 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. ^1H (500 and 200 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on Varian VXR-500 and Varian Gemini-200 spectrometers with TMS as an internal standard. MS and HRMS were obtained with a Hitachi M-4100 mass spectrometer. Glycerol was used for SIMS and HR-SIMS as the matrix. MPLC was carried out with Wakogel LP-40 C18. TLC was performed on precoated Kieselgel $60F_{254}$ plates (Merck).

Plant Material. The aerial parts of *Rhazya orientalis* were collected at Kyoto Botanical Garden, Kyoto, Japan. A voucher specimen (KPU-012) is deposited in the laboratory of Kobe Pharmaceutical University.

Extraction and Isolation. Dried aerial parts (61.8 g) of *R. orientalis* were extracted with hot MeOH, the extracts were concentrated in vacuo, and the resulting residue (19.0 g) was resuspended in H_2O and extracted successively with $CHCl_3$ and *n*-BuOH. The residue (3.1 g) from the *n*-BuOH layers was fractionated on reversed-phase MPLC. Elution with H_2O−MeOH mixtures of the indicated MeOH content gave 11 fractions, 1 (20%, 151 mg), 2 (20%, 135 mg), 3 (20%, 60.8 mg), 4 (25%, 74.2 mg), 5 (25%, 150 mg), 6 (25%, 238 mg), 7 (30%,

Table 2. ^{13}C NMR Spectral Data of **1**−**7** at 125 MHz.

C	**1**[a]	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[b]
flavonol								
2	156.9	159.5	159.0	159.1	159.0	159.3	159.3	159.1
3	132.8	134.6	134.7	134.6	134.5	134.5	134.6	134.9
4	177.2	179.4	179.3	179.3	179.3	179.3	179.3	179.5
5	160.8	162.9	163.0	162.9	163.0	163.0	163.0	163.0
6	99.3	100.4	100.4	100.4	100.4	100.5	100.5	100.5
7	161.5	163.4	163.4	163.4	163.5	163.5	163.5	163.5
8	94.3	95.6	95.6	95.7	95.6	95.7	95.7	95.6
9	155.8	157.9	157.9	157.9	158.0	158.0	158.0	158.0
10	105.5	107.5	107.6	107.6	107.6	107.6	107.7	107.6
1′	120.9	123.4	123.0	123.2	123.0	123.1	123.2	123.1
2′	116.2	117.5	117.5	114.8	114.8[o]	114.7	115.1	117.5
3′	144.9	146.1	145.9	148.4	148.5	148.4	148.6	146.0
4′	148.7	149.9	150.0	151.0	150.9	151.1	151.2	150.0
5′	115.0	116.0	116.3	116.2	116.0	116.1	116.2	116.3
6′	121.8	123.5	123.2	123.9	123.5	123.8	124.0	123.3
OMe-3′				57.3	57.2	57.2	57.3	
galactose								
1	98.6	100.6	100.9	100.9	100.6	100.9	100.8	101.1
2	75.2	78.1	75.5	78.2	75.9	77.9	78.2	77.5
3	71.7[c]	74.0[e]	77.8	73.7[k]	77.7	74.0[r]	74.0[t]	75.8
4	70.5[d]	72.4[f]	68.0	72.4[l]	67.7	72.4[s]	72.4[u]	70.9
5	71.5[c]	74.1[e]	74.9	74.0[k]	75.1	74.2[r]	74.0[t]	75.4
6	64.4	66.8	66.5	66.9	66.5	67.2	67.0	67.2
rhamnose-I								
1	98.4	99.9	100.0	99.9	99.9	99.9	99.9	100.0
2	70.2[d]	72.1[f]	72.0[i]	72.1[l]	72.0[p]	72.1[s]	72.1[u]	72.2[x]
3	70.5[d]	72.3[f]	72.5[i]	72.4[l]	72.5[p]	72.4[s]	72.4[u]	72.5[x]
4	71.7[c]	73.8[e]	73.9[j]	73.9[k]	73.8[q]	74.0[r]	74.0[t]	73.7
5	69.8[d]	71.3[g]	70.2	71.3[m]	70.1	71.3	71.3[v]	71.3
6	17.8	18.1[h]	18.1	18.2[n]	18.1	18.2	18.0[w]	18.1
rhamnose-II								
1	100.7	102.8	102.8	103.0	102.9	102.9	103.0	102.7
2	70.0[d]	72.0[f]	71.8[i]	72.2[l]	71.7[p]	72.1[s]	72.1[u]	72.1[x]
3	70.3[d]	72.1[f]	72.2[i]	72.1[l]	72.3[p]	72.2[s]	72.2[u]	72.4[x]
4	71.5[c]	73.7[e]	73.9[j]	73.9[k]	73.8[q]	73.8[r]	73.8[t]	74.1
5	68.3	70.1[g]	69.7	70.0[m]	69.7	70.0	70.0[v]	69.9
6	17.2	17.6[h]	17.5	17.6[n]	17.4	17.5	17.6[w]	17.5
rhamnose-III								
1	100.2	102.1	101.8	102.2	101.8	102.2	102.3	101.9
2	70.2[d]	71.7[f]	71.3[i]	71.7[l]	71.3[p]	71.8[s]	71.8[u]	71.8[x]
3	70.2[d]	72.1[f]	72.1[i]	72.0[l]	72.2[p]	72.1[s]	72.2[u]	72.3[x]
4	71.6[c]	73.6[e]	73.6[j]	73.8[k]	73.6[q]	73.7[r]	73.7[t]	74.0
5	68.3	69.9	69.7	69.9[m]	69.7	70.0	70.0[v]	69.8
6	17.7	17.9	18.0	18.0	18.0	17.9	18.1	18.0
acyl								
1	125.0	127.2	127.1	127.2	127.1	127.5	127.7	
2	130.2	131.5	131.5	131.4	131.4	134.0	111.6	
3	115.8	116.9	116.9	117.0	116.8	116.0	149.5	
4	159.7	161.3	161.5	161.4	161.5	160.3	150.9	
5	115.8	116.9	116.9	117.0	116.8	116.0	116.6	
6	130.2	131.5	131.5	131.4	131.4	134.0	124.6	
α	114.0	115.0	114.8	114.7	114.6[o]	115.8	115.1	
β	144.9	147.3	147.6	147.6	147.5	145.8	147.8	
CO	165.9	168.8	168.3	168.7	168.3	167.7	168.7	
OMe-3							56.7	

[a] Measured in DMSO-d_6. [b] Measured in CD_3OD. [c−x] Values with the same superscript are interchangeable.

121 mg), 8 (30%, 339 mg), 9 (40%, 49.2 mg), 10 (40%, 149 mg), and 11 (50%, 96.2 mg). Fraction 1 was purified by preparative HPLC (*μ*Bondasphere 5 *μ* C18-100 Å, MeOH−H_2O, 9:11) to afford loganic acid (36.9 mg). Fraction 2 was recrystalized from MeOH to afford 1-*O*-sinapoyl-*β*-D-glucose (28.0 mg), and the mother liquor was purified by preparative HPLC (MeOH−H_2O, 3:7), giving rise to sweroside (5.4 mg). Fractions 3−11 were further purified by a combination of HPLC (MeOH−H_2O, 3:7, 7:13, 2:3, 9:11, or 3:2) and PTLC ($CHCl_3$−MeOH−H_2O, 70:30:0.3), respectively. Fraction 3 yielded 1-*O*-sinapoyl-*β*-D-glucose (5.6 mg); fraction 4, quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (4.2 mg); fraction 5, **1** (20.9 mg) and quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (36.6 mg); fraction 6, **1** (41.7 mg), **2** (15.8 mg), (5*S*)-5-carboxystrictosidine (12.6 mg), quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (45.1 mg), kaempferol 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (1.1 mg), and isorhamnetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (1.3 mg); fraction 7, **1** (13.6 mg), **3** (4.7 mg), **4** (2.1 mg), quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-glucopyranoside (rutin) (2.5 mg), quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (13.5 mg), kaempferol 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (2.4 mg), and isorhamnetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (15.9 mg); fraction 8, **3** (29.7 mg), **4** (5.3 mg), **6** (6.1 mg), kaempferol 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (2.3 mg), and isorhamnetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (69.6 mg); fraction 9, isorhamnetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galactopyranoside (6.4 mg) and isorhamnetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-glucopyranoside (1.9 mg); fraction 10, **5** (16.7 mg), isorhamnetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-*β*-D-galac-

topyranoside (3.3 mg), and isorhamnetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (2.0 mg); fraction 11, strictosamide (6.2 mg).

Quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (1): yellow powder; $[\alpha]^{24}_D$ −193° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (log ε) 227sh (4.35), 256.5 (4.31), 270sh (4.27), 299sh (4.33), 315.5 (4.40), 364sh (4.14); IR ν_{max}^{KBr} cm^{-1} 3394, 1699, 1655, 1605, 1516, 1495; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; SIMS *m*/*z* 1047 [M − H]$^-$, 901, 755; ROESY H-6/H-1 of rhamnose-I, H-8/H-1 of rhamnose-I, H-1 of rhamnose-II/H-2 of galactose, H-1 of rhamnose-III/H_2-6 of galactose; HMBC H-2′→C-2, H-6′→C-2, H-1 of rhamnose-I→C-7, H-1 of galactose→C-3, H-1 of rhamnose-II→C-2 of galactose, H-1 of rhamnose-III→C-6 of galactose, H-4 of galactose→CO (δ 165.9), H-α→CO (δ 165.9), H-β→CO (δ 165.9), H-α→C-1″; HR-SIMS *m*/*z* 1047.2951 (calcd for $C_{48}H_{55}O_{26}$, 1047.2983).

Quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (2): yellow powder; $[\alpha]^{21}_D$ −160° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (log ε) 228sh (4.42), 255.5 (4.40), 270sh (4.34), 300sh (4.42), 317.5 (4.49), 361sh (4.24); IR ν_{max}^{KBr} cm^{-1} 3410, 1695, 1655, 1605, 1516, 1493; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; SIMS *m*/*z* 1047 [M − H]$^-$, 901; ROESY H-6/H-1 of rhamnose-I, H-8/H-1 of rhamnose-I, H-α/H_2-2″, 6″, H-β/H_2-2″, 6″; HMBC H-2′→C-2, H-6′→C-2, H-1 of rhamnose-I→C-7, H-2 of galactose→C-1 of rhamnose-II, H-1 of rhamnose-II→C-2 of galactose, H_2-6 of galactose→C-1 of rhamnose-III, H-1 of rhamnose-III→C-6 of galactose, H-3 of galactose→CO (δ 168.3), H-α→CO (δ 168.3), H-β→CO (δ 168.3), H-α→C-1″, H_2-2″, 6″→C-β, H-β→C_2-2″, 6″; HR-SIMS *m*/*z* 1047.3003 (calcd for $C_{48}H_{55}O_{26}$, 1047.2983).

Isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (3): yellow powder; $[\alpha]^{22}_D$ −222° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (log ε) 230sh (4.36), 254 (4.39), 268 (4.32), 300sh (4.39), 317.5 (4.46), 362sh (4.22); IR ν_{max}^{KBr} cm^{-1} 3421, 1653, 1603, 1516; ^{1}H NMR Table 1; ^{13}C NMR Table 2; SIMS *m*/*z* 1061 [M − H]$^-$, 915, 769; ROESY H-2′/OMe, H-6/H-1 of rhamnose-I, H-8/H-1 of rhamnose-I, H-α/H_2-2″, 6″, H-β/H_2-2″, 6″; HMBC H-2′→C-2, H-1 of rhamnose-I→C-7, H-1 of galactose→C-3, H-2 of galactose →C-1 of rhamnose-II, H-1 of rhamnose-II→C-2 of galactose, H_2-6 of galactose→C-1 of rhamnose-III, H-1 of rhamnose-III→C-6 of galactose, H-4 of galactose→CO (δ 168.7), H-α→CO (δ 168.7), H-β→CO (δ 168.7), H-α→C-1″, H_2-2″, 6″→C-β, H-β→C_2-2″, 6″; HR-SIMS *m*/*z* 1061.3155 (calcd for $C_{49}H_{57}O_{26}$, 1061.3140).

Isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (4): yellow powder; $[\alpha]^{25}_D$ −92° (*c* 0.38, MeOH); UV λ_{max}^{MeOH} nm (log ε) 230sh (4.30), 253.5 (4.34), 267.5 (4.24), 299sh (4.32), 318.5 (4.41), 360sh (4.17); IR ν_{max}^{KBr} cm^{-1} 3421, 1653, 1602, 1516; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; SIMS *m*/*z* 1061 [M − H]$^-$, 915, 769; ROESY H-2′/OMe, H-6/H-1 of rhamnose-I, H-8/H-1 of rhamnose-I, H-α/H_2-2″, 6″, H-β/H_2-2″, 6″; HMBC H-2′→C-2, H-6′→C-2, H-1 of rhamnose-I→C-7, H-1 of rhamnose-II→C-2 of galactose, H-2 of galactose→C-1 of rhamnose-II, H-1 of rhamnose-III→C-6 of galactose, H-3 of galactose→CO (δ 168.3), H-α→CO (δ 168.3), H-β→CO (δ 168.3), H-α→C-1″, H_2-2″, 6″→C-β, H-β→C_2-2″, 6″; HR-SIMS *m*/*z* 1061.3158 (calcd for $C_{49}H_{57}O_{26}$, 1061.3140).

Isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*cis*-*p*-coumaroyl)-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (5): yellow powder; $[\alpha]^{20}_D$ −147° (*c* 0.45, MeOH); UV λ_{max}^{MeOH} nm (log ε) 230sh (4.37), 254 (4.44), 267 (4.36), 297sh (4.31), 320 (4.39), 355sh (4.31); IR ν_{max}^{KBr} cm^{-1} 3421, 1655, 1601, 1516, 1493, 1456; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; SIMS *m*/*z* 1061 [M − H]$^-$, 915, 769, 461, 315; ROESY H-2′/OMe, H-6/H-1 of rhamnose-I, H-8/H-1 of rhamnose-I, H-β/H_2-2″, 6″; HMBC H-2′→C-2, H-6′→C-2, H-1 of rhamnose-II→C-2 of galactose, H-2 of galactose→C-1 of rhamnose-II, H_2-6 of galactose→C-1 of rhamnose-III, H-1 of rhamnose-III→C-6 of galactose, H-β→CO (δ 167.7), H_2-2″, 6″→C-β, H-β→C_2-2″, 6″, H-α→C-1″; HR-SIMS *m*/*z* 1061.3098 (calcd for $C_{49}H_{57}O_{26}$, 1061.3140).

Isorhamnetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-feruloyl)-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (6): yellow powder; $[\alpha]^{26}_D$ −185° (*c* 0.34, MeOH); UV λ_{max}^{MeOH} nm (log ε) 250 (4.43), 270sh (4.27), 290sh (4.23), 332 (4.43); IR ν_{max}^{KBr} cm^{-1} 3421, 1697, 1653, 1599, 1516; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; SIMS *m*/*z* 1091 [M − H]$^-$, 945, 915; ROESY H-2′/OMe (δ 4.04), H-2″/OMe (δ 3.96), H-6/H-1 of rhamnose-I, H-8/H-1 of rhamnose-I, H-β/H-2″, H-β/H-6″; HMBC H-2′→C-2, H-6′→C-2, H-1 of rhamnose-I→C-7, H-1 of rhamnose-II→C-2 of galactose, H-1 of rhamnose-III→C-6 of galactose, H-4 of galactose→CO (δ 168.7), H-α→CO (δ 168.7), H-β→CO (δ 168.7), H-α→C-1″, H-β→C-2″, H-β→C-6″, H-2″→C-β, H-6″→C-β; HR-SIMS *m*/*z* 1091.3256 (calcd for $C_{50}H_{59}O_{27}$, 1091.3245).

Alkaline Methanolysis of 1. A solution of compound **1** (20.0 mg) in 0.05 M NaOMe (0.2 mL) was stirred at room temperature for 7 h. After neutralization with Amberlite IR-120 (H^+ form), the reaction mixture was concentrated and the residue was subjected to preparative HPLC (MeOH−H_2O, 9:11) to afford quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**7**) (4.5 mg) and methyl *trans*-*p*-coumarate (2.1 mg).

Quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[α-L-rhamnopyranosyl(1→2)]-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (7): yellow powder; $[\alpha]^{28}_D$ −132° (*c* 0.34, MeOH); UV λ_{max}^{MeOH} nm (log ε) 256 (4.28), 270sh (4.14), 297sh (3.81), 356 (4.14); IR ν_{max}^{KBr} cm^{-1} 3405, 1655, 1601, 1493; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; SIMS *m*/*z* 901 [M − H]$^-$; ROESY H-6/H-1 of rhamnose-I, H-8/H-1 of rhamnose-I; HMBC H-2′→C-2, H-1 of rhamnose-I→C-7, H-1 of rhamnose-II→C-2 of galactose, H-2 of galactose→C-1 of rhamnose-II, H-1 of rhamnose-III→C-6 of galactose, H_2-6 of galactose→C-1 of rhamnose-III; HR-SIMS *m*/*z* 901.2583 (calcd for $C_{39}H_{49}O_{24}$, 901.2615).

Methyl *trans*-*p*-coumarate: ^{1}H NMR (CD_3OD, 200 MHz) δ 3.80 (3H, s, OMe), 6.30 (1H, d, *J* = 16.0 Hz, H-α), 6.84 (2H, d, *J* = 8.5 Hz, H-3, H-5), 7.44 (2H, d, *J* = 8.5 Hz, H-2, H-6), 7.64 (1H, d, *J* = 16.0 Hz, H-β); EIMS *m*/*z* 178 [M]$^+$ (100%), 147.

Acid Hydrolysis of Compound 7. Compound **7** (1 mg) was heated at 95 °C with dioxane (0.5 mL) and 5% H_2SO_4 (0.5 mL) for 1 h. After neutralization with Amberlite IRA-400 (OH^- form), the reaction mixture was concentrated and the residue was passed through a Sep-Pak C_{18} cartridge with H_2O. The eluate was concentrated, and the residue was treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.125 mL) at 60 °C for 1 h. The solution was then treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.05 mL) at 60 °C for 1 h. The supernatant was applied to GLC; GLC conditions: column, Supelco SPB-1, 30 m × 0.25 mm; column temperature, 230 °C; N_2 flow rate, 0.8 mL/min; t_R of derivatives, D-galactose 13.7 min, L-galactose 14.6 min, L-rhamnose 8.9 min. D-Galactose and L-rhamnose were detected from **7**.

Acknowledgment. We thank Dr. M. Sugiura (Kobe Pharmaceutical University) for the NMR spectra and Dr. K. Saiki (Kobe Pharmaceutical University) for the MS measurements.

References and Notes

(1) Atta-ur-Rahman; Qureshi, M. M.; Zaman, K.; Malik, S.; Ali, S. S. *Fitoterapia* **1989**, *60*, 291−322.
(2) Chatterjee, A.; Banerji, J.; Banerji, A. *J. Indian Chem. Soc.* **1974**, *51*, 156−170.
(3) Ferrari, F.; Messana, I.; Botta, B. *J. Nat. Prod.* **1986**, *49*, 1150−1151.
(4) Erdelmeier, C. A. J.; Wright, A. D.; Orjala, J.; Baumgartner, B.; Rali, T.; Sticher, O. *Planta Med.* **1991**, *57*, 149−152.
(5) El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod.* **1980**, *43*, 649−707.
(6) Ishimaru, K.; Sudo, H.; Satake, M.; Shimomura, K. *Phytochemistry* **1990**, *29*, 3823−3825.

(7) Rastrelli, L.; Saturnino, P.; Schettino, O.; Dini, A. *J. Agric. Food Chem*. **1995**, *43*, 2020−2024.
(8) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1986**, *34*, 1843−1845.
(9) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389−1397.
(10) Liu, Q.; Liu, M.; Mabry, T. J.; Dixon, R. A. *Phytochemistry* **1994**, *36*, 229−231.
(11) Andersen, W. K.; Omar, A. A.; Christensen, S. B. *Phytochemistry* **1987**, *26*, 291−294.

NP010518W