A New Acylated Flavonol Glycoside from the Leaves of *Eriobotrya japonica*

Nobuo KAWAHARA,*[,a] Motoyoshi SATAKE,[b] and Yukihiro GODA[a]

[a] *National Institute of Health Sciences (NIHS); 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan: and* [b] *Japan Pharmacists Education Center (JPEC); Toranomon 19 Mori Bldg., 1–2–20 Toranomon, Minato-ku, Tokyo 105–0001, Japan.* Received July 24, 2002; accepted September 28, 2002

A new acylated flavonol glycoside was isolated from the leaves of *Eriobotrya japonica* along with two known flavonol glycosides. Their structures were determined by extensive spectroscopic investigation.

Key words *Eriobotrya japonica*; Biwayo; Chinese crude drug; Rosaceae; flavonol glycoside

The leaves of *Eriobotrya japonica* LINDLEY (Rosaceae) have been traditionally used as a Chinese crude drug (known as "Pipaye" in Chinese and "Biwayo" in Japanese) to treat stomachache and promote antidiarrheal, antitussive, anti-inflammatory and diuretic effects. A number of triterpenoids with anti-inflammatory[1)] and antiviral[2)] effects, sesquiterpene glycosides,[3,4)] a small amount of amygdalin[1)] and sevral polyphenolic constituents[5,6)] have been reported from the above medicinal plant. Biwayo was first registered in the "Japanese Herbal Medicines Codex 1989" (JHMC),[7)] and ursolic acid, widely distributed in plant, was registered as a standard compound for TLC based identification. The Japanese Pharmacopoeia Committee recently began to consider registering this crude drug in the "Supplement I to The Japanese Pharmacopoeia Fourteenth Edition," and also began to study of new standard compound for TLC based identification. Finally, nerolidol 3-*O*-{[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)][α-L-rhamnopyranosyl-(1→6)]}-β-D-glucopyranoside, which was originally isolated from this crude drug,[3)] was registered as a new standard compound.[8)] During our chemical investigation of the above compound, we isolated a new acylated flavonol glycoside (**1**), along with two known flavonol glycosides, kaempherol 3-*O*-α-L-(2″,4″-di-*E*-*p*-coumaroyl)-rhamnoside (**2**) and kaempherol 3-*O*-α-L-(2″,4″-di-*Z*-*p*-coumaroyl)-rhamnoside (**3**). In the present paper, we report the structural elucidation of compound **1**.

The methanolic extract (8.8 g) of the leaf (100 g) was partitioned with CH_2Cl_2, EtOAc and H_2O. The EtOAc extract (1.25 g) was fractionated by silica gel column chromatography using a $CHCl_3$–MeOH gradient. The $CHCl_3$–MeOH (10 : 1) eluate was then subjected to HPLC with a $CHCl_3$–MeOH (20 : 1) solvent system, eluting compounds **1**—**3**. Compounds **2** and **3** were identified as kaempherol 3-*O*-α-L-(2″,4″-di-*E*-*p*-coumaroyl)-rhamnoside and kaempherol 3-*O*-α-L-(2″,4″-di-*Z*-*p*-coumaroyl)-rhamnoside, respectively, based on ^{1}H- and ^{13}C-NMR spectra and comparison with previously published data.[9,10)]

Compound **1** was a pale yellow amorphous powder, $[\alpha]_D$ −19.4° (c=0.16, MeOH), and gave a quasi-molecular ion at m/z 785 $[M+H]^+$ in positive ion FAB-MS. High-resolution (HR)-FAB-MS showed the molecular formula to be $C_{41}H_{37}O_{16}$ ($[M+H]^+$; m/z 785.2075). UV absorption bands at 265 and 328 nm suggested it to be a flavonoid.[11)] IR spectrum revealed the presence of hydroxyl groups and carbonyl groups conjugated with double bonds. ^{13}C-NMR data of **1** (Table 1) indicated thirty-three sp^2 carbons, including three carbonyl groups. ^{1}H-NMR spectrum of **1** (Table 1) showed characteristic of a kaempherol moiety.[10)] Signals at δ 6.21 (d, J=2.1 Hz) and 6.39 (d, J=2.1 Hz), correlated with the carbons at δ 99.9 and 94.8, respectively, in the heteronuclear multiple quantum coherence (HMQC) spectrum, were assigned to H-6 and H-8 on the A ring. Signals at δ 7.05 (2H, d, J=7.9 Hz) and 7.80 (2H, d, J=7.9 Hz), showed cross peaks with signals at δ 122.5 (C′-1) and 159.5 (C-2), respectively, in the heteronuclear multiple-bond connectivity (HMBC) spectrum, were attributed to H_2-3′,5′ and H_2-2′,6′ on the B ring, respectively. ^{1}H-NMR spectrum showed a characteristic signal assignable to an anomeric proton at δ 5.80 (d, J= 1.5 Hz). The spin system of a sugar unit, starting from anomeric proton at δ 5.80 in the ^{1}H–^{1}H shift correlation spectroscopy (COSY) and HMQC spectra, showed clear coupling connections around the α-rhamnopyranoside ring.[10)] HMBC correlation between δ 5.80 (H-1″) and δ 134.5 (C-3) indicated that **1** has a kaempherol-3-*O*-α-rhamnopyranosyl (afzelin)[12)] moiety. Furthermore, the appearance of two sets of ABX-type aromatic proton signals at δ 6.81 (d, J=7.9 Hz), 7.10 (dd, J=2.1, 7.9 Hz), 7.24 (d, J=2.1 Hz) and δ 6.83 (d, J=7.9 Hz), 7.14 (dd, J=2.1, 7.9 Hz), 7.27 (d, J=2.1 Hz), two pairs of *trans*-coupled olefinic proton signals at δ 6.33 (d, J=15.9 Hz), 7.56 (d, J=15.9 Hz) and δ 6.46 (d, J=15.9 Hz), 7.68 (d, J=15.9 Hz), and two methoxyl signals at δ 3.90 (3H, s) and 3.94 (3H, s) in the COSY spectrum of **1** indicated the presence of two sets of feruloyl groups.[13)] Two downfield shifts (1.32 and 1.64 ppm) in signal due to H-2″ and H-4″, respectively, suggested that two feruloyl groups

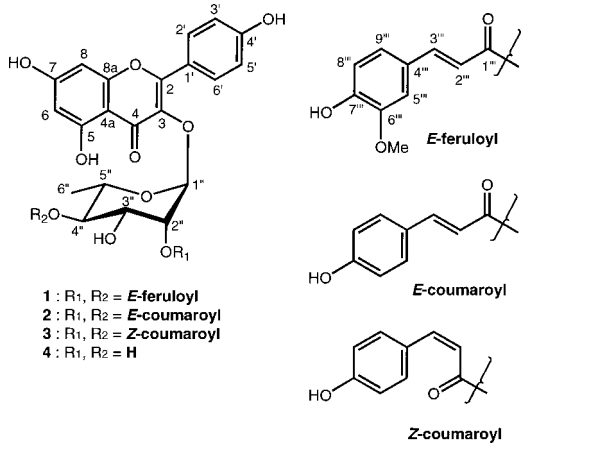

Chart 1

* To whom correspondence should be addressed. e-mail: kawahara@nihs.go.jp

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts of Compound **1** in CD_3OD

Position	$^{1}H^{a)}$	$^{13}C^{b)}$
2		159.5 s
3		134.5 s
4		179.2 s
4a		105.9 s
5		163.3 s
6	6.21 (d, 2.1)	99.9 d
7		165.9 s
8	6.39 (d, 2.1)	94.8 d
8a		158.6 s
1′		122.5 s
2′,6′	7.80 (d, 7.9)	131.9 d
3′,5′	7.05 (d, 7.9)	116.7 d
4′		161.8 s
1″	5.80 (d, 1.5)	98.9 d
2″	5.53 (dd, 1.5, 3.4)	73.0 d
3″	4.14 (dd, 3.4, 9.8)	68.4 d
4″	4.96 (t, 9.8)	74.6 d
5″	3.23 (dq, 6.4, 9.8)	69.7 d
6″	0.84 (d, 6.4)	17.6 q
1‴		168.2 s
2‴	6.46 (d, 15.9)	114.9 d
3‴	7.68 (d, 15.9)	147.7 d
4‴		127.71 s
5‴	7.24 (d, 2.1)	111.6 d
6‴		149.4 s
7‴		150.8 s
8‴	6.81 (d, 7.9)	116.44 d
9‴	7.10 (dd, 2.1, 7.9)	124.5 d
1⁗		168.3 s
2⁗	6.33 (d, 15.9)	115.3 d
3⁗	7.56 (d, 15.9)	147.2 d
4⁗		127.67 s
5⁗	7.27 (d, 2.1)	111.9 d
6⁗		149.4 s
7⁗		150.7 s
8⁗	6.83 (d, 7.9)	116.38 d
9⁗	7.14 (dd, 2.1, 7.9)	124.1 d
OMe	3.90 (s)	56.4 q
OMe	3.94 (s)	56.5 q

a) *J* values (in Hz) in parentheses. *b*) Multiplicities and assignments made by the heteronuclear multiple quantum coherence (HMQC) techniques.

were attached to C-2″ and C-4″ of **4**. The two feruloyl groups were determined to be attached at the C-2″ and C-4″ positions of the α-rhamnosyl moiety based on correlations between the H-2″ (δ 5.53) and H-4″ (δ 4.96) signals, and the respective carbonyl signals at δ 168.2 and 168.3 ppm, seen in the HMBC spectrum.

To confirm the structure of **1** as 4-hydroxy-3-methoxycinnamate, deacylation with sodium hydroxide was carried out. The products were analyzed by TLC and HPLC and identified as afzelin and ferulic acid. Therefore, the structure of **1** was determined to be kaempherol 3-*O*-α-L-(2″,4″-di-*E*-feruloyl)-rhamnoside.

Although numerous acylated flavonol glycosides have been isolated from plant materials, including kaempherol 3-*O*-α-L-(2″,4″-di-*E*-*p*-coumaroyl)-rhamnoside (**2**) and kaempherol 3-*O*-α-L-(2″,4″-di-*Z*-*p*-coumaroyl)-rhamnoside (**3**), **1** is the first example of a feruloyl afzelin to be isolated from plant materials.

Experimental

General Procedures Optical rotation was measured with a JASCO DIP-370 (Tokyo, Japan) spectrometer. FAB-MS and HR-FAB-MS spectra were obtained on a JEOL JMS-SX102 (Tokyo, Japan) spectrometer. UV and IR spectra were recorded on a Shimadzu UV-2550 (Kyoto, Japan) spectrophotometer and a JASCO IR-5300 spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL A-500 spectrometer at 500 MHz and at 125 MHz, respectively, using tetramethylsilane as an internal standard. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck, Darmstadt, Germany). HPLC was performed on a column of LiChrospher Si 60 (250×10 mm i.d., Merck) and Inertsil ODS-3 (250×4.6 mm i.d., GL Science Inc., Tokyo, Japan). TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected under UV light.

Plant Material Leaves of *Eriobotrya japonica* were purchased from Shibata Co., Ltd. (Tokyo, Japan). A voucher specimen was deposited at the National Institute of Health Sciences, Japan.

Extraction and Isolation Leaves of *Eriobotrya japonica* (100 g) were crushed and subjected to extraction with MeOH (0.5 l×3), giving a crude extract (8.8 g). This crude extract was partitioned between CH_2Cl_2 and H_2O, and then EtOAc and H_2O, giving an EtOAc extract (1.25 g). The EtOAc extract was then fractionated by silica gel column chromatography using a $CHCl_3$–MeOH gradient. The $CHCl_3$–MeOH (10 : 1) eluate was subjected to HPLC with a $CHCl_3$–MeOH (20 : 1) solvent system, eluting compound **1** (2.5 mg), kaempherol 3-*O*-α-L-(2″,4″-di-*E*-*p*-coumaroyl)-rhamnoside (**2**, 2.5 mg) and kaempherol 3-*O*-α-L-(2″,4″-di-*Z*-*p*-coumaroyl)-rhamnoside (**3**, 1.5 mg).

Compound **1**: Pale yellow amorphous powder. $[\alpha]_D^{23}$ −19.4° (*c*=0.16, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3429 (OH), 1705 (CO) and 1649 (CO). UV λ_{max}^{MeOH} (log ε): 265 (3.87), 300sh (3.97), 328 (4.09). Positive FAB-MS *m*/*z*: 785 $[M+H]^+$, HR-FAB-MS *m*/*z*: Calcd for $C_{41}H_{37}O_{16}$: 785.2075, Found: 785.2081. ^{1}H- and ^{13}C-NMR: Table 1.

Alkaline Hydrolysis of 1 Compound **1** (0.3 mg) was dissolved in 5% sodium hydroxide (0.5 ml) and incubated at room temperature for 12 h. The reaction mixture was neutralized with 10% H_2SO_4 and subjected to extraction with EtOAc. The EtOAc layer underwent TLC ($CHCl_3$–MeOH, 5 : 1) and HPLC analysis in order to identify ferulic acid (t_R, 8.9 min) and afzelin (t_R, 12.7 min), respectively. HPLC conditions: column, Inertsil ODS-3 (GL Science Inc. Tokyo, Japan), 250×4.6 mm (i.d.); solvent, 0.1% trifluoroacetic acid in CH_3CN–H_2O (1 : 3, v/v); flow rate, 1.0 ml/min; detection, 254 nm.

Acknowledgments The authors would like to thank Dr. K. Masuda (Showa College of Pharmaceutical Sciences) for assistance with obtaining FAB-MS measurements, Dr. M. Kuroyanagi (School of Bioresources, Hiroshima Prefectural University), who kindly provided the afzelin reference standard.

References and Notes

1) Shimizu M., Fukumura H., Tsuji H., Tanaami S., Hayashi T., Morita N., *Chem. Pharm. Bull.*, **34**, 2614—2617 (1986).
2) De Tommaasi N., De Simone F., Pizza C., Mahmood N., Orsi N., Stein M. L., *J. Nat. Prod.*, **55**, 1067—1073 (1992).
3) De Tommaasi N., De Simone F., Aquino R., Pizza C., *J. Nat. Prod.*, **53**, 810—815 (1990).
4) De Tommaasi N., Aquino R., De Simone F., Pizza C., *J. Nat. Prod.*, **55**, 1025—1032 (1992).
5) Jung H. A., Park J. C., Chung H. Y., Kim J., Choi J. S., *Arch. Pharm. Res.*, **22**, 213—218 (1999).
6) Ito H., Kobayashi E., Takamatsu Y., Li S. H., Hatano T., Sakagami H., Kusama K., Satoh K., Sugita D., Shimura S., Itoh Y., Yoshida T., *Chem. Pharm. Bull.*, **48**, 687—693 (2000).
7) Ministry of Health and Welfare, "Japanese Herbal Medicines Codex 1989," Yakuji Nippo Sha, Tokyo, 1989, p. 74.
8) Detail of this new standard compound will be published elsewhere.
9) Bloor S. J., *Phytochemistry*, **38**, 1033—1035 (1995).
10) Fiorini C., David B., Fouraste I., Vercauteren J., *Phytochemistry*, **47**, 821—824 (1998).
11) Voirin B., *Phytochemistry*, **22**, 2107—2145 (1983).
12) Masuda T., Jitoe A., Kato S., Nakatani N., *Phytochemistry*, **30**, 2391—2392 (1991).
13) Reference standard was purchased from Kanto Kagaku Co., Ltd.