Labdane-Type Diterpene Glycosides from Fruits of *Rubus foliolosus*

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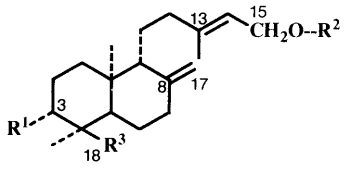
From fruits of *Rubus foliolosus* (Rosaceae), a traditional medicine used in Yunnan, China, seven labdane-type diterpene glycosides were isolated. Of these glycosides, five were identified as goshonosides-F1—5 (1—5) which have been isolated from leaves of *R. chingii*. Structures of two new glycosides, goshonosides-F6 (6) and -F7 (8), were elucidated as α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside of 13(*E*)-*ent*-labda-8(17),13-diene-3β,15,18-triol and 3β,15-di-*O*-β-D-glucopyranoside of 13(*E*)-*ent*-labda-8(17),13-diene-3β,15-diol. Goshonosides-F6 (6) and -F7 (8) were also isolated from the leaves of *R. chingii*.

Keywords Chinese traditional medicine; *Rubus foliolosus*; *Rubus chingii*; Rosaceae; diterpene glycoside; labdane type diterpene; goshonoside; fu-pen-zi

Previously, rubusoside (13,19-di-*O*-β-D-glucosyl-steviol), a sweet kaurane-type diterpene glycoside had been isolated from leaves and fruits of *Rubus suavissimus* S. LEE[1,2] which grows in Guang-xi and Guang-dong, southern China. Very recently, another sweet kaurane-type diterpene glucoside, named suavioside, has also been isolated from the leaves of this plant.[3]

In China and Korea, fruits of some of the *Rubus* spp. have been used as a tonic for aged people (覆盆子 Chinese name: fu-pen-zi; Korean name: bog-bun-ja). In relation to the chemical identification of the source plant of this crude drug, chemotaxonomical studies on a number of *Rubus* spp. growing in Eastern Asia have been conducted. *R. chingii* HU which grows in An-fui, Jiang-su, Zhe-jiang, Jiang-xi and Fu-jien, China and also in Yamaguchi, Ohita and Kochi, Japan (Japanese name: gosho-ichigo), is morphologically very similar to *R. suavissimus*. From the leaves of *R. chingii*, no rubusoside but several labdane type diterpene glycosides, named goshonosides-F1 (**1**), -F2 (**2**), -F3 (**3**), -F4 (**4**) and -F5 (**5**) were isolated in yields of 0.2, 0.2, 0.4, 0.5 and 5.7%, respectively,[4] all of which do not taste sweet (yields in the previous paper[4] are erroneous and must be amended as above). Goshonoside-F5 (**5**) was isolated from the fruits of this plant and also from commercial fu-pen-zi purchased in Kuang-zhou.[5] This indicates that the plant source of this drug in southern China is *R. chingii*.



	R^1	R^2	R^3
1	OH	Glc	CH_2OH
2	OH	H	CH_2O-Glc
3	H	Glc	COO-Glc
4	H	Glc	CH_2O-Glc
5	OH	Glc	CH_2O-Glc
6	OH	H	CH_2O-Glc-(6←1)-Ara*f*
7	OH	H	CH_2OH
8	O-Glc	Glc	CH_3
9	OH	H	CH_3

Glc: β-D-glucopyranosyl Ara*f*: α-L-arabinofuranosyl

Chart 1

We have further studied the glycosides of a number of *Rubus* spp. other than *R. suavissimus* and *R. chingii*. However, no diterpene glycoside has been isolated but several 19α-hydroxyursane-type triterpene glycosides have been isolated.[6] In continuing these serial studies, a characteristic dimeric triterpene glycoside, named coreanoside F1 was very recently isolated from the leaves and fruits of *R. coreanus* MIQ. which has been used as one of the plant sources of fu-pen-zi in Korea and northern China.[7] Coreanoside F1 was also isolated from commercial Korean bog-bun-ja but not identified in the leaves and fruits of *R. crataegifolius* BUNGE. and *R. parvifolius* LINN., both of which are also described as the plant sources of this crude drug. The present paper reports on the glycosides of the fruits of *R. foliolosus* D. DON. which is also used as fu-pen-zi in Yunnan, south-western China.

The fruits collected in Dali, Yunnan were extracted with methanol. A suspension of the methanolic extract in water was washed with ethyl acetate and then chromatographed as described in the Experimental, affording seven glycosides. Of these, five were identified as goshonosides-F1—5 (**1**—**5**) already obtained from *R. chingii*.

A new glycoside, **6** yielded D-glucose and L-arabinose on acid hydrolysis. It was revealed that signals assigned to the aglycone moiety of **2**[4] appeared at almost the same positions in the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of **6**. This indicated that **6** must be a 18-*O*-glycoside of 13(*E*)-*ent*-labda-8(17),13-diene-3β,15,18-triol (**7**) which is the common aglycone of **1**, **2** and **5**. Furthermore, the ^{13}C-NMR spectrum of **6** exhibited signals due to a terminal α-arabinofuranoside unit[8] and a 6-linked β-glucopyranoside unit. It follows that **6** can be formulated as 18-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside of **7**.

Another new glycoside, **8** afforded D-glucose on acid hydrolysis. On hydrolysis with β-glucosidase, **8** yielded D-glucose and an aglycone which was identified as 13(*E*)-*ent*-labda-8(17),13-diene-3β,15-diol (**9**) by comparison of the optical rotation with literature[9] and the NMR with the corresponding enantiomeric compound which has been isolated from the leaves of *Acacia* spp.[10] The ^{13}C-NMR spectrum of **8** showed carbon signals due to two terminal β-D-glucopyranosyl units and the glucosylation shift[11] was observed for the signals due to carbons around C-3 and

TABLE I. ^{13}C-NMR Data for Compounds **6**, **7**, **8** and **9** (δ from TMS in C_5D_5N)

	6	7	8	9
Aglycone				
C- 1	38.1	38.3	38.4	39.5
C- 2	27.8	28.3	24.5	27.8
C- 3	71.8	72.8	84.8	79.6
C- 4	43.4	43.3	38.9	40.1
C- 5	46.9	47.6	55.6	56.6
C- 6	24.3	24.3	24.4	24.3
C- 7	37.1	37.3	37.0	37.1
C- 8	148.9	148.8	148.6	148.6
C- 9	56.5	56.4	55.1	55.1
C-10	39.5	39.6	39.5	39.5
C-11	22.5	22.4	21.8	21.9
C-12	38.9	38.7	38.5	38.7
C-13	137.6	137.4	140.9	137.4
C-14	125.8	125.9	121.5	125.9
C-15	59.0	58.9	65.3	58.9
C-16	16.5	16.4	16.3	16.5
C-17	106.5	106.6	106.9	106.7
C-18	74.2	67.3	28.7	28.7
C-19	12.8	12.9	17.0	16.9
C-20	15.3	15.2	14.7	14.8
3-*O*-Glc				
1			102.5	
2			75.3	
3			78.4	
4			72.2	
5			78.7	
6			63.4	
15-*O*-Glc				
1			102.9	
2			75.2	
3			78.6	
4			71.8	
5			78.7	
6			62.9	
18-*O*-Glc				
1	105.5			
2	74.9			
3	78.4			
4	72.3			
5	76.7			
6	68.9			
Ara*f*				
1	110.2			
2	83.2			
3	78.5			
4	86.0			
5	62.7			

-15. Based on these results, **8** can be formulated as 3,15-di-*O*-β-D-glucopyranoside of **9**. Since **6** and **8**, were also isolated from the leaves of *R. chingii* in the present study, the names, goshonosides-F6 and -F7 were proposed for **6** and **8**, respectively. Both **6** and **8** were also detected in commercial fu-pen-zi purchased in guang-zhou.

The present study demonstrated that the chemical distinction between commercial fu-pen-zi prepared from the fruits of *R. foliolosus* and those from *R. chingii* is difficult by using glycosides as the marker. It is noteworthy that fruits of *R. foliolosus* can be readily morphologically distinguished from those of *R. chingii*.

Experimental

Melting points were uncorrected. Optical rotations were measured with a Union PM-101. ^{1}H-NMR (400 MHz, in C_5D_5N or $CDCl_3$) and ^{13}C-NMR (100 MHz, in C_5D_5N) spectra were run on a JEOL JMN-GX400 using tetramethylsilane as an internal standard. Acid hydrolysis of glycosides followed by identification of the resulting monosaccharide including absolute configuration,[12] and the methylation analysis of the sugar moieties monitored by gas chromatography–mass spectrometry (GC-MS) were carried out as described in the previous paper.[13]

Extraction and Isolation The dried fruit (500 g) of *Rubus foliolosus* D. DON, collected in Dali, Yunnan, China, was extracted with hot MeOH. The MeOH extract (32.8 g) was suspended in H_2O and defatted with EtOAc, and the aqueous layer was applied on a column of Diaion HP-20. The column was washed with H_2O and then eluted with 40% MeOH, 80% MeOH, MeOH and acetone. The 80% MeOH eluate (3.3 g) contained goshonosides, and this fraction was chromatographed on a Si-gel column using AcOEt–EtOH–H_2O [16:2:1, 8:2:1 and 5:2:1] to give seven fractions (frs. 1—7). Fraction 2 was purified by high performance liquid chromatography (HPLC) [TSKgel ODS-120T (21.5 mm × 30.0 cm), MeOH–H_2O (70:30)] to give **1** (0.0012%) and **2** (0.0014%). Fraction 3 was separated by HPLC [YMC-Pack ODS (20.0 mm × 25.0 cm), MeOH–H_2O (78:22)] to give **6**, **3**, **8** and **4** in yields of 0.0012, 0.0016, 0.0027 and 0.0014%, respectively. Fraction 5 was purified by HPLC [TSKgel ODS-120T (21.5 mm × 30.0 cm), MeOH–H_2O (65:35)] to give **5** (0.036%). **1**: Colorless prisms from MeCN–H_2O, mp 95—98 °C, $[\alpha]_D^{18}$ −55.6° (c=1.91, MeOH). **2**: A white powder, $[\alpha]_D^{18}$ −30.4° (c=0.96, MeOH). **3**: A white powder, $[\alpha]_D^{18}$ −31.5° (c=0.65, MeOH). **4**: A white powder, $[\alpha]_D^{18}$ −36.5° (c=0.75, MeOH). **5**: A white powder, $[\alpha]_D^{18}$ −45.2° (c=1.85, MeOH).

Goshonoside F6 (6) A white powder, $[\alpha]_D^{18}$ −28.4° (c=0.56, MeOH). *Anal.* Calcd for $C_{31}H_{52}O_{12}\cdot H_2O$: C, 58.66; H, 8.56. Found: C, 58.72; H, 8.52%. ^{1}H-NMR (in C_5D_5N) δ: 0.71 (3H, s, H_3-20), 0.88 (3H, s, H_3-19), 1.66 (3H, s, H_3-16), 3.53, 4.36 (each 1H, each d, J=9.8 Hz, H_2-18), 4.21 (1H, dd, J=4.4, 12.4 Hz, H-3), 4.47 (2H, br d, J=6.3 Hz, H_2-15), 4.57, 4.88 (each 1H, br s, H_2-17), 5.77 (1H, t, J=6.3 Hz, H-14), 4.84 (1H, d, J=7.8 Hz, H-1 of Glc), 5.69 (1H, d, J=2.0 Hz, H-1 of Ara). ^{13}C-NMR data is listed in Table I.

Enzymatic Hydrolysis of 6 Goshonoside F6 (**6**, 20 mg) was treated with crude hesperidinase at 40 °C for 16 h, and then extracted with EtOAc to give **7** (12 mg).

13(*E*)-*ent*-Labda-8(17),13-diene-3β,15,18-triol (7) Colorless prisms from $CHCl_3$, mp 141—143 °C, $[\alpha]_D^{18}$ −29.0° (c=0.98, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.73 (3H, s, H_3-20), 0.82 (3H, s, H_3-19), 1.66 (3H, s, H_3-16), 3.36, 3.65 (each 1H, each d, J=10.0 Hz, H_2-18), 3.65 (1H, dd, J=4.5, 12.0 Hz, H-3), 4.13 (2H, br d, J=6.3 Hz, H_2-15), 4.53, 4.85 (each 1H, each br s, H_2-17), 5.37 (1H, t, J=6.3 Hz, H-14). ^{13}C-NMR data is listed in Table I.

Goshonoside F7 (8) A white powder, $[\alpha]_D^{18}$ −47.7° (c=0.55, MeOH). *Anal.* Calcd for $C_{32}H_{54}O_{12}$: C, 60.93; H, 8.63%. Found: C, 61.01; H, 8.59%. ^{1}H-NMR (in C_5D_5N) δ: 0.69 (3H, s, H_3-20), 0.91 (3H, s, H_3-19), 1.26 (3H, s, H_3-18), 1.69 (3H, s, H_3-16), 3.69 (1H, dd, J=4.4, 11.7 Hz, H-3), 4.54 (1H, dd, J=7.8, 12.2 Hz, H_a-15), 4.61 (1H, d, J=5.3, 12.2 Hz, H_b-15), 4.60, 4.90 (each 1H, each br s, H_2-17), 5.58 (1H, dd, J=5.3, 7.8 Hz, H-14), 4.96 (1H, d, J=7.3 Hz, H-1 of 15-*O*-Glc), 4.99 (1H, d, J=7.8 Hz, H-1 of 3-*O*-Glc). ^{13}C-NMR data is listed in Table I.

Enzymatic Hydrolysis of 8 An aqueous solution (2 ml) of **8** (15 mg) was incubated with β-glucosidase (10 unit, from sweet almonds, Sigma) at 40 °C for 24 h, and then extracted with EtOAc to give **9** (10 mg).

13(*E*)-*ent*-Labda-8(17),13-diene-3β,15-diol (9) Colorless needles from $CHCl_3$, mp 161—163 °C, $[\alpha]_D^{18}$ −29° (c=0.56, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.69 (3H, s, H_3-20), 0.77 (3H, s, H_3-19), 0.99 (3H, s, H_3-18), 1.67 (3H, s, H_3-16), 3.35 (1H, dd, J=4.5, 12.1 Hz, H-3), 4.14 (2H, br d, J=7.1 Hz, H_2-15), 4.53, 4.85 (each 1H, each br s, H_2-17), 5.39 (1H, dd, J=5.3, 7.8 Hz, H-14). ^{13}C-NMR data is listed in Table I.

Acknowledgement This study was supported by a Grant-in-Aid (Monbusho International Scientific Program between Hiroshima University and Kunming Institute of Botany, Chinese Academy of Science, 1988—1989 and 1991—1993) from the Ministry of Education, Science and Culture to which our thanks are due.

References

1) T. Tanaka, H. Kohda, O. Tanaka, F. Chen, W. Chou and J. Leu, *Agric. Biol. Chem.*, **45**, 2165 (1981).
2) S. Lee, *Guihaia* (China), **1**, 17 (1981).
3) S. Hirono, W. Chou, R. Kasai, O. Tanaka and T. Tada, *Chem. Pharm. Bull.*, **38**, 1743 (1990).
4) T. Tanaka, K. Kawamura, T. Kitahara, H. Kohda and O. Tanaka, *Phytochemistry*, **23**, 615 (1984).

5) W. Chou, T. Oinaka, F. Kanamaru, K. Mizutani, F. Chen and O. Tanaka, *Chem. Pharm. Bull.*, **35**, 3021 (1987).
6) T. Seto, T. Tanaka, O. Tanaka and N. Naruhashi, *Phytochemistry*, **23**, 2829 (1984).
7) K. Ohtani, C. Miyajima, T. Takahashi, R. Kasai, O. Tanaka, D. Hahn and N. Naruhashi, *Phytochemistry*, **29**, 3275 (1990).
8) K. Mizutani, R. Kasai, M. Nakamura, O. Tanaka and H. Matsuura, *Carbohydr. Res.*, **185**, 27 (1989).
9) J. R. Mahazan and L. A. L. Ferreira, *An. Acad. Bras. Cienc.*, **43**, 145 (1977).
10) P. G. Forster, E. L. Ghisalberti and P. R. Jefferris, *Phytochemistry*, **24**, 2991 (1985).
11) R. Kasai, M. Suzuo, J. Asakawa and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175.
12) R. Ohshima, J. Kumanotani and C. Watanabe, *J. Chromatogr.*, **259**, 159 (1983).
13) H. Bjoendal, B. Lindberg, A. Pilotti and S. Svensson, *Carbohydr. Res.*, **15**, 339 (1970).