Note

Flavonoid Glycosides from *Sedum bulbiferum*Tsutomu Warashina^{*a} and Toshio Miyase^b^aSchool of Food and Nutritional Sciences, University of Shizuoka; 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan; and ^bSchool of Pharmaceutical Sciences, University of Shizuoka; 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan.

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The MeOH extract from dried whole *Sedum bulbiferum* MAKINO (Crassulaceae) plants yielded 34 compounds, including six new flavonoid glycosides and 28 known compounds. The structures of new compounds were established using NMR, Mass spectroscopic analysis and chemical evidence.

Key words *Sedum bulbiferum*; Crassulaceae; flavonoid glycoside; kaempferol

The genus *Sedum* are classified into the Crassulaceae family, and recently, some *Sedum* species were reported to be useful in the cosmetic industry, for example, skin moisturizing and lightening effects, improvement and protection of rough skin, and proliferation of skin fibroblasts.¹⁾ *Sedum bulbiferum* MAKINO is also one of the Crassulaceae plants and is widely distributed in the Honshu, Shikoku, and Kyushu Islands of Japan, the Korean Peninsula, and China. This plant has not been used as a folk medicine, but its extract was reported to exhibit the anti-tumor activity.²⁾ There have been no detailed reports about the constituents of *S. bulbiferum*, so in the course of research to determine the phytochemicals in the Crassulaceae family we investigated the constituents of this plant. We were particularly interested in efficacious agents from this family in the cosmetic field.

MeOH extract from dried whole *S. bulbiferum* plants was suspended in 90% MeOH in water. The solution was extracted with *n*-hexane and partitioned into a *n*-hexane-soluble fraction and a 90% MeOH in water-soluble fraction. The 90% MeOH in water-soluble fraction was dried *in vacuo*, and the residue was suspended in water. The suspension was extracted with diethylether and partitioned into an ether-soluble fraction and a water-soluble fraction. The residues of the water-soluble fraction and the ether-soluble fraction were subjected to silica gel column chromatography and/or semi-preparative HPLC to give three flavonoids (**1–3**), and thirty-one flavonoid glycosides (**4–34**). Compounds **1**,³⁾ **2**,³⁾ **3**,³⁾ **4**,⁴⁾ **5**,⁵⁾ **6**,⁵⁾ **7**,⁶⁾ **8**,⁷⁾ **9**,⁸⁾ **10**,⁹⁾ **11**,¹⁰⁾ **12**,¹¹⁾ **13**,⁴⁾ **14**,⁴⁾ **15**,^{5,12)} **16**,¹³⁾ **17**,¹²⁾ **18**,¹⁴⁾ **20**,¹⁵⁾ **22**,¹⁶⁾ **23**,¹⁷⁾ **26**,¹⁸⁾ **27**,¹⁹⁾ **28**,²⁰⁾ **29**,²¹⁾ **30**,²¹⁾ **31**,²¹⁾ and **34**²²⁾ were identified as shown in Chart 1 based on the ¹H and/or ¹³C-NMR spectroscopic data.

The molecular formula of bulbiferumside I (**19**), C₃₃H₄₀O₁₈, was established based on high resolution (HR)-FAB-MS. The aglycone of **19** was identified as kaempferol, according to observations of 15 carbon signals including twelve aromatic carbons (δ 163.7, 163.1, 161.9, 158.2, 132.0 $\times$ 2, 116.7 $\times$ 2, 122.4, 107.2, 100.6, 95.7), two olefin carbons (δ 159.8, 137.1), and one carbonyl carbon (δ 179.9), and the AX-type aromatic proton [δ 6.74 (1H, d, J =2.0 Hz), and 6.48 (1H, d, J =2.0 Hz)] and AA'XX'-type aromatic proton signals [δ 7.82 (2H, brd, J =8.5 Hz), and 6.96 (2H, brd, J =8.5 Hz)] in the ¹³C- and ¹H-NMR spectroscopic data. Moreover, in the ¹H- and ¹³C-NMR spectra of **19**, three anomeric proton and carbon

signals were observed at δ 5.61 (1H, d, J =1.5 Hz), 5.56 (1H, d, J =1.5 Hz), 4.36 (1H, d, J =8.0 Hz) and δ 107.2, 103.2, 100.0. Thus, **19** was considered to be a kaempferol triglycoside. Acid hydrolysis of **19** afforded L-rhamnose and D-quinovose with kaempferol. On the basis of ¹H-detected heteronuclear multiple quantum coherency (HMQC) and ¹H-¹H shift correlation spectroscopy (COSY) measurements, the signals at δ 5.61, 103.2, δ 5.56, 100.0, and δ 4.36, 107.2 were assigned at the anomeric proton and carbon of α -L-rhamnopyranoses and β -D-quinovopyranose, whose conformations were judged from the ¹H-chemical shift or coupling constant of each anomeric proton signal, respectively. In the ¹H-detected heteronuclear multiple-bond connectivity (HMBC) measurements, long-range correlations were exhibited between C-3 of the aglycone (δ 137.1) and H-1 of α -L-rhamnopyranose (δ 5.61), C-2 of α -L-rhamnopyranose (δ 83.0) and H-1' of β -D-quinovopyranose (δ 4.36), and C-7 of the aglycone (δ 163.7) and H-1' of α -L-rhamnopyranose (δ 5.56). In addition, the rotating frame nuclear Overhauser effect (ROE) difference experiment exhibited ROEs between H-1' of β -D-quinovopyranose and H-2 of α -L-rhamnopyranose [δ 4.26 (1H, dd, J =3.5, 1.5 Hz)], and H-1''' of α -L-rhamnopyranose and H-6 (δ 6.48), H-8 (δ 6.74) of the aglycone. Hence, **19** was determined to be kaempferol 3-O- β -D-quinovopyranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranoside-7-O- α -L-rhamnopyranoside.

The molecular formula of bulbiferumside II (**21**) was proposed to be C₃₂H₃₈O₁₉, based on HR-FAB-MS. The aglycone of **21** was determined to be quercetin, following observation of 15 carbon signals including twelve aromatic carbons (δ 163.6, 163.0, 158.1, 150.1, 146.5, 122.9, 122.7, 116.9, 116.5, 107.5, 100.6, 95.6), two olefin carbons (δ 159.7 and 137.1), one carbonyl carbon (δ 179.9), and the AX-type aromatic protons [δ 6.71 (1H, d, J =2.0 Hz) and 6.46 (1H, d, J =2.0 Hz)] derived from the A-ring and the AMX-type proton signals [δ 7.38 (1H, d, J =2.0 Hz), 7.33 (1H, dd, J =8.0, 2.0 Hz), and 6.93 (1H, d, J =8.0 Hz)] derived from the B-ring in the ¹³C- and ¹H-NMR spectroscopic data. Moreover, in the ¹H- and ¹³C-NMR spectra of **21**, three anomeric proton and carbon signals were also observed at δ 5.37 (1H, d, J =1.5 Hz), 5.56 (1H, d, J =1.5 Hz), 4.27 (1H, d, J =7.5 Hz) and δ 107.8, 103.3, 100.0. Thus, **21** was considered to be a quercetin triglycoside. Acid hydrolysis of **21** afforded L-rhamnose and D-xylose with quercetin. Because the ¹³C-NMR spectroscopic data of the sugar moiety

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Chart 1. Structure of Compounds 1–34

of **21** were consistent with those of **20**, the structure of **21** was identified as quercetin 3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranoside-7-*O*- α -L-rhamnopyranoside. This sugar linkage was confirmed by consequences of the HMBC measurement and ROE difference experiments irradiating each anomeric proton.

The molecular formulae of bulbiferumosides III (**24**) and IV (**25**) were identified as C₄₂H₄₆O₂₁ by HR-FAB-MS. The NMR spectroscopic data for **24** and **25** were similar to those of **22**, and signals due to the (*E*)-*p*-coumaroyl and (*Z*)-*p*-coumaroyl groups were observed in **24** and **25**, respectively. Additionally, alkaline hydrolysis yielded (*E*)-*p*-coumaric acid from **24** and (*Z*)-*p*-coumaric acid from **25** together with **22**. In the homonuclear Hahn–Hahn (HOHAHA) difference experiment of **24**, irradiation of H-1' of β -D-glucopyranose [δ 4.47 (1H, d, *J*=8.0 Hz)] exhibited signal enhancement for H-6' of β -D-glucopyranose [δ 4.58

(1H, dd, *J*=12.0, 2.0 Hz) and 4.09 (1H, dd, *J*=12.0, 7.0 Hz)]. Moreover, HMBC measurements in **24** revealed long-range correlations between H-6' of β -D-glucopyranose (δ 4.58, 4.09) and C- α' of the (*E*)-*p*-coumaroyl group (δ 168.7). Similarly, the HOHAHA difference experiments of **25** showed signal enhancement for H-6' of β -D-glucopyranose [δ 4.29 (1H, dd, *J*=12.0, 2.0 Hz) and 4.25 (1H, dd, *J*=12.0, 6.5 Hz)] on irradiating H-1' of the β -D-glucopyranosyl groups [δ 4.41 (1H, d, *J*=8.0 Hz)]. HMBC correlations were observed between H-6' of the β -D-glucopyranosyl group (δ 4.29, 4.25) and C- α' of the (*Z*)-*p*-coumaroyl groups (δ 167.8) in **25**. Thus, **24** and **25** were established to be kaempferol 3-*O*- β -D-6-*O*-[4-hydroxy-(*E*)-cinnamoyl]-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside-7-*O*- α -L-rhamnopyranoside and kaempferol 3-*O*- β -D-6-*O*-[4-hydroxy-(*Z*)-cinnamoyl]-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside-7-*O*- α -L-rhamnopyranoside, respectively.

Chart 2. Key ROEs, HOHAHAs, and HMBC Correlations of Compounds **19** and **33**

Bulbiferumosides **V** (**32**) and **VI** (**33**) showed the molecular formulae, $C_{35}H_{42}O_{21}$ and $C_{44}H_{48}O_{23}$ by HR-FAB-MS. The NMR spectroscopic data for **32** were similar to those of **27**, and signals due to the acetyl group were observed at δ 172.3, 20.4, and δ 1.75 (3H, s). Two-dimensional (2D) NMR measurements and ROE experiments irradiating each anomeric proton were showed the presence of **27** as a part of **32**, which was supported by alkaline hydrolysis affording **27** from **32**. HOHAHA difference experiments irradiating H-1 (δ 5.41) and H-1' (δ 4.78) of the β -D-glucopyranosyl groups showed signal enhancement at H-6s (δ 4.18, 4.03) and H-6's (δ 3.82, 3.71) of the β -D-glucopyranosyl groups. Moreover, in the HMBC measurement, the long-range correlations were observed at the above carbonyl carbon signal (δ 172.3) and H-6s of β -D-glucopyranose. Thus, **32** was determined to be kaempferol 3-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-(6-O-acetyl)-glucopyranoside-7-O- α -L-rhamnopyranoside. The NMR spectroscopic data for **33** suggested the presence of an acetyl group and a (*E*)-*p*-coumaroyl group with **27**. Based on the results of the HMBC measurement and HOHAHA difference experiments irradiating the anomeric protons of the β -D-glucopyranosyl groups, the acetyl group and the (*E*)-*p*-coumaroyl group were attached at the C-6 and C-6' positions of the β -D-glucopyranosyl groups, respectively. Hence, **33** was identified as kaempferol 3-O- β -D-[6-O-4-hydroxy-(*E*)-cinnamoyl]-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-(6-O-acetyl)-glucopyranoside-7-O- α -L-rhamnopyranoside (Chart 2).

The present investigation of the constituents in *Sedum bulbiferum* afforded six new flavonoid glycosides together with 28 known compounds. In our previous paper,²³⁾ we reported some kaempferol glycosides from *Botrychium ternatum*, that did not show proliferation on human skin fibroblasts or a tyrosinase inhibitory effect. Since the flavonoid glycosides from *S. bulbiferum* are similar to those of *B. ternatum*, they are not expected to be useful in the cosmetic field. In the literature,^{24–26)} some flavonoid glycosides from other *Sedum* species were reported to have lipid accumulation inhibitory, antinociceptive, and anti-inflammatory activities, and we were interested in these effects in the flavonoid glycosides afforded by *S. bulbiferum*.

Experimental

General Procedures Optical rotations and UV spectra were measured on a JASCO-P2200 polarimeter and JASCO V-630 spectrophotometer, respectively. FAB-MS spectra

were collected on a JEOL JMS-700 spectrometer in the positive mode using a *m*-nitrobenzyl alcohol matrix. Both ^1H - and ^{13}C -NMR spectra were recorded a JEOL α -400 (400, 100.40 MHz, respectively). Chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) as an internal standard. Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{\text{C-H}}=145\text{ Hz}$) and HMBC (optimized for $^nJ_{\text{C-H}}=8\text{ Hz}$) pulse sequences with a pulse field gradient. The ROE difference spectra were recorded with the mixing time set at 250 ms. The HOHAHA difference spectra were recorded with the spin locking time set at 180 ms. A Hitachi-G-3000 gas chromatograph with flame ionization detector (FID) was utilized for CG. JASCO 800 and 900 system instruments were used for HPLC (column: Nomura Chemical Co., Ltd., Aichi, Japan, Develosil-ODS 15/30 50 mm i.d. $\times$ 100 cm and YMC Co., Ltd., Kyoto, Japan, YMC-ODS 20 mm i.d. $\times$ 25 cm).

Plant Materials Whole *S. bulbiferum* plants were collected in Shizuoka city, Japan in May 2011 and 2012, and identified by Dr. T. Warashina. The dried materials were stored in a herbarium of the University of Shizuoka (voucher number, 918-M).

Extraction and Isolation Dried whole plants of *S. bulbiferum* (390 g) were extracted twice with MeOH (3.5 L) under reflux for 3 h. The extract (29.8 g) was concentrated under reduced pressure and the residue was dissolved in the MeOH–H₂O (9:1) solution (1 L). This solution was successively extracted with *n*-hexane (1 L). The MeOH–H₂O (9:1) extract was evaporated dry, and the resulting residue was partitioned between the diethylether-soluble fraction and H₂O-soluble fraction. The ether-soluble fraction was concentrated, and the residue (1.47 g) was subjected to silica gel column chromatography with a CHCl₃–MeOH (99:1 $\rightarrow$ 85:15, v/v) system to obtain eight fractions. (A (188 mg), B (82 mg), C (65 mg), D (177 mg), E (150 mg), F (218 mg), G (72 mg), and H (307 mg)). Using semi-preparative HPLC (YMC-ODS 20 mm i.d. $\times$ 25 cm (MeCN–H₂O (1:4, 22.5:77.5, 25:75, 3:7, 32.5:67.5, v/v), and MeOH–H₂O (35:65, 1:1, v/v), fraction C afforded **2** (10 mg). Fraction D yielded **1** (5 mg), **3** (5 mg), and **34** (8 mg). Fractions F and G produced **13** (10 mg) and **4** (9 mg), respectively.

The H₂O layer of the MeOH–H₂O (9:1) extract was passed through a porous polymer gel (Diaion HP-20, Mitsubishi Chemical Co., Tokyo, Japan) column with absorbed material being eluted with MeOH–H₂O 1:1 (2.7 L), 7:3 (2.5 L), and MeOH (2.0 L). The MeOH–H₂O 1:1 and 7:3 fractions

Table 1. ^{13}C -NMR Spectroscopic Data of Compounds **19**, **21**, **24**, **25**, **32**, and **33**

	19	21	24	25	32	33
Aglycone moiety						
C-2	159.8	159.7	158.9	159.6	159.8	159.0
-3	137.1	137.1	137.1	137.8	134.9	135.1
-4	179.9	179.9	179.7	179.9	179.7	179.8
-5	163.1	163.0	162.9	163.1	162.9	162.7
-6	100.6	100.6	100.6	100.6	100.6	100.5
-7	163.7	163.6	163.4	163.5	163.5	163.3
-8	95.7	95.6	95.6	95.7	95.7	95.5
-9	158.2	158.1	157.8	158.1	158.0	157.7
-10	107.2	107.5	107.7	108.0	107.4	107.3
-1'	122.4	122.7	122.4	122.4	122.7	122.4
-2'	132.0	116.9	132.0	132.1	132.4	132.6
-3'	116.7	146.5	116.5	116.4	116.2	116.3
-4'	161.9	150.1	161.6	161.6	161.7	161.7
-5'	116.7	116.5	116.5	116.4	116.2	116.3
-6'	132.0	122.9	132.0	132.1	132.4	132.6
Sugar moieties						
3- <i>O</i> -Sugars						
	Rha	Rha	Rha	Rha	Glc	Glc
-1	103.2	103.3	102.6	103.5	100.8	100.8
-2	83.0	82.6	83.6	83.8	82.3	84.9
-3	71.9	72.1 ^{a)}	72.1 ^{a)}	71.8 ^{a)}	77.7 ^{a)}	77.5
-4	73.5 ^{a)}	73.7 ^{b)}	73.1 ^{b)}	73.7	71.3 ^{b)}	71.4
-5	71.9	71.8 ^{c)}	71.7 ^{c)}	71.8 ^{a)}	75.5 ^{c)}	75.4
-6	17.6	17.7	17.7	17.7	64.0	64.0
	Qui	Xyl	Glc	Glc	Glc	Glc
-1'	107.2	107.8	107.0	107.1	104.6	106.3
-2'	75.7	75.3	75.2 ^{d)}	75.2	75.4 ^{c)}	76.2
-3'	77.7	77.8	77.7	77.7	78.3	77.9
-4'	76.9	71.0	72.3	71.8 ^{a)}	71.3 ^{b)}	72.2 ^{a)}
-5'	73.7 ^{a)}	67.1	75.4 ^{d)}	75.6	77.9 ^{a)}	75.7
-6'	18.1	—	64.5	64.4	62.7	65.0
7- <i>O</i> -Sugars						
	Rha	Rha	Rha	Rha	Rha	Rha
-1''	100.0	100.0	99.9	99.9	99.9	99.8
-2''	71.7	71.7 ^{c)}	71.8 ^{c)}	71.7 ^{a)}	71.7	71.7
-3''	72.1	72.0 ^{a)}	72.0 ^{a)}	72.1	72.1	72.1 ^{a)}
-4''	73.7 ^{a)}	73.6 ^{b)}	73.5 ^{c)}	73.7	73.6	73.7
-5''	71.3	71.3	71.3	71.3	71.4 ^{b)}	71.2
-6''	18.1	18.1	18.1	18.1	18.1	18.1
Ester moieties						
- α	—	—	—	—	172.3	172.2
- β	—	—	—	—	20.4	20.2
- α'	—	—	168.7	167.8	—	168.9
- β'	—	—	114.8	115.8	—	114.7
- γ'	—	—	146.5	144.8	—	146.4
-1'	—	—	126.9	127.3	—	126.8
-2',6'	—	—	131.0	133.7	—	130.7
-3',5'	—	—	116.7	115.9	—	116.6
-4'	—	—	161.1	160.0	—	161.0

Measured in MeOH- d_4 solution at 35°C. a-d) Interchangeable in each column. Rha: α -L-rhamnopyranose, Qui: β -D-quinovopyranose, Xyl: β -D-xylopyranose, Glc: β -D-glucopyranose.

were dried *in vacuo*, yielding 3.0 and 1.5 g, respectively. The residue of the MeOH-H₂O (7:3) fraction (1.5 g) was subjected to semi-preparative HPLC (Develosil-ODS-15/30 50 mm i.d.×100 cm (MeCN-H₂O 17:83→2:8, v/v), YMC-ODS 20 mm i.d.×25 cm (MeCN-H₂O (15: 85, 17.5:82.5, v/v),

and MeOH-H₂O (4:6, 45:55, v/v)). This residue yielded **4** (15 mg), **5** (8 mg), **6** (9 mg), **7** (18 mg), **8** (15 mg), **10** (6 mg), **11** (16 mg), **12** (7 mg), **14** (31 mg), **15** (7 mg), **17** (5 mg), **19** (2 mg), **20** (35 mg), **22** (24 mg), **24** (87 mg), **25** (9 mg), **29** (48 mg), and **33** (16 mg). The residue of the MeOH-H₂O (1:1) fraction (3.0 g) was subjected to semi-preparative HPLC (Develosil-ODS-15/30 50 mm i.d.×100 cm (MeCN-H₂O 14:86→19:81, v/v), YMC-ODS 20 mm i.d.×25 cm (MeCN-H₂O (1:9, 12.5:87.5, 15:85, 17.5: 82.5, v/v), MeOH-H₂O (25:75, 3:7, 32.5:67.5, 35:65, 4:6, v/v)). This residue provided **9** (14 mg), **16** (5 mg), **18** (4 mg), **21** (10 mg), **23** (5 mg), **26** (6 mg), **27** (30 mg), **28** (18 mg), **30** (15 mg), **31** (5 mg), **32** (25 mg), and **34** (8 mg).

Bulbiferumoside I (**19**)

Yellow amorphous powder. $[\alpha]_D^{20}$ -214 ($c=0.21$, MeOH). FAB-MS m/z : 747 [M+Na]⁺, 725 [M+H]⁺. HR-FAB-MS m/z : 747.2136 (Calcd for C₃₃H₄₀O₁₈Na: 747.2112). UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 266 (4.39), 344 (4.24). ¹H-NMR data (measured in MeOH- d_4 solution at 35°C): aglycone moiety, δ 7.82 (2H, brd, $J=8.5$ Hz, H-2', 6'), 6.96 (2H, brd, $J=8.5$ Hz, H-3', 5'), 6.74 (1H, d, $J=2.0$ Hz, H-8), 6.48 (1H, d, $J=2.0$ Hz, H-6). 3-*O*-Sugar moiety, δ 5.61 (1H, d, $J=1.5$ Hz, Rha-1), 4.36 (1H, d, $J=8.0$ Hz, Qui-1'), 4.26 (1H, dd, $J=3.5$, 1.5 Hz, Rha-2), 3.81 (1H, dd, $J=9.5$, 3.5 Hz, Rha-3), 3.57 (1H, dq, $J=9.5$, 6.0 Hz, Rha-5), 3.33 (overlapping, Rha-4), 3.30 (overlapping, Qui-3'), 3.24 (1H, dq, $J=9.5$, 6.0 Hz, Qui-5'), 3.22 (1H, dd, $J=9.0$, 8.0 Hz, Qui-2'), 2.95 (1H, t, $J=9.0$ Hz, Qui-4'), 1.16 (3H, d, $J=6.0$ Hz, Qui-6'), 0.98 (3H, d, $J=6.0$ Hz, Rha-6). 7-*O*-Sugar moiety, δ 5.56 (1H, d, $J=1.5$, Rha-1''), 4.02 (1H, dd, $J=3.5$, 1.5 Hz, Rha-2''), 3.83 (1H, dd, $J=9.5$, 3.5 Hz, Rha-3''), 3.60 (1H, dq, $J=9.5$, 6.0 Hz, Rha-5''), 3.48 (1H, t, $J=9.5$ Hz, Rha-4''), 1.26 (3H, d, $J=6.0$ Hz, Rha-6'').

Bulbiferumoside II (**21**)

Yellow amorphous powder. $[\alpha]_D^{20}$ -191 ($c=0.92$, MeOH). FAB-MS m/z : 749 [M+Na]⁺, 727 [M+H]⁺. HR-FAB-MS m/z : 749.1923, 727.2088 (Calcd for C₃₂H₃₈O₁₉Na: 749.1905, C₃₂H₃₉O₁₉: 727.2086). UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 258 (4.37), 267 (sh), 351 (4.22). ¹H-NMR data (measured in MeOH- d_4 solution at 35°C): aglycone moiety, δ 7.38 (1H, d, $J=2.0$ Hz, H-2'), 7.33 (1H, dd, $J=8.0$, 2.0 Hz, H-6'), 6.93 (1H, d, $J=8.0$ Hz, H-5'), 6.71 (1H, d, $J=2.0$ Hz, H-8), 6.46 (1H, d, $J=2.0$ Hz, H-6). 3-*O*-Sugar moiety, δ 5.37 (1H, d, $J=1.5$ Hz, Rha-1), 4.27 (1H, d, $J=7.5$ Hz, Xyl-1'), 4.20 (1H, dd, $J=3.5$, 1.5 Hz, Rha-2), 3.88 (1H, dd, $J=9.5$, 3.5 Hz, Rha-3), 3.85 (overlapping, Rha-5), 3.65 (1H, dd, $J=11.5$, 5.0 Hz, Xyl-5'), 3.39 (1H, m, Xyl-4'), 3.34 (1H, t, $J=9.5$ Hz, Rha-4), 3.29 (1H, t, $J=9.0$ Hz, Xyl-3'), 3.19 (1H, dd, $J=9.0$, 7.5 Hz, Xyl-2'), 3.06 (1H, dd, $J=11.5$, 10.5 Hz, Xyl-5'), 1.03 (3H, d, $J=6.0$ Hz, Rha-6). 7-*O*-Sugar moiety, δ 5.56 (1H, $J=1.5$ Hz, Rha-1''), 4.02 (1H, $J=3.5$, 1.5 Hz, Rha-2''), 3.83 (1H, dd, $J=9.5$, 3.5 Hz, Rha-3''), 3.60 (1H, dq, $J=9.5$, 6.0 Hz, Rha-5''), 3.48 (1H, t, $J=9.5$ Hz, Rha-4''), 1.26 (3H, d, $J=6.0$ Hz, Rha-6'').

Bulbiferumoside III (**24**)

Yellow amorphous powder. $[\alpha]_D^{23}$ -137 ($c=0.69$, MeOH). FAB-MS m/z : 909 [M+Na]⁺. HR-FAB-MS m/z : 909.2417 (Calcd for C₄₂H₄₆O₂₁Na: 909.2429). UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 226 (4.38), 268 (4.38), 316 (4.48). ¹H-NMR data (measured in MeOH- d_4 solution at 35°C): aglycone moiety, δ 7.66 (2H, brd, $J=8.5$ Hz, H-2', 6'), 6.91 (2H, brd, $J=8.5$, H-3', 5'), 6.49 (1H, d, $J=2.0$ Hz, H-8), 6.40 (1H, d, $J=2.0$ Hz, H-6). 3-*O*-Sugar moiety, δ 5.79 (1H, d, $J=1.5$ Hz, Rha-1), 4.58 (1H,

dd, $J=12.0, 2.0\text{ Hz}$, Glc-6'), 4.47 (1H, d, $J=8.0\text{ Hz}$, Glc-1'), 4.39 (1H, dd, $J=3.5, 1.5\text{ Hz}$, Rha-2), 4.09 (1H, dd, $J=12.0, 7.0\text{ Hz}$, Glc-6'), 3.84 (1H, dd, $J=9.5, 3.5\text{ Hz}$, Rha-3), 3.57 (1H, dq, $J=9.5, 6.0\text{ Hz}$, Rha-5), 3.49 (overlapping, Glc-5'), 3.43 (1H, t, $J=9.0\text{ Hz}$, Glc-3'), 3.37 (1H, t, $J=9.5\text{ Hz}$, Rha-4), 3.30 (overlapping, Glc-2'), 3.28 (1H, t, $J=9.0\text{ Hz}$, Glc-4'), 1.06 (3H, $J=6.0\text{ Hz}$, Rha-6). 7-*O*-Sugar moiety, δ 5.51 (1H, d, $J=1.5\text{ Hz}$, Rha-1''), 4.04 (1H, dd, $J=3.5, 1.5\text{ Hz}$, Rha-2''), 3.83 (1H, dd, $J=9.5, 3.5\text{ Hz}$, Rha-3''), 3.64 (1H, dq, $J=9.5, 6.0\text{ Hz}$, Rha-5''), 3.49 (1H, t, $J=9.5\text{ Hz}$, Rha-4''), 1.27 (3H, d, $J=6.0\text{ Hz}$, Rha-6''). Ester moiety, δ 7.38 (1H, d, $J=16.0\text{ Hz}$, H- γ'), 7.16 (2H, brd, $J=8.5\text{ Hz}$, H-2', 6'), 6.66 (2H, brd, $J=8.5\text{ Hz}$, H-3', 5'), 5.94 (1H, d, $J=16.0\text{ Hz}$, H- β').

Bulbiferumoside IV (25)

Yellow amorphous powder. $[\alpha]_D^{23} -157$ ($c=0.89$, MeOH). FAB-MS m/z : 909 $[M+Na]^+$. HR-FAB-MS m/z : 909.2430 (Calcd for $C_{42}H_{46}O_{21}Na$: 909.2429). UV λ_{max} (MeOH) nm (log ϵ): 228 (sh), 267 (4.39), 315 (4.38). $^1\text{H-NMR}$ data (measured in MeOH- d_4 solution at 35°C): aglycone moiety, δ 7.68 (2H, brd, $J=9.0\text{ Hz}$, H-2', 6'), 6.90 (2H, brd, $J=9.0$, H-3', 5'), 6.68 (1H, d, $J=2.0\text{ Hz}$, H-8), 6.43 (1H, d, $J=2.0\text{ Hz}$, H-6). 3-*O*-Sugar moiety, δ 5.61 (1H, d, $J=1.5\text{ Hz}$, Rha-1), 4.41 (1H, d, $J=8.0\text{ Hz}$, Glc-1'), 4.40 (overlapping, Rha-2), 4.29 (1H, dd, $J=12.0, 2.0\text{ Hz}$, Glc-6'), 4.25 (1H, dd, $J=12.0, 6.5\text{ Hz}$, Glc-6'), 3.83 (1H, dd, $J=9.5, 3.5\text{ Hz}$, Rha-3), 3.58 (1H, dq, $J=9.5, 6.0\text{ Hz}$, Rha-5), 3.44 (1H, m, Glc-5'), 3.39 (1H, t, $J=9.0\text{ Hz}$, Glc-3'), 3.36 (1H, t, $J=9.5\text{ Hz}$, Rha-4), 3.26 (1H, dd, $J=9.0, 8.0\text{ Hz}$, Glc-2'), 3.22 (1H, t, $J=9.0\text{ Hz}$, Glc-4'), 1.02 (3H, $J=6.0\text{ Hz}$, Rha-6). 7-*O*-Sugar moiety, δ 5.52 (1H, d, $J=1.5\text{ Hz}$, Rha-1''), 4.02 (1H, dd, $J=3.5, 1.5\text{ Hz}$, Rha-2''), 3.83 (1H, dd, $J=9.5, 3.5\text{ Hz}$, Rha-3''), 3.61 (1H, dq, $J=9.5, 6.0\text{ Hz}$, Rha-5''), 3.48 (1H, t, $J=9.5\text{ Hz}$, Rha-4''), 1.25 (3H, d, $J=6.0\text{ Hz}$, Rha-6''). Ester moiety, δ 7.44 (2H, brd, $J=8.5\text{ Hz}$, H-2', 6'), 6.67 (2H, brd, $J=8.5\text{ Hz}$, H-3', 5'), 6.36 (1H, d, $J=13.0\text{ Hz}$, H- γ'), 5.34 (1H, d, $J=13.0\text{ Hz}$, H- β'').

Bulbiferumoside V (32)

Yellow amorphous powder. $[\alpha]_D^{20} -126$ ($c=0.84$, MeOH). FAB-MS m/z : 821 $[M+Na]^+$. HR-FAB-MS m/z : 821.2133 (Calcd for $C_{35}H_{42}O_{21}Na$: 821.2116). UV λ_{max} (MeOH) nm (log ϵ): 267 (4.32), 349 (4.24). $^1\text{H-NMR}$ data (measured in MeOH- d_4 solution at 35°C): aglycone moiety, δ 8.02 (2H, brd, $J=9.0\text{ Hz}$, H-2', 6'), 6.90 (2H, brd, $J=9.0$, H-3', 5'), 6.74 (1H, d, $J=2.0\text{ Hz}$, H-8), 6.45 (1H, d, $J=2.0\text{ Hz}$, H-6). 3-*O*-Sugar moiety, δ 5.41 (1H, d, $J=8.0\text{ Hz}$, Glc-1), 4.78 (1H, d, $J=8.0\text{ Hz}$, Glc-1'), 4.18 (1H, dd, $J=12.0, 2.0\text{ Hz}$, Glc-6), 4.03 (1H, dd, $J=12.0, 5.5\text{ Hz}$, Glc-6), 3.82 (1H, dd, $J=12.0, 2.0\text{ Hz}$, Glc-6'), 3.75 (1H, dd, $J=9.0, 8.0\text{ Hz}$, Glc-2), 3.71 (1H, dd, $J=12.0, 5.0\text{ Hz}$, Glc-6'), 3.62 (1H, t, $J=9.0\text{ Hz}$, Glc-3), 3.42 (overlapping, Glc-2'), 3.39 (overlapping, Glc-3', 4'), 3.38 (overlapping, Glc-5), 3.33 (overlapping, Glc-4, 5'). 7-*O*-Sugar moiety, δ 5.57 (1H, d, $J=1.5\text{ Hz}$, Rha-1''), 4.03 (overlapping, Rha-2''), 3.83 (1H, dd, $J=9.5, 3.5\text{ Hz}$, Rha-3''), 3.59 (1H, dq, $J=9.5, 6.0\text{ Hz}$, Rha-5''), 3.48 (1H, t, $J=9.5\text{ Hz}$, Rha-4''), 1.26 (3H, d, $J=6.0\text{ Hz}$, Rha-6''). Ester moiety, δ 1.75 (3H, s, H- β).

Bulbiferumoside VI (33)

Yellow amorphous powder. $[\alpha]_D^{23} -83$ ($c=0.79$, MeOH). FAB-MS m/z : 967 $[M+Na]^+$. HR-FAB-MS m/z : 967.2513 (Calcd for $C_{44}H_{48}O_{23}Na$: 967.2484). UV λ_{max} (MeOH) nm (log ϵ): 225 (sh), 267 (4.40), 317 (4.48). $^1\text{H-NMR}$ data (measured in MeOH- d_4 solution at 35°C): aglycone moiety, δ 8.05 (2H, brd, $J=8.5\text{ Hz}$, H-2', 6'), 6.89 (2H, brd, $J=8.5$, H-3', 5'),

6.49 (1H, d, $J=2.0\text{ Hz}$, H-8), 6.38 (1H, d, $J=2.0\text{ Hz}$, H-6). 3-*O*-Sugar moiety, δ 5.17 (1H, d, $J=8.0\text{ Hz}$, Glc-1), 4.72 (1H, d, $J=8.0\text{ Hz}$, Glc-1'), 4.46 (1H, dd, $J=12.0, 2.5\text{ Hz}$, Glc-6'), 4.37 (1H, dd, $J=12.0, 7.0\text{ Hz}$, Glc-6'), 4.13 (1H, dd, $J=12.0, 2.0\text{ Hz}$, Glc-6), 4.02 (1H, dd, $J=12.0, 6.0\text{ Hz}$, Glc-6), 3.69 (1H, dd, $J=9.0, 8.0\text{ Hz}$, Glc-2), 3.68 (overlapping, Glc-5'), 3.56 (1H, t, $J=9.0\text{ Hz}$, Glc-3), 3.48 (1H, t, $J=9.0\text{ Hz}$, Glc-3'), 3.41 (1H, dd, $J=9.0, 8.0\text{ Hz}$, Glc-2'), 3.36 (1H, t, $J=9.0\text{ Hz}$, Glc-4'), 3.29 (overlapping, Glc-4, 5). 7-*O*-Sugar moiety, δ 5.52 (1H, d, $J=1.5\text{ Hz}$, Rha-1''), 4.04 (overlapping, Rha-2''), 3.83 (1H, dd, $J=9.5, 3.5\text{ Hz}$, Rha-3''), 3.60 (1H, dq, $J=9.5, 6.0\text{ Hz}$, Rha-5''), 3.49 (1H, t, $J=9.5\text{ Hz}$, Rha-4''), 1.26 (3H, d, $J=6.0\text{ Hz}$, Rha-6''). Ester moiety, δ 7.34 (1H, d, $J=16.0\text{ Hz}$, H- γ'), 7.06 (2H, brd, $J=9.0\text{ Hz}$, H-2', 6'), 6.59 (2H, brd, $J=9.0\text{ Hz}$, H-3', 5'), 5.99 (1H, d, $J=16.0\text{ Hz}$, H- β'), 1.66 (3H, s, H- β).

Acid Hydrolysis of Compounds 19 and 21 Compounds **19** and **21** (*ca.* 1 mg) was dissolved in 2 M HCl (200 μL) and 1,4-dioxane (20 μL). The solutions were heated at 100°C for 1 h. After hydrolysis, each reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was concentrated dry, and the residue from each compound was analyzed using HPLC through comparison with an authentic sample. HPLC conditions: column, YMC-ODS-AM 4.6 mm i.d. $\times$ 25 cm; flow rate, 1.0 mL/min; 30% MeCN in water +0.05% trifluoroacetic acid (TFA); t_R , 15.2 min (quercetin (**3**)), 29.4 min (kaempferol (**2**)). **2** and **3** were detected in **19** and **21**, respectively. The H_2O layer was neutralized with an Amberlite IRA-60E column, and the eluate was concentrated dry. The residue was stirred with D-cysteine methyl ester hydrochloride, hexamethyldisilazane and trimethylsilylchloride in pyridine using the same procedures as in previous reports.^{27,28} After the reactions, the supernatant was subjected to GC. GC conditions: column, TC-1 (GL Science Inc., Tokyo, Japan) 0.25 mm i.d. $\times$ 30 m; carrier gas, N_2 ; column temperature, 215°C ; t_R , 22.2 min (L-rhamnose (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan)), 21.7 min (D-rhamnose), 21.6 min (D-quinovose (Sigma Chem. Co., St. Louis, U.S.A.)), 20.5 min (L-quinovose), 19.1 min (D-xylose (Tokyo Kasei Kogyo Co., Ltd.)), 17.6 min (L-xylose). The t_R s for D-rhamnose, L-quinovose and L-xylose were obtained from their enantiomers (L-rhamnose+L-cysteine, D-quinovose+L-cysteine, D-xylose+L-cysteine). L-Rhamnose and D-quinovose were found in **19** and L-rhamnose and D-xylose were detected in **21**.

Alkaline Hydrolysis of Compounds 24, 25, 32, and 33 Compounds **24**, **25**, **32**, and **33** (*ca.* 1 mg) were dissolved in 0.05 M NaOH (100 μL), and stirred at room temperature for 3.5 h under an N_2 atmosphere. After the reactions, each mixture was neutralized with an Amberlite IR-120B column with the eluate concentrated dry. The residue was partitioned between EtOAc and H_2O , and both layers were concentrated dry. The residue from the EtOAc layer was analyzed using HPLC through comparison with an authentic sample. HPLC conditions: column, YMC-ODS-AM 4.6 mm i.d. $\times$ 25 cm; flow rate, 1.0 mL/min; 17.5% MeCN in water +0.05% TFA; t_R , 16.0 min [(*E*)-*p*-coumaric acid (Tokyo Kasei Kogyo Co., Ltd.)], 17.6 min [(*Z*)-*p*-coumaric acid (The authentic sample was provided by Prof. T. Miyase.)]. (*E*)-*p*-Coumaric acid was detected in **24** and **33**, and (*Z*)-*p*-coumaric acid was identified in **25**. The residues from the H_2O layers of compounds **24**, **25**, **32**, and **33** were also analyzed using HPLC through comparison with compounds **22** and **27**. HPLC conditions: column, YMC-ODS-

AM 4.6 mm i.d. $\times$ 25 cm; flow rate, 1.0 mL/min; 17.5% MeCN in water; t_R , 14.8 min (**22**), 12.5% MeCN in water; t_R , 7.2 min (**27**). **22** was detected in **24** and **25**, and **27** was found in **32** and **33**.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials. Table S1. ^1H -NMR spectroscopic data of compounds **19**, **21**, **24**, **25**, **32**, and **33**. Figure S1. The ^{13}C - and ^1H -NMR spectra of compounds **19**, **21**, **24**, **25**, **32**, and **33**. Figure S2. The HOHAHA spectra of compounds **24**, **25**, **32**, and **33**.

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