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C,*O*-Bisglycosylapigenins from the leaves of *Rhamnella inaequilatera*

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Abstract

From the leaves of *Rhamnella inaequilatera*, three flavone *C*,*O*-bisglycosides, rhamnellaflavosides A, B and C, were isolated and their structures were elucidated based on their spectral data and chemical evidence.

Keywords: *Rhamnella inaequilatera*; Rhamnaceae; Rhamnellaflavosides A, B and C; Apigenin; Oliose; Boivinose

1. Introduction

Some Rhamnaceae plants are used as traditional medicines in Japan and China (Kitagawa et al., 1988). For example, *Rhamnella gilgitica* is used as a remedy for rheumatism, arthritis, skin diseases and leprosy in Chinese medicine (Xiao, 1993). During the course of the studies on the constituents of the plants grown under subtropical climate, we investigated the glycosidic constituents of the leaves of *Rhamnella inaequilatera* Ohwi (Hatusima et al., 1994) harvested in Okinawa Prefecture, Japan and isolated three new apigenin *C*,*O*-bisglycosides, rhamnellaflavosides A (**1**), B (**2**) and C (**3**) together with the known 2′-*O*-α-L-rhamnopyranosyl-6-*C*-β-D-xylopyranosylapigenin (Bouillant et al., 1978, Mabry et al., 1971) and 2′-*O*-α-L-rhamnopyranosylisovitexin (Mastenbroek et al., 1986). This paper deals with the isolation and structure elucidation of the new compounds.

2. Results and discussion

Rhamnellaflavosides A (**1**), B (**2**) and C (**3**) were isolated from the *n*-BuOH soluble fraction of the methanolic extract of the leaves of *R. inaequilatera* by combination of chromatographic procedures using highly-porous synthetic resin, Diaion HP-20 and silica gel, and reversed phase HPLC, respectively.

Rhamnellaflavoside A (**1**), $[\alpha]_D$ +51.4°(MeOH), was obtained as a yellow colored powder and formulated as $C_{27}H_{30}O_{13}$ based on its HR-FAB-MS. It showed absorption maxima characteristic of a flavone skeleton at 272 and 323 nm, which shifted to 283 and 343 nm, and to 278 and 371 nm on addition of aluminum chloride and sodium acetate, respectively. The ^{1}H NMR spectrum showed the presence of a *p*-disubstituted benzene ring [δ_H 7.44 and 7.88 (each 2H, *d*, J = 8.8 Hz)], hydrogen bonded hydroxyl group (δ_H 14.06) and two olefinic protons [δ_H 6.74 and 6.88 (each 1H, *s*)]. In the sp^2-carbon region, the ^{13}C NMR spectrum (Table 2) showed signals due to six carbon atoms having a hydrogen atom and nine quaternary carbon atoms. The above mentioned data suggested the presence of monosubstituted apigenin moiety in the structure. In addition to the signals due to the β-glucopyranosyl moiety, the

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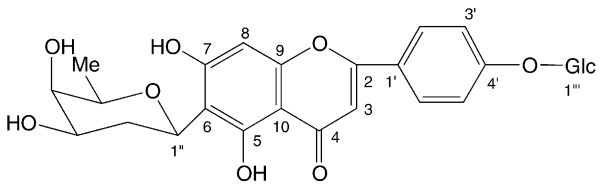
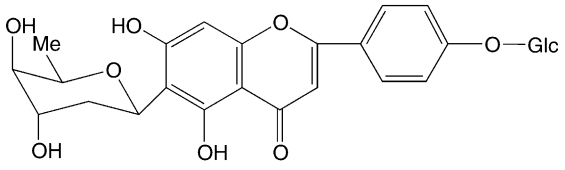
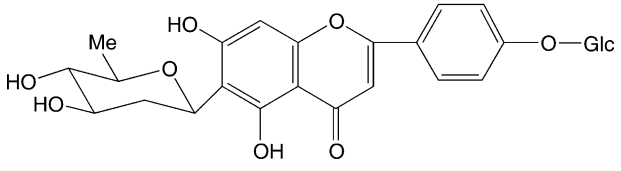
^{13}C NMR spectrum also showed signals due to six carbon atoms at δ 70.4, 33.5, 71.3, 73.0, 76.7 and 17.7 ppm. The signals were deduced to arise from a β-*C*-oliose moiety based on the following rationals. The axial proton at C-2″ appeared as a quartet [δ 2.85 (J = 12 Hz)] which coupled with the axial protons at C-1″ and C-3″ in addition to the geminal proton. On the other hand, the signals due to H-5″ appeared as a quartet (J = 6.4 Hz) at δ 3.79 and no coupling with H-4″. The results indicated that the hydroxyl groups at C-3″ and C-4″ have equatorial and axial orientations, respectively. Enzymatic hydrolysis with β-glucosidase from almond gave **1b** and acidic hydrolysis gave D-glucose. The former was identified using torosaflavone A (apigenin 6-*C*-β-D-olioside) (Kitanaka et al., 1989) as a reference compound. The location of the additional glucose moiety was inferred to be on *O*-4′ from the above mentioned UV data and the fact that the octaacetate (**1a**) obtained by acetylation showed H-8 signals at δ 7.26 which had a downfield shift relative to that in **1**. The presumption was confirmed by the fact that a differential NOE was observed for H_2-3′,5′ (δ 7.44) on irradiation at δ 5.77 (J = 7.6 Hz) assigned as the anomeric proton of glucose moiety. Judged from the coupling constant of the anomeric proton of D-glucose moiety, the glucosidic linkage has a β-orientation. Thus, rhamnellaflavoside A was elucidated to be 6-*C*-β-D-oliopyranosyl-4′-*O*-β-D-glucopyranosylapigenin (**1**).



(**1**)



(**2**)



(**3**)

Glc : β-D-glucopyranoside

Rhamnellaflavoside B (**2**), $[\alpha]_D$ +4.1°(MeOH), was obtained as a yellow colored powder. The molecular formula was the same as that of rhamnellaflavoside A (**1**) and the UV spectral behavior is essentially the same as in **1**. Acetylation of **2** gave the octaacetate (**2a**). The ^{1}H and ^{13}C NMR spectra also were essentially the same as those in **1**, except for the signals arising from the 2,6-dideoxyhexopyranose moiety. Thus, the signal due to H_2-2″ resonated at δ 2.29 (1H, *br.d-like*, J = *ca* 13 Hz) and 2.98 (1H, *br.t-like*, J = *ca* 12 Hz). The coupling pattern of the protons suggested that the proton at C-3″ takes an equatorial orientation. The proton H-5″ resonated at δ 4.70 (1H, *quart*., J = 6.4 Hz), suggesting that H-4″ also takes an equatorial orientation. The proton signals assigned to anomeric proton [δ 6.24 (1H, *dd*, J = 2.0, 11.6 Hz)] and H-5″ were downfield by 0.66 and 0.91 ppm, respectively, this being ascribed to a 1,3-diaxial relationship between 3″-OH, and H-1″ and H-5″. Thus, the 2,6-deoxyhexopyranose moiety in **2** was deduced to be boivinopyranose. Enzymatic hydrolysis of **2** gave **2b** together with D-glucose. The ^{13}C NMR spectroscopic data of **2b** and alternanthin (**2c**) (Zhou et al., 1988) were essentially the same except for signals arising from the B ring. Considering the optical rotation of **2b**, compound **2b** was elucidated as apigenin 6-*C*-β-D-boivinopyranose. On irradiation at δ 5.77 (J = 7.2 Hz) (anomeric proton of D-glucose moiety), a NOE was observed for the signal at δ 7.44 (H_2-3′,5′), indicating that β-D-glucose moiety bound on *O*-4′. Thus, rhamnellaflavoside B was elucidated to be 6-*C*-β-D-boivinopyranosyl-4′-*O*-β-D-glucopyranosylapigenin (**2**).

Rhamnellaflavoside C (**3**), $[\alpha]_D$ +33.6°(MeOH), was also obtained as a yellow colored powder. The molecular formula, $C_{27}H_{30}O_{13}$, was again the same as rhamnellaflanosides A (**1**) and B (**2**), and the spectral data were similar to those of **1** and **2** except for the signal arising from the 2,6-dideoxyhexopyranose moiety. The ^{1}H NMR spectrum of **3** showed signals corresponding to methylene groups at δ 2.73 (1H, *ddd*, J = 2.0, 4.4 and 12.0 Hz) and 2.86 (1H, *quart*., J = 12.0 Hz) due to H_2-2″, an anomeric proton at δ 5.68 (1H, *dd*, J = 2.0 and 11.6 Hz), an methine proton geminal to a hydroxyl group [δ 3.78 (1H, *quart*., J = 5.6 Hz)] due to H-5″ and a secondary methyl group [δ 1.67 (3H, *d*, J = 5.6 Hz), H_3-6″]. Acetylation with acetic anhydride and pyridine gave the octaacetate (**3a**) and enzymatic hydrolysis gave **3b** and D-glucose. Analysis of ^{1}H NMR signals clearly demonstrated that all substituents in 2,6-dideoxyhexopyranose moiety take equatorial orientations. Thus, **3b** was elucidated to be 6-*C*-β-D-4-epioliosylapigenin. The position of β-D-glucopyranosyl residue was determined on *O*-4′, since an NOE was observed for δ 5.76 (1H,d, J = 6.8 Hz, H-1‴) on irradiation at δ 7.42 (2H, d, J = 8.8 Hz, H_2-3′,5′). Thus, the structure of rhamnellaflavoside C (**3**) was elucidated to be 6-*C*-β-D-4-epioliosyl-4″-*O*-β-D-glucopyranosylapigenin (**3**).

3. Experimental

3.1. General

^{1}H and ^{13}C NMR (400 MHz and 100 MHz, respectively) spectra were recorded on a JEOL JNM EX-400 spectrometer, using tetramethylsilane as an internal standard. FABMS were obtained on a JEOL JMS SX-102 spectrometer, using PEG-400, PEG-600 or *m*-nitrobenzyl alcohol as matrix. IR and UV specra were taken on a Perkin Elmer 1720 Infrared FT and JASCO V-530SR spectrophotometers. Specific rotations were determined using a JASCO DIP-360 digital polarimeter. The following were used for compound purification: the highly porous synthetic resin, Diaion HP-20 (Mitsubishi Chemical Co., Ltd., Tokyo), silica gel 60 (Merck, 230–400 mesh), cosmosil 75 C_{18}-OPN (Nacalai Tesque, Kyoto Japan), packed column for HPLC (Cosmosil 10 C_{18}, 20 × 250 mm), silica gel 60 F_{254} TLC plates (Merck, 0.25 and 0.5 mm in thickness) and β-glucosidase from almond (Nacalai Tesque, Kyoto, Japan).

3.2. Plant material

Leaves of *Rhamnella inaequilatera* Ohwi were collected in July, 1994, in Nago-city, Okinawa Prefecture, Japan. A specimen was authenticated by Anki Takushi of the Okinawa Prefectural Station of Forestry, whom the authors acknowledge, and a voucher herbarium specimen (94-RI-Okinawa-0712) is deposited in the Herbarium of Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University.

3.3. Isolation

Dried leaves (5.0 kg) of *R. inaequilatera* were extracted with MeOH (45 l) at room temp for 3 weeks. Concentration of the MeOH extract in vacuo gave a residue which was dissolved in 90% ag MeOH (2.1 l). After washing with *n*-hexane (1 l × 3), the 90% MeOH solution was concectrated in vacuo. The residue was suspended in H_2O (1 l) and the suspension was extracted with EtOAc (1 l × 3) and *n*-BuOH (1 l × 3), successively. The *n*-BuOH extract was concentrated in vacuo to give a residue (48 g) which was applied on Diaion HP-20 (ϕ = 70 mm, L = 420 mm) eluted with stepwise increases of MeOH contents in H_2O [0 (3 l), 20 (3 l), 40 (3 l), 50 (3 l), 70 (3 l) and 100% (3 l)], fractions of 1 l being collected. Fractions 17–19 were combined and evaporated in vacuo to give a residue (14.0 g) which was applied on a silica gel column (360 g) eluted with a mixture of $CHCl_3$ and MeOH with increasing amounts of MeOH content. $CHCl_3$ (1.5 l), $CHCl_3$–MeOH (19:1, 1.5 l), $CHCl_3$–MeOH (9:1, 1.5 l), $CHCl_3$–MeOH(17:3, 2.5 l), $CHCl_3$–MeOH (4:1, 2.5 l), $CHCl_3$–MeOH (3:1, 1 l) and $CHCl_3$–MeOH (7:3, 1.5 l) were eluted successively, fractions of 100 ml being collected. Fractions 68–69, 70–75, 76–78, 79–81 and 82–98 gave residues (126 mg, 462 mg, 329 mg, 328 mg and 1.63 g, respectively). Each residue was separated by repeated preparative HPLC (solvent: MeOH–H_2O 1:1) to give rhamnellaflavosides A (1) (406.5 mg), B (2) (22.5 mg) and C (3) (46.1 mg).

Fractions 14–16 from HP-20 chromatography gave a residue (11.4 g) which was applied on a silica gel column (360 g) eluted with a mixture of $CHCl_3$ and MeOH with increasing amounts of MeOH content. $CHCl_3$ (2l), $CHCl_3$–MeOH (9:1, 1.5 l), $CHCl_3$–MeOH (17: 3, 1.5 l), $CHCl_3$–MeOH (4:1, 4.5 l), $CHCl_3$–MeOH (3:1, 2 l), $CHCl_3$–MeOH (7:3, 3.5 l), $CHCl_3$–MeOH (3:2, 1 l), $CHCl_3$–MeOH (1:1, 1 l) were passed successively, fractions of 100 ml being collected. Fractions 64–68 and 69–78 gave residues (728 mg and 1.85 g, respectively) which were subjected to a 75 C_{18}-OPN column eluted (octadecylsilica gel)(ϕ 2.2 × L = 28 cm) with linear gradient of H_2O and MeOH [20% (500 ml) and 80% MeOH (500 ml) to give 2′-*O*-α-L-rhamnopyranosyl-6-*C*-β-D-xylopyranosylapigenin (1.12 g) which showed a spot (R_f 0.21) on TLC (solvent: $CHCl_3$–MeOH–H_2O 15:6:1). Fractions 115–170 gave a residue (2.79 g) which was applied to a 75 C_{18}-OPN column eluted with a linear gradient of H_2O (1 l) and 60% MeOH–H_2O (1 l) to give 2′-*O*-α-L-rhamnopyranosyl- isovitexin (660 mg) which showed a spot (R_f 0.12) on TLC (solvent: as above).

3.4. Rhamnellaflavoside A (**1**)

Yellow amorphous powder, $[\alpha]_D^{26}$ +51.4°(MeOH; *c* 1.50). UV λ_{max} (MeOH) nm (log ϵ): 272 (4.41), 323 (4.36), λ_{max} (MeOH + $AlCl_3$) nm: 283, 343, λ_{max} (MeOH + CH_3COONa) nm: 278, 371; IR ν_{max} (film) cm^{-1}: 3325, 1655, 1628, 1581, 1500, 1367, 1242, 1072; for ^{1}H NMR (C_5D_5N): and ^{13}C NMR (CD_3OD), see Tables 1 and 2; HRFABMS (negative) *m*/*z* 561.1608 $[M–H]^-$ ($C_{27}H_{29}O_{13}$ requires 561.1608).

3.5. Rhamnellaflavoside B (**2**)

Yellow amorphous powder, $[\alpha]_D^{26}$ +4.1°(MeOH; *c* 0.50). UV λ_{max} (MeOH) nm (ϵ): 272 (4.31), 325 (4.26), λ_{max} (MeOH + $AlCl_3$) nm: 283, 345, λ_{max} (MeOH + CH_3COONa) nm: 278, 371; IR ν_{max} (film) cm^{-1}:3325, 1655, 1628, 1581, 1502, 1359, 1244, 1074; for ^{1}H NMR (C_5D_5N) and ^{13}C NMR (CD_3OD) see Tables 1 and 2; HR FABMS (negative) *m*/*z*: 561.1641 $[M–H]^-$($C_{27}H_{29}O_{13}$ requires 561.1608).

3.6. Rhamnellaflavoside C (**3**)

Yellow amorphous powder, $[\alpha]_D^{26}$ +33.6°(MeOH; *c* 0.83). UV λ_{max} (MeOH) nm (log ϵ): 272 (4.29), 325 (4.35), λ_{max} (MeOH + $AlCl_3$) nm: 281, 348, λ_{max} (MeOH + CH_3COONa) nm: 278, 363; IR ν_{max} (film)

Table 1
^{1}H NMR spectroscopic data for rhamnellaflavosides A (**1**), B (**2**) and C (**3**) (measured for d_5-pyridine solution at 400 MHz)

	1	2	3
3	6.88 (*s*)	6.88 (*s*)	6,89 (*s*)
8	6.74 (*s*)	6.78 (*s*)	6.77 (*s*)
2′,6′	7.88 (*d*, *J* = 8.8 Hz)	7.89 (*d*, *J* = 8.8 Hz)	7.86 (*d*, *J* = 8.8 Hz)
3′,5′	7.44 (*d*, J = 8.8 Hz)	7.44 (*d*, *J* = 8.8 Hz)	7.42 (*d*, *J* = 8.8 Hz)
1″	5.58 (*dd*, *J* = 3.2, 12.0 Hz)	6.24 (*dd*, *J* = 2.0, 11.6 Hz)	5.68 (*dd*, *J* = 2.0, 11.6 Hz)
2″	2.85 (*quart*., *J* = 12.0 Hz)	2.98 (*br. t-like*, *J* = *ca*12 Hz)	2.86 (*q*, J = 12.0)
	2.39 (*ddd*, *J* = 3.2, 3.6, 12.0 Hz)	2.29 (*br. d-like*, *J* = *ca* 13 Hz)	2.73 (*ddd*, J = 2.0, 4.4, 12.0 Hz)
3″-4″	Overlapped	Overlapped	Overlapped
5″	3.79 (*quart*., *J* = 6.4 Hz)	4.70 (*quart*., *J* = 6.4 Hz)	3.78 (*quart*., *J* = 5.6 Hz)
6″	1.15 (*d*, *J* = 6.4 Hz)	1.53 (*d*, *J* = 6.8 Hz)	1.67 (*d*, *J* = 5.6 Hz)
1‴	5.77 (*d*, *J* = 7.6 Hz)	5.77 (*d*, *J* = 7.2 Hz)	5.76 (*d*, *J* = 6.8 Hz)
2‴-5‴	Overlapped	Overlapped	Overlapped
6‴	4.63 (*brd*, *J* = 11.6 Hz)	Overlapped	4.63 (*brd*, *J* = 12.4 Hz)
5-OH	14.06	13.40	14.23

Table 2
^{13}C NMR spectroscopic data (δ) for rhamnellaflavosides A (**1**), B (**2**) and C (**3**) (measured for CD_3OD solution at 100 MHz)

C	1	2	3
2	165.6	165.7	163.8
3	104.8	104.8	104.7
4	184.0	184.1	182.9
5	158.8	158.8	159.8
6	111.2	111.7	110.7
7	164.5	164.8	163.7
8	96.1	96.1	95.1
9	158.3	158.3	157.1
10	105.0	105.0	104.8
1′	126.3	126.1	124.8
2′	129.2	129.3	128.5
3′	118.0	118.1	117.1
4′	162.1	162.1	161.2
5′	118.0	118.1	117.1
6′	129.2	129.3	128.5
1″	70.4	70.6	70.4
2″	33.5	32.8	39.0
3″	71.3	71.3	72.5
4″	73.0	72.6	73.3
5″	76.7	68.8	79.2
6″	17.7	17.4	18.9
1‴	101.7	101.7	101.6
2‴	74.8	74.8	74.8
3‴	78.3	78.3	78.5
4‴	71.3	71.3	71.2
5‴	77.9	77.9	78.2
6‴	62.4	62.5	62.3

cm^{-1}: 3322, 1653, 1628, 1581, 1502, 1367, 1244, 1074; for ^{1}H NMR (C_5D_5N) and ^{13}C NMR (CD_3OD) see Tables 1 and 2; HRFABMS (negative) *m*/*z* 561.1616 $[M–H]^-$ ($C_{27}H_{29}O_{13}$ requires 561.1608)

3.7. Rhamnellaflavoside A octaacetate (*1a*)

Rhamnellaflavoside A (**1**) (10.4 mg) was dissolved in a mixture of pyridine (0.1 ml) and Ac_2O (0.1 ml) and the solution was allowed to stand for 47 h at room temp. Excess MeOH was added to the mixture and the solvent was removed in vacuo to give a residue which was purified by prep. TLC (solvent: $CHCl_3$–Me_2CO 17:3) to give octaacetate (**1a**) (8.5 mg) as a colourless powder. ^{1}H NMR ($CDCl_3$) δ 1.19 (3H, *d*, *J* = 6.4 Hz, H-6″), 1.68 (1H, *m*, H-2″), 2.49 (1H, *m*, H-2″), 3.79 (1H, *q*, *J* = 6.4 Hz, H-5″), 3.90 (1H, *ddd*, *J* = 2.4, 5.2, 10.0 Hz, H-5‴), 4.19 (1H, *dd*, *J* = 2.4, 12.0 Hz, H-6‴a), 4.30 (1H, *dd*, *J* = 5.2, 12.0 Hz, H-6‴b), 4.81 (1H, *brd*, *J* = 11.2 Hz, H-1″), 5.09 (1H, *m*, H-3″), 6.58 (1H, *s*, H-3), 7.26 (1H, *s*, H-8), 7.10 (2H, *d*, *J* = 8.8 Hz, H-3′, 5′), 7.80 (2H, *d*, *J* = 8.8 Hz, H-2′, 6′), 2.00, 2.05, 2.06, 2.08, 2.09, 2.21, 2.44, 2.51 (each 3H, *s*, OAc); HRFABMS (positive, +NaI) *m*/*z*: 921.2426 $[M+Na]^+$ ($C_{43}H_{46}O_{21}Na$ requires 921.2429).

3.8. Acidic hydrolysis of rhamnellaflavoside A (*1*)

Rhamnellaflavoside A (**1**) (30.8 mg) was dissolved in 2 N H_2SO_4 (5 ml) and the solution was refluxed for 3.5 h. The resulting precipitates were removed by filtration and the filtrate neutralized with 2 N $Ba(OH)_2$. The precipitates were again removed by filters, with the filtrate evapourated in vacuo to give a residue (10.6 mg), $[\alpha]_D^{25}$ +28.9°(H_2O, *c* 0.53), which was identified as D-glucose by co-chromatography on silica gel plate with an authetic sample (solvent: *n*-BuOH:Me_2CO; H_2O 4:5:1, R_f 0.47).

3.9. Enzymatic hydrolysis of rhamnellaflavoside A (*1*)

Rhamnellaflavoside A (**1**) (30.6 mg) and β-glucosidase from almond (11.6 mg) was dissolved in H_2O (10 ml) and the solution was allowed to stand at 37 °C for 25 h. After addition of H_2O (10 ml), the reaction mixture was extracted with EtOAc (20 ml × 3). After drying, the EtOAc extract was concentrated in vacuo to give **1b** (20.1 mg). $[\alpha]_D^{26}$ +92.5°(C_5H_5N; *c* 1.50). UV λ_{max}

(MeOH) nm (log ϵ): 270 (4.46), 334 (4.52), λ_{max} (MeOH + $AlCl_3$) nm: 279, 304, 353, λ_{max} (MeOH + CH_3COONa) nm: 279, 382; ^{1}HNMR (DMSO-d_6): δ 1.19 (3H, *d*, J = 6.0 Hz, H-6″), 1.61 (1H, *brd*, J = 11.6 Hz, H-2″eq), 2.06 (1H, *q*, J = 11.6 Hz, H-2″ax), 5.00 (1H, *d*, J = 10.4 Hz, H-1″), 6.55 (1H, *s*, H-8), 6.81 (1H, *s*, H-3), 6.93 (2H, *d*, J = 8.4 Hz, H-3′, 5′), 7.94 (2H, *d*, J = 8.4 Hz, H-2′, 6′), 13.51 (5-OH); ^{13}C NMR (DMSO-d_6):δ17.4 (C-6″), 32.2 (C-2″), 68.4 (C-3″), 69.5 (C-4″), 70.0 (C-1″), 74.3 (C-5″), 94.6 (C-8), 102.6 (C-3), 103.2 (C-10), 109.9 (C-6), 115.9 (C-3′,5′), 120.9 (C-1′), 128.4 (C-2′,6′), 156.0 (C-9), 157.3 (C-5), 161.2 (C-4′), 162.4 (C-7), 163.8 (C-2), 181.8 (C-4); HRFABMS (negative) *m*/*z*: 399.1106 [M-H] ($C_{21}H_{19}O_8$ requires 399.1080).

3.10. Rhamnellaflavoside B octaacetate (**2a**)

Rhamnellaflavoside B (**2**) (1.7 mg) was acetylated and purified as described above to give the octaacetate (**2a**) (1.8 mg). ^{1}H NMR ($CDCl_3$): δ 1.16 (3H, *d*, J = 6.8 Hz, H-6″), 3.92 (1H, *m*, H-5‴), 4.04 (1H, *br quart.*, J = 6.4 Hz, H-5″), 4.19 (1H, *brd*, J = 12.0 Hz, H-6‴a), 4.30 (1H, *dd*, J = 5.2, 12.0 Hz, H-6‴b), 5.08 (1H, *m*, H-3″), 6.58 (1H, *s*, H-3), 7.25 (1H, *s*, H-8), 7.10 (2H, *d*, J = 8.8 Hz, H-3′,5′), 7.80 (2H, *d*, J = 8.8 Hz, H-2′, 6′), 2.05, 2.06, 2.08, 2.09, 2.15, 2.19, 2.41, 2.47 (each 3H, *s*, OAc); HRFABMS (positive, + NaI) *m*/*z*: 921.2427 $[M + Na]^+$ ($C_{43}H_{46}O_{21}Na$ requires 921.2429).

3.11. Enzymatic hydrolysis of rhamnellaflavoside B (**2**)

Rhamnellaflavoside B (**2**) (10.9 mg) and β-glucosidase from almond (9.1 mg) was dissolved in a mixture of DMSO (0.5 ml) and H_2O (5 ml) and the solution was treated as above to give a residue which was applied on a silica gel column (5 g) eluted with a mixture of $CHCl_3$ and MeOH with increasing amounts of MeOH content. The 10% MeOH eluate gave **2b** (2.6 mg). $[\alpha]_D^{26}$ +33.3°(C_5H_5N; *c* 0.11). UV_{max} (MeOH) nm (log ϵ): 271 (4.11), 333 (4.17), λ_{max} (MeOH + $AlCl_3$) nm: 280, 304, 353, λ_{max} (MeOH + CH_3COONa) nm: 273, 382; ^{1}H NMR (d_6-DMSO) δ: 1.15 (3H, *d*, J = 6.4 Hz, H-6″), 1.48 (1H, *brd*, J = 13.8 Hz, H-2″eq), 2.23 (1H, *brt*, J = 11.6 Hz, H-2″ax), 3.23 (1H, *d*, J = 3.6 Hz, H-4″), 3.84 (1H, *brs*, H-3″), 4.03 (1H, *q*, J = 6.4 Hz, H-5″), 5.31 (1H, *dd*, J = 2.4, 12.0 Hz, H-1″), 6.53 (1H, *s*, H-8), 6.80 (1H, *s*, H-3), 6.92 (2H, *d*, J = 8.8 Hz, H-3′, 5′), 7.93 (2H, *d*, J = 8.8 Hz, H-2′, 6′), 13.46 (1H, *s*, 5-OH); ^{13}C NMR (DMSO-d_6) δ: 17.0 (C-6″), 31.2 (C-2″), 67.2 (C-3″), 68.4 (C-4″), 70.5 (C-1″), 66.3 (C-5″), 94.6 (C-8), 102.7 (C-3), 103.3 (C-10), 110.2 (C-6), 115.8 (C-3′, 5′), 121.0 (C-1′), 128.4 (C-2′,6′), 156.0 (C-9), 159.9 (C-5), 161.0 (C-4′), 162.3 (C-7), 163.7 (C-2), 181.8 (C-4); HRFABMS (negative) *m*/*z*: 399.1055 [M–H] ($C_{21}H_{19}O_8$ requires 399.1080).

The H_2O layer was concentrated in vacuo to give a residue which was purified by chromatography over silica gel (5 g) with a mixture of $CHCl_3$–MeOH to give D-glucose (1.2 mg) (identified as above), $[\alpha]_D^{24}$ +45.0°(H_2O; *c* 0.06).

3.12. Rhamnellaflavoside C octaacetate (**3a**)

Rhamnellaflavoside C (**3**) (3.0 mg) was acetylated and processed as above to give octaacetate (**3a**) (3.7 mg). ^{1}H NMR ($CDCl_3$): δ 1.20 (3H, *d*, J = 6.8 Hz, H-6″), 3.57 (1H, *m*, H-5‴), 3.92 (1H, *ddd*, J = 2.4, 5.2, 10.4 Hz, H-5″), 4.19 (1H, *dd*, J = 2.0, 12.4 Hz, H-6‴a), 4.30 (1H, *dd*, J = 5.2, 12.4 Hz, H-6‴b), 4.77 (1H, *t*, J = 9.2 Hz, H-2‴), 4.85 (1H, *brd*, J = 12.0 Hz, H-1″), 5.11 (1H, *m*, H-3″), 6.58 (1H, *s*, H-3), 7.26 (1H, *s*, H-8), 7.10 (2H, *d*, J = 9.2 Hz, H-3′,5′), 7.80 (2H, *d*, J = 9.2 Hz, H-2′, 6′), 2.02, 2.03, 2.05, 2.06, 2.08, 2.08, 2.40, 2.49 (each 3H, *s*, OAc); HRFABMS (positive, + NaI) *m*/*z*:921.2424 $[M + Na]^+$ ($C_{43}H_{46}O_{21}Na$ requires 921.2429).

3.13. Enzymatic hydrolysis of rhamnollaflavoside C (**3**)

Rhamnellaflavoside C (**3**) (22.9 mg) and β-glucosidase from almond (16.0 mg) was incubated as in Section 3.11 to give **3b** (5.6 mg). $[\alpha]_D^{26}$ +39.1°(C_5H_5N; *c* 0.19). UV λ_{max} (MeOH) nm (log ϵ): 270 (4.26), 334 (4.29), λ_{max} (MeOH + $AlCl_3$) nm: 278, 303, 353, λ_{max} (MeOH + CH_3COONa) nm: 272, 357; ^{1}H NMR (DMSO-d_6): δ 1.19 (3H, *d*, J = 6.4 Hz, H-6″), 1.78 (1H, *ddd*, J = 2.0, 4.8, 12.8 Hz, H-2″eq), 2.28 (1H, *q*, J = 11.6 Hz, H-2″ax), 2.90 (1H, *t*, J = 8.8 Hz, H-4″), 3.25 (1H, *dq*, J = 9.2, 6.0 Hz, H-5″), 3.45 (1H, *m*, H-3″), 4.91 (1H, *brd*, J = 10.0 Hz, H-1″), 6.52 (1H, *s*, H-8), 6.78 (1H, *s*, H-3), 6.93 (2H, *d*, J = 8.8 Hz, H-3′,5′), 7.92 (2H, *d*, J = 8.8 Hz, H-2′, 6′), 13.59 (1H, *s*, OH); ^{13}C NMR (DMSO-d_6): δ 18.2 (C-6″), 36.9 (C-2″), 69.3 (C-1″), 72.1 (C-3″), 76.4 (C-4″), 76.8 (C-5″), 93.8 (C-8), 102.7 (C-3), 103.3 (C-10), 109.8 (C-6), 115.9 (C-3′,5′)121.0 (C-1′), 128.4 (C-2′,6′), 155.9 (C-9), 159.2 (C-5), 161.1 (C-4′), 162.3 (C-7), 163.6 (C-2), 181.9 (C-4); HRFABMS (negative) *m*/*z*: 399.1060 [M–H] ($C_{21}H_{19}O_8$ requires 399.1080).

The aqueous layer was purified as above to give D-glucose (2.4 mg), $[\alpha]_D^{24}$ +40.0°(H_2O; *c* 0.24).

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