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TWO FLAVONOL GLYCOSIDES FROM SEEDS OF *CAMELLIA SINENSIS*

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Key Word Index—*Camellia sinensis*; Theaceae; tea-seed cake; flavonol glycosides; camelliaside A; camelliaside B.

Abstract—Two novel flavonol triglycosides, camelliaside A and B, have been isolated from seeds of *Camellia sinensis*. The structures were determined to be kaempferol 3-*O*-[2-*O*-*β*-D-galactopyranosyl-6-*O*-*α*-L-rhamnopyranosyl]-*β*-D-glucopyranoside and kaempferol 3-*O*-[2-*O*-*β*-D-xylopyranosyl-6-*O*-*α*-L-rhamnopyranosyl]-*β*-D-glucopyranoside on the basis of spectroscopic, chemical and enzymatic studies. These types of interglycosidic linkages, Gal(1→2)[Rha(1→6)]Glc and Xyl(1→2)[Rha(1→6)]Glc, have not been reported previously in flavone and flavonol glycosides.

INTRODUCTION

Camellia sinensis O. Kuntze is known to contain caffeine, vitamin C, tannins and saponins and has been cultivated widely in Asia for centuries as a source of green, black and oolong teas. In the course of our investigations on the biologically active constituents of Thai traditional medicines [1–5], we have isolated two new flavonol glycosides, camelliaside A (**1**) and B (**2**), from the Thai crude drug, 'tea seed cake'. The latter is prepared from the seeds of *C. sinensis* by defatting and is used for skin diseases and as a fertilizer [6]. More than 30 flavonoid constituents, including kaempferol, quercetin, myricetin and their glycosides have been identified previously from the leaves and shoots of *C. sinensis* [7–14]. The present paper describes the isolation and structural determination of the novel flavonol triglycosides **1** and **2**.

RESULTS AND DISCUSSION

Camelliaside A (**1**) gave a positive coloration in the Mg–HCl test and was positive to $FeCl_3$ reagent on TLC. The IR spectrum suggested the presence of hydroxyl (3400 and 1075 cm^{-1}), carbonyl (1650 cm^{-1}) and phenyl (1610 and 840 cm^{-1}) groups. The UV spectrum in methanol displayed λ_{max} at 347.8 and 265.2 nm, suggesting that **1** was a flavone or flavonol [15, 16]. The bathochromic shifts of the UV bands of **1** with NaOAc (λ_{II} = +7.6 nm) and $AlCl_3$ (λ_I = +46.6 nm) suggested the presence of free 7- and 5-dihydroxyl groups. Acid hydrolysis of **1** with 3% H_2SO_4 gave kaempferol, galactose, glucose and rhamnose. Compound **1** showed a quasi-molecular ion $[M+H]^+$ at *m/z* 757 in its positive-ion FAB mass spectrum, and ion $[M-H]^-$ at *m/z* 755 in the negative-ion FAB mass spectrum. Two significant fragment ion peaks at *m/z* 609 [755 (M−H)−146 (deoxyhexose unit)]$^-$ and at *m/z* 593 [755 (M−H)−162 (hexose unit)]$^-$ were observed in the negative-ion FAB mass spectrum. The per-*O*-acetate of **1** prepared in the usual manner (Ac_2O–pyridine) showed peaks at *m/z* 331 [(terminal hexose) Ac_4]$^+$ and at *m/z* 273 [(terminal deoxyhexose) Ac_3]$^+$ in its EI mass spectrum. These results indicate that **1** is a kaempferol 3-*O*-triglycoside with a branched sugar moiety. This was confirmed by the MS/MS spectrum of **1** (the data is shown in the Experimental). The general features of the NMR spectra also support this idea. Thus, the ^1H NMR spectrum showed four kinds of aromatic protons [δ8.02 (2H, *d*, *J* = 8.8 Hz), 6.90 (2H, *d*, *J* = 8.8 Hz), 6.38 (1H, *s*) and 6.19 (1H, *s*)] due to the kaempferol skeleton together with three anomeric protons [δ5.33 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 4.76 (1H, *d*, *J* = 7.4 Hz, Gal H-1) and 4.47 (1H, *d*, *J* = 1.4 Hz, Rha H-1). In addition, 31 carbon signals were observed in the ^{13}C NMR spectrum (Table 1). Among them, 13 carbon signals were assigned to the kaempferol skeleton and three carbon signals [δ104.6, 102.3 and 101.2] to anomeric carbons of the sugar moiety. Interglycosidic linkage points of **1** were determined from the identification of the partial hydrolysates (**3**–**5**) obtained by enzymatic and acid hydrolyses.

On enzymatic hydrolysis with crude hesperidinase, **1** furnished a partial hydrolysate **3** (68%) (Scheme 1). On the other hand, enzymatic hydrolysis of **1** with cellulase under the same conditions yielded a different partial hydrolysate **4** (74%). Enzymatic hydrolysis of **1** with *β*-glucosidase under similar conditions gave kaempferol. Inspection of the NMR spectra of **3** and **4** indicated that they are both kaempferol diglycosides. The structure of **3** was determined to be kaempferol 3-*O*-*β*-galactopyranosyl(1→2)-*β*-glucopyranoside by the spectroscopic and chemical (acid hydrolysis) evidence. This is the first characterization of **3**, although kaempferol 3-*O*-galactosylglucoside has been isolated in the course of chemotaxonomic studies [17, 18]. The structure of **4** was identified as kaempferol 3-*O*-*α*-L-rhamnopyranosyl (1→6)-*β*-D-glucopyranoside (nicotiflorin) by direct comparison of physical, chemical and HPLC data with those

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Table 1. ^{13}C NMR data of compounds **1**–**5** (100 MHz, CD_3OD, TMS)

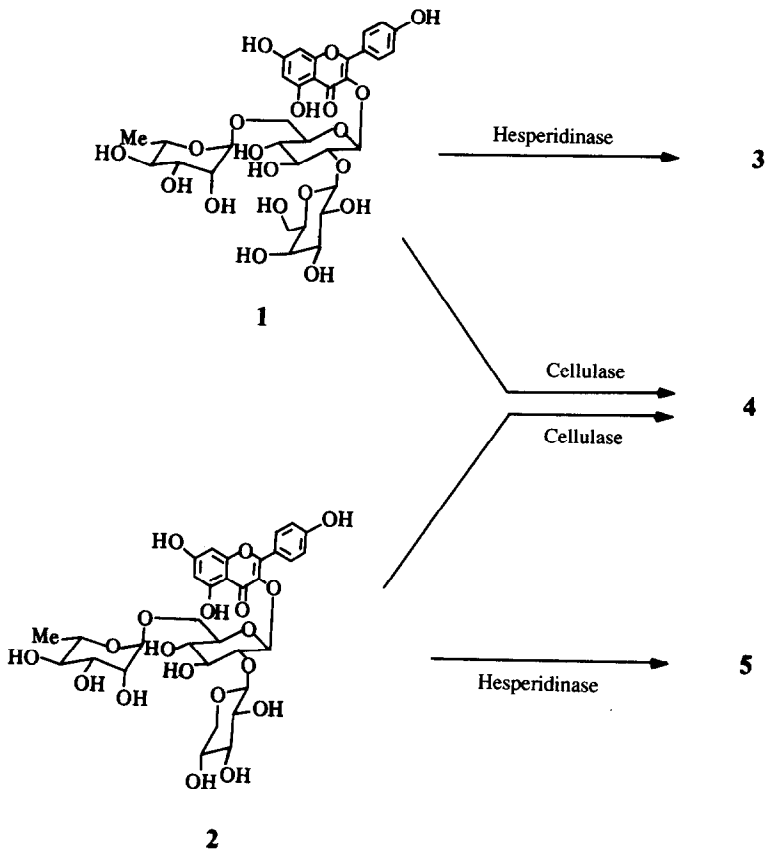
C	1	2	3	4	5
Kaempferol					
2	158.7	158.5	158.5	158.5	158.5
3	134.5	134.5	134.9	135.5	135.0
4	179.2	179.1	179.6	174.4	179.5
5	161.6	161.1	163.0	163.0	160.0
6	101.0	100.7	100.1	100.1	100.3
7	162.8	162.5	166.5	166.3	167.2
8	95.7	95.5	94.9	95.0	95.0
9	159.3	158.5	158.9	159.4	158.3
10	105.0	105.1	105.6	105.6	105.5
1′	123.0	122.9	122.8	122.8	122.8
2′,6′	132.4	132.3	132.4	132.4	132.3
3′,5′	116.3	116.3	116.3	116.2	116.2
4′	159.3	158.7	161.5	161.5	161.5
Glucose					
1	101.2	100.7	101.1	104.9	100.9
2	82.3	81.9	82.2	75.7	82.3
3	78.3	77.9	78.1[a]	78.1	78.1[a]
4	72.1[a]	71.2[a]	71.2[b]	71.4[a]	71.0[b]
5	77.8[b]	76.7	78.1[a]	77.2	77.1
6	68.3	68.0	62.4[c]	68.5	62.3
Rhamnose					
1	102.3	102.0		102.5	
2	72.3[a]	72.1		72.3[a]	
3	73.9	73.6		72.1[a]	
4	71.4[c]	71.8[a]		73.9[a]	
5	69.7	69.7		69.7	
6	17.9	17.8		18.0	
Galactose					
1	104.6		104.5		
2	75.4		75.4		
3	76.9[b]		77.8		
4	71.2[c]		71.0[b]		
5	77.8[b]		77.8[a]		
6	62.4		62.5[c]		
Xylose					
1		105.0			105.4
2		74.7			74.9
3		76.9			78.2[a]
4		70.8[a]			71.0[b]
5		66.5			66.7

[a–c]Assignments may be interchanged in each column.

of an authentic sample. Nicotiflorin (**4**) has been isolated from many plants [e.g. 19–21] including *C. sinensis* [8, 9, 11, 13]. The anomeric configuration of the galactopyranoside and glucopyranoside linkages were both determined to be β from the *J* values of their anomeric proton signals; Gal H-1 ($J = 6.2$ Hz); Glc H-1 ($J = 7.3$ Hz). Therefore, crude hesperidinase and cellulase selectively cleaved the rhamnosyl and galactosyl moiety from **1** to give partial hydrolysates, **3** and **4**, respectively. Furthermore, controlled acid hydrolysis of **1** under mild conditions using 0.1, 0.01 M HCl or 50% acetic acid provided mainly nicotiflorin (**4**). In a comparison of the ^{13}C NMR spectrum of **1** with those of **3** and **4**, the glycosylation shifts were observed for the carbon signals due to C-2 (+6.6 ppm) and C-6 (+5.9 ppm) of the glucopyranosyl moiety (Table 1). These data, coupled with the results of the partial hydrolyses, suggest that the galactose in **1** is linked to the C-2 position of the glucosyl moiety, and that the rhamnose is linked to the C-6 position.

From the spectroscopic, chemical and enzymatic evidence, the structure of camelliaside A (**1**) was determined to be kaempferol-3-*O*-[2-*O*-β-D-galactopyranosyl-6-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside. The absolute configurations of glucose, galactose and rhamnose residues in **1** were presumed to be D, D and L, respectively, from the natural occurrence of these sugars.

Camelliaside B (**2**) displayed less polar behaviour on TLC [solvent A] than camelliaside A (**1**). Compound **2** gave a positive coloration in the Mg–HCl test and was positive to $FeCl_3$ reagent on TLC. The IR and UV spectra of **2** were very similar to those of **1**, suggesting that **2** is also a kaempferol 3-*O*-glycoside. On acid hydrolysis, **2** liberated kaempferol, glucose, rhamnose and xylose. Positive- and negative-ion FAB mass spectra of **2** gave



Scheme 1.

quasi-molecular ions, $[M+H]^+$ at *m/z* 727 and $[M-H]^-$ at *m/z* 725, respectively. Furthermore, two significant fragment ion peaks at *m/z* 579 [725 (M − H) − 146 (deoxyhexose unit)]$^-$ and at *m/z* 593 [725 (M − H) − 132 (pentose unit)]$^-$ were observed in the negative-ion FAB mass spectrum. The per-*O*-acetate of **2** showed peaks at *m/z* 273 [(terminal deoxyhexose) Ac_3]$^+$ and at *m/z* 259 [(terminal pentose)Ac_3]$^+$ in its EI mass spectrum. Therefore, **2** was also considered to be a kaempferol 3-*O*-triglycoside which has a branched sugar moiety. Inspection of the NMR spectra of **2** indicated it has kaempferol and three monosaccharide units. Namely, the ^{1}H NMR spectrum showed four kinds of aromatic protons [δ8.05 (2H, *d*, *J* = 8.8 Hz), 6.89 (2H, *d*, *J* = 8.8 Hz), 6.34 (1H, *s*) and 6.16 (1H, *s*)] due to kaempferol together with three anomeric protons [δ5.37 (1H, *d*, *J* = 7.5 Hz, Glc H-1), 4.78 (1H, *d*, *J* = 6.8 Hz, Xyl H-1) and 4.49 (1H, *s*, Rha H-1). Furthermore, 30 carbon signals were observed in the ^{13}C NMR spectrum (Table 1). Among them, 13 carbon signals were assigned to the kaempferol skeleton and three carbon signals [δ105.0, 102.0, 100.7] were due to the anomeric carbons of the sugar moiety.

Enzymatic hydrolysis of **2** with crude hesperidinase furnished a partial hydrolysate **5** (74%) (Scheme 1). On the other hand, it was observed that **2** gave a different partial hydrolysate **4**, on TLC and HPLC, on enzymatic hydrolysis with cellulase under the same conditions, and on acid hydrolysis under mild conditions. The NMR spectra of **5** indicated that it is a kaempferol diglycoside. The structure of **5** was identified as kaempferol 3-*O*-β-xylopyranosyl(1→2)-β-glucopyranoside (leucoside) from the spectroscopic and chemical evidence. Leucoside (**5**) has been characterized from *Lilium candidum* L. [22]. Therefore, crude hesperidinase and cellulase selectively cleaved the rhamnosyl and xylosyl moiety from **2** to give **5** and **4**, respectively. Both C-2 and C-6 of the glucosyl moiety in **2** were shifted downfield by 6.2 and 5.7 ppm, respectively, compared with those of **4** and **5** (Table 1). The above observations suggest that the xylose in **2** is linked to the C-2 position of the glucosyl moiety, and the rhamnose to the C-6 position.

Based on the above and by assuming that glucose, xylose and rhamnose residues have D, D and L configurations, respectively, the structure of camelliaside B was concluded to be kaempferol 3-*O*-[2-*O*-β-D-xylopyranosyl-6-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**2**).

Kaempferol triglycosides containing glucose, galactose, xylose or rhamnose previously isolated include a kaempferol 3-*O*-xylosylrhamnosylglucoside from the pollen of a garden tulip [23], and a 3-*O*-rhamnosylglucosylgalactoside from the leaves of *Peltophorum africanum* [24]. As far as we know, the kaempferol triglycosides presented in this paper have not been previously characterized. Furthermore, the branched triglycoside moieties,

Gal (1→2) [Rha (1→6)]Glc and Xyl(1→2) [Rha(1→6)]Glc are reported for the first time in flavone and flavonol glycosides [25].

EXPERIMENTAL

General. Mps: uncorr. IR were measured in KBr disks. FABMS and MS/MS (collisionally activated decomposition spectra) were recorded on JEOL HX-110 of EBE geometry, and EIMS at 70 eV. ^{1}H and ^{13}C NMR were all recorded on 270, 400 and 500 MHz spectrometers with TMS as int. standard, and chemical shifts are given in δ (ppm) from TMS. TLC were performed on silica gel (Merck, Kieselgel 60 F_{254}) using *n*-BuOH–EtOAc–H_2O (4:1:4, upper phase, solvent A) or $CHCl_3$–MeOH–H_2O (13:7:2, lower phase, solvent B) as eluents.

Extraction and separation. Tea seed cake was obtained at Chiang Mai, Thailand (1988). The tea seed cake (300 g) cut into small pieces was macerated with boiling H_2O (1.2 l) for 30 min. The concd H_2O extract (300 ml) was extracted successively with EtOAc (300 ml, ×3) and *n*-BuOH (400 ml, ×5). A portion (7.12 g) of the concd *n*-BuOH extract (15.12 g) was chromatographed repeatedly over Sephadex LH-20 (Pharmacia Fine Chemicals, 25–100 μm) and eluted with a H_2O–MeOH gradient to yield camelliaside A (410 mg, 0.29%/dry wt) and camelliaside B (454 mg, 0.32%/dry wt). The R_f values of silica gel TLC of camelliaside A and B were 0.34 and 0.40, respectively, using solvent A.

Camelliaside A (**1**). Yellow amorphous; mp 203–205°; $[\alpha]_D$ −39.6° (MeOH; *c* 0.35); UV λ_{max}^{MeOH} nm (log ε): 347.8 (4.12), 265.2 (4.22); (NaOH) 396.4, 324.4, 273.4; ($AlCl_3$) 394.4, 350.2, 303.2, 272.8; ($AlCl_3$ + HCl) 393.4, 346.4, 301.8, 273.8; (NaOAc) 369.0, 301.4, 272.4; (NaOAc–H_3BO_3) 349.8, 265.8; IR ν_{max} cm^{-1}: 3400, 2920, 1650, 1610, 1075, 845, 810; positive-ion FABMS [glycerol] *m/z* (rel. int.) 757 (8) $[M+H]^+$, 286 (22) [kaempferol]$^+$, 287 (33) [kaempferol + H]$^+$; negative-ion FABMS [thioglycerol + glycerol] *m/z* (rel. int.) 755 (71) $[M-H]^-$, 609 (10) $[M-Rha-H]^-$, 593 (17) $[M-Gal-H]^-$, 285 (100) [kaempferol − H]$^-$; EIMS of per-*O*-acetate *m/z* (rel. int.) 331 (6.3) [(Gal)Ac_4]$^+$, 273 (5.3) [(Rha)Ac_3]$^+$, 286 (29.7) [kaempferol]$^+$; MS/MS *m/z* (rel. int.) 757 (100) $[M+H]^+$, 611 (0.8), 595 (2.4), 449 (1.5), 286 (8.5); ^{1}H NMR δ8.02 (2H, *d*, *J* = 8.8 Hz, H-2′, H-6′), 6.90 (2H, *d*, *J* = 8.8 Hz, H-3′, H-5′), 6.38 (1H, *s*, H-8), 6.19 (1H, *s*, H-6), 5.33 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 4.76 (1H, *d*, *J* = 7.4 Hz, Gal H-1), 4.47 (1H, *d*, *J* = 1.4 Hz, Rha H-1), 3.8–3.2 (16H, *m*), 1.10 (3H, *d*, *J* = 6.3 Hz, Rha H-6); ^{13}C NMR see Table 1.

Camelliaside B (**2**). Yellow amorphous; mp 194–196°; $[\alpha]_D$ −57.4° (MeOH; *c* 0.36); UV λ_{max}^{MeOH} nm (log ε): 347.6 (4.06), 265.2 (4.16); (NaOH) 395.0, 323.4, 273.6; ($AlCl_3$) 395.8, 350.2, 273.0; ($AlCl_3$ + HCl) 395.0, 345.6, 301.6, 274.2; (NaOAc) 371.4, 301.8, 272.8; (NaOAc–H_3BO_3) 350.0, 266.0, 210.2; IR λ_{max} cm^{-1}: 3450, 2920, 1660, 1610, 1070, 840, 810; positive-ion FABMS [glycerol] *m/z* (rel. int.) 727 (12) $[M+H]^+$, 286 (20) [kaempferol]$^+$, 287 (32) [kaempferol + H]$^+$; negative-ion FABMS [thioglycerol + glycerol] *m/z* (rel. int.) 725 (35) $[M-H]^-$, 593 (7) $[M-Xyl-H]^-$, 579 (3.8) $[M-Rha-H]^-$, 285 (100) [kaempferol − H]$^-$; EIMS of per-*O*-acetate *m/z* (rel. int.) 286 (30.8) [kaempferol]$^+$, 273 (6.5) [(Rha)Ac_3]$^+$, 259 (7.1) [(Xyl)Ac_3]$^+$; MS/MS *m/z* (rel. int.) 727 (100) $[M+H]^+$, 595 (4.9), 581 (1.3), 449 (2.6), 286 (11.2); ^{1}H NMR δ8.05 (2H, *d*, *J* = 8.8 Hz, H-2′, H-6′), 6.89 (2H, *d*, *J* = 8.8 Hz, H-3′, H-5′), 6.34 (1H, *s*, H-8), 6.16 (1H, *s*, H-6), 5.37 (1H, *d*, *J* = 7.5 Hz, Glc H-1), 4.78 (1H, *d*, *J* = 6.8 Hz, Xyl H-1), 4.49 (1H, *s*, Rha H-1), 4.0–3.2 (15H, *m*), 1.10 (3H, *d*, *J* = 6.3 Hz, Rha H-6); ^{13}C NMR see Table 1.

Acid hydrolysis of camelliaside A and B. Camelliaside A or B (10 mg) in 3% H_2SO_4 (3 ml) was heated at 100° for 1 hr. Extraction with EtOAc gave kaempferol; yellow needles; mp 275–277°; UV λ_{max}^{MeOH} nm: 365.0, 266.0; ^{1}H NMR δ8.09 (2H, *d*, *J* = 8.8 Hz, H-2′, H-6′), 6.91 (2H, *d*, *J* = 8.8 Hz, H-3′, H-5′), 6.38 (1H, *s*, H-8), 6.19 (1H, *s*, H-6). The water layer was neutralized with 5% NH_4OH and concd *in vacuo*. The residue was trimethylsilylated with TMSCl and analysed by GLC [Shimazu GC 8A system equipped with a glass column (3 mm × 2 m) containing 2% OV-17 on Gas Chrom Q]. Authentic sugar samples were treated in the same manner and R_t values compared with those from **1** and **2**.

Enzymatic hydrolysis of camelliaside A (**1**) *with crude hesperidinase.* Compound **1** (100 mg) with crude hesperidinase (200 mg, provided by Tanabe Pharm. Co., Ltd, Osaka, Japan), in citrate–Pi buffer (pH 5.0, 10 ml) was incubated at 37° for 4 hr. The reaction mixt., diluted with H_2O was extracted with *n*-BuOH. The washed concd extract was subjected to CC on Sephadex LH-20 using H_2O as eluent to afford **3** (55.1 mg, 68.2%), R_f on silica gel TLC for **3**, 0.54 (solvent A); yellow amorphous; mp 199–201°; $[\alpha]_D$ −47.5° (MeOH; *c* 0.44); UV λ_{max}^{MeOH} nm (log ε): 348.0 (4.19), 265.8 (4.30); IR λ_{max} cm^{-1}: 3350, 2930, 1660, 1600, 1060, 890, 820; EIMS of per-*O*-acetate *m/z* (rel. int.) 331 (2.9) [(Gal)Ac_4]$^+$, 286 (15.5) [kaempferol]$^+$; ^{1}H NMR δ8.03 (2H, *d*, *J* = 8.8 Hz, H-2′, H-6′), 6.90 (2H, *d*, *J* = 8.8 Hz, H-3′, H-5′), 6.35 (1H, *s*, H-8), 6.15 (1H, *s*, H-6), 5.40 (1H, *d*, *J* = 7.3 Hz, Glc H-1), 4.78 (1H, *d*, *J* = 6.2 Hz, Gal H-1), 3.8–3.2 (12H, *m*); ^{13}C NMR see Table 1.

Enzymatic hydrolysis of camelliaside B (**2**) *with crude hesperidinase.* Compound **2** (100 mg) with crude hesperidinase (200 mg) in citrate-Pi buffer (pH 5, 10 ml) was incubated at 37° for 4 hr. Work-up as described above gave leucoside (**5**) (58.8 mg, 73.6%). The R_f on silica gel TLC for **5** is 0.66 (solvent A); yellow amorphous; mp 203–205°; $[\alpha]_D$ −75.6° (MeOH; *c* 0.47) (lit. [22] $[\alpha]_D$ −52.5°); UV λ_{max}^{MeOH} nm (log ε): 347.8 (4.22), 265.4 (4.31); IR λ_{max} cm^{-1}: 3350, 2990, 1660, 1610, 1070, 890, 840, 810; EIMS of per-*O*-acetate *m/z* (rel. int.) 286 (13.0) [kaempferol]$^+$, 259 (3.2) [(Xyl)Ac_3]$^+$; ^{1}H NMR δ8.06 (2H, *d*, *J* = 8.8 Hz, H-2′, H-6′), 6.89 (2H, *d*, *J* = 8.8 Hz, H-3′, H-5′), 6.33 (1H, *s*, H-8), 6.14 (1H, *s*, H-6), 5.42 (1H, *d*, *J* = 7.6 Hz, Glc H-1), 4.76 (1H, *d*, *J* = 6.9 Hz, Xyl H-1), 4.0–3.2 (11H, *m*); ^{13}C NMR see Table 1.

Enzymatic hydrolysis of camelliaside A (**1**) *with cellulase.* Compound **1** (100 mg) with cellulase (Onozuka R-10, 100 mg, Yakult Co., Ltd, Japan) in citrate–Pi buffer (pH 5.0, 10 ml) was incubated at 37° for 10 days. The ppt. was recrystallized from H_2O to give nicotiflorin (**4**) (58.2 mg, 74.0%). R_f on silica gel TLC for **4** is 0.56 (solvent A); yellow needles; mp 186–188° (lit. [11] mp 181–184°); $[\alpha]_D$ −13.6° (MeOH; *c* 0.51); UV λ_{max}^{MeOH} nm (log ε): 349.0 (4.17), 265.0 (4.24); IR ν_{max} cm^{-1}: 3400, 2940, 1660, 1610, 1060, 845, 815; EIMS of per-*O*-acetate *m/z* (rel. int.) 561 (3.1) [(Glc-Rha)Ac_6]$^+$, 286 (43.3) [kaempferol]$^+$, 273 (100) [(Rha)Ac_3]$^+$; ^{1}H NMR δ8.06 (2H, *d*, *J* = 8.8 Hz, H-2′, H-6′), 6.88 (2H, *d*, *J* = 8.8 Hz, H-3′, H-5′), 6.39 (1H, *d*, *J* = 2.0 Hz, H-8), 6.20 (1H, *d*, J = 2.0 Hz, H-6), 5.12 (1H, *d*, *J* = 7.6 Hz, Glc H-1), 4.51 (1H, *d*, *J* = 1.7 Hz, Rha H-1), 3.8–3.2 (10H, *m*), 1.11 (3H, *d*, *J* = 5.9 Hz, Rha H-6); ^{13}C NMR see Table 1.

Enzymatic hydrolysis of camelliaside B (**2**) *with cellulase.* Compound **2** (1 mg) with cellulase (1 mg) in citrate–Pi buffer (pH 5.0, 0.3 ml) was incubated at 37° for 10 days. The product was identified as nicotiflorin by TLC and HPLC analysis [Inertsil ODS-2 (5 μm), 40 × 10 mm) (Gasukuro Kogyo Inc.), H_2O–MeOH, 1:1].

Controlled acid hydrolyses of camelliaside A and B. Compounds **1** or **2** (1 mg) were dissolved in 0.1 or 0.01 M HCl [or 50% HOAc] (0.3 ml) and the soln heated at 80–90° for 15–120 min [8–24 hr]. After cooling, the reaction mixt. was extracted with *n*-BuOH. Nicotiflorin (**4**) from the *n*-BuOH extract was identified by TLC and HPLC analysis as described above.

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