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FOUR KAEMPFEROL GLYCOSIDES FROM LEAVES OF *CINNAMOMUM SIEBOLDII*

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Key Word Index—*Cinnamomum sieboldii*; Lauraceae; leaves; flavonoids; kaempferol glycosides.

Abstract—Four flavonol glycosides were isolated from *Cinnamomum sieboldii* and characterized as kaempferol 7-*O*-α-L-rhamnopyranosides having 3-*O*-α-L-rhamnopyranosyl, 3-*O*-α-L-arabinofuranosyl, 3-*O*-β-D-apiofuranosyl-(1 → 2)-α-L-arabinofuranosyl and 3-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl residues, respectively. The latter three are new compounds.

INTRODUCTION

In the course of our research on *Cassia* diterpenes [1–3], which were obtained from the fraction exhibiting anti-allergic activity of the bark of *C. cassia*, we have now isolated four flavonol glycosides, **1**–**4**, from the leaves of *C. sieboldii*. This paper deals with their chemical characterization.

RESULTS AND DISCUSSION

Glycoside **1** gave a brown colour with ferric chloride. Its IR spectrum suggested the presence of hydroxyl and carbonyl groups and an aromatic ring. On acid hydrolysis with 2 N hydrochloric acid–methanol, **1** gave kaempferol (**5**) and rhamnose. The mass spectrum of the octaacetate (**6**) of **1** showed fragments due to the terminal per-acetylated methylpentose (*m/z* 273) and the aglycone moiety (*m/z* 558, 328 and 286). The ^{1}H NMR spectrum ($CDCl_3$) showed eight acetoxy methyl groups (δ 1.98–2.42), two secondary methyl groups (each 3H, *d*, *J* = 7 Hz, at δ 0.88 and 1.16), six aromatic protons (each 1H, *d*, *J* = 2 Hz at δ 6.76 and 7.09, and each 2H, *d*, *J* = 9 Hz at δ 7.28 and 7.90). These observations indicated that **1** was a kaempferol bisrhamnoside. The location of two rhamnosyl groups in **1** was shown to be at the C-3 and C-7 hydroxyl groups on **5** by comparison of the ^{13}C NMR spectrum (pyridine-d_5) of **1** with those of kaempferol and methyl α-L-rhamnopyranoside. That is, as shown in Table 1, the signals ascribable to C-2, C-3 and C-7 in **1** were shifted by +9.2, +1.1 and −2.8 ppm, respectively, compared with those of **5**, suggesting the presence of rhamnosyl linkages to the hydroxyl groups at both C-3 and C-7 on **5** [4].

Enzymic hydrolysis of **1** using crude hesperidinase readily afforded a desmonorhamnosyl compound (**7**), which was identified as kaempferol 3-*O*-α-L-rhamnopyranoside by ^{13}C NMR. Accordingly, **1** is kaempferol 3,7-*O*-bis-α-L-rhamnopyranoside, being assumed to be identical with kaempferitrin [5].

Glycoside **2** on acid hydrolysis yielded rhamnose and arabinose together with **5**. Enzymic hydrolysis of **2** with crude hesperidinase gave a product (**8**), whose ^{13}C NMR spectrum showed the arabinofuranosyl moiety bound to the hydroxyl group at C-3 on **5**. Moreover, the ^{13}C NMR spectrum of **2** suggested that an additional sugar, the rhamnopyranosyl residue, was attached to the hydroxyl group at C-7 on **5**. The structure for **2** was therefore deduced to be 3-*O*-α-L-arabinofuranosyl kaempferol 7-*O*-α-L-rhamnopyranoside.

Glycoside **3** consisted of rhamnose, arabinose, apiose and **5**. Enzymic hydrolysis of **3** with crude hesperidinase afforded a product (**9**), whose ^{13}C NMR spectrum showed that the apiofuranosyl moiety was bound to the hydroxyl group at C-2 of the inner arabinofuranosyl moiety [6, 7] with its diglycoside linked to the hydroxyl at C-3 on **5**. Moreover, the ^{13}C NMR spectrum of **3** suggested that an additional sugar, the rhamnopyranosyl residue, was attached to the hydroxyl at C-7 on **5**. Consequently, **3** can be represented as 3-*O*-β-D-apiofuranosyl-(1 → 2)-α-L-arabinofuranosyl kaempferol 7-*O*-α-L-rhamnopyranoside.

Glycoside **4** was composed of rhamnose, glucose and **5**. On enzymic hydrolysis in the same way as **1** it yielded a product (**10**). Its ^{13}C NMR spectrum revealed that the glucopyranosyl residue was attached to the hydroxyl at C-3 of the rhamnopyranosyl residue with its diglycoside linked to the hydroxyl at C-3 on **5**. Furthermore, the ^{13}C NMR spectrum suggested that one additional rhamnopyranosyl residue was bound to the hydroxyl at C-7 on **5**. Therefore, **4** can be represented as 3-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl kaempferol 7-*O*-α-L-rhamnopyranoside.

All the flavonol glycosides here isolated from the leaves of *C. sieboldii* possess one rhamnopyranosyl moiety on the hydroxyl group at C-7 on **5** and their rhamnopyranosyl moieties are selectively hydrolysable with the aid of crude hesperidinase. The *Cassia* diterpene could not be found in this plant.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR were recorded at 100 MHz, ^{13}C NMR at 50 MHz.

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Table 1. ^{13}C NMR chemical shifts of compounds **5**, **1**, **7**, **2**, **8**, **3**, **9**, **4** and **10** in C_5D_5N

Carbon		5	1	7	2	8	3	9	4	10
	2	147.5	158.4	157.9	158.2	157.6	158.2	157.6	158.3	157.7
	3	137.7	136.0	135.9	135.8	134.6	134.7	134.4	136.2	136.0
	4	177.2	179.1	179.0	179.3	179.1	179.1	179.0	179.1	178.9
	5	162.4	162.4*	162.9	162.3*	162.8	162.3*	162.8	162.4*	162.9
	6	99.2	99.9	99.8	100.0	99.7	100.0	99.7	100.0	99.8
	7	165.5	162.7*	166.0	162.8*	165.8	162.7*	165.8	162.8*	165.9
	8	94.3	94.8	94.6	94.9	94.5	94.8	94.5	94.9	94.6
	9	157.4	157.0	157.7	157.0	157.6	157.0	157.6	157.1	157.6
	10	104.4	107.0	105.2	106.9	105.2	106.9	105.3	107.0	105.4
	1′	123.2	121.5	121.9	121.8	121.9	121.7	121.9	121.4	121.7
	2′	130.5	131.5	131.4	131.7	131.5	131.7	131.5	131.5	131.3
	3′	116.3	116.4	116.4	116.5	116.3	116.5	116.4	116.5	116.4
	4′	160.7	161.8	161.6	161.8	161.5	161.8	161.6	161.9	161.7
	5′	116.3	116.4	116.4	116.5	116.3	116.5	116.4	116.5	116.4
	6′	130.5	131.5	131.4	131.7	131.5	131.7	131.5	131.5	131.3
7-Rha	1		100.3		100.4		100.3		100.4	
	2		71.8†		71.6		71.5		71.6	
	3		72.3†		72.4		72.3		72.4	
	4		73.1‡		73.6		73.5		73.5	
	5		71.4§		71.4		71.4		71.4	
	6		18.6‖		18.6		18.6		18.2	
3-Rha	1		103.8	103.7					103.8	103.8
	2		72.0†	71.9					70.3	70.3
	3		72.4†	72.5					84.2	84.2
	4		73.5‡	73.2					72.4	72.3
	5		71.5§	71.9					71.4	71.4
	6		18.3‖	18.3					18.2	18.2
3-Ara	1				110.0	109.8	107.6	107.5		
	2				83.5	83.2	89.5	89.4		
	3				78.9	78.8	77.2	77.1		
	4				88.7	88.6	87.5	87.5		
	5				62.5	62.4	62.2	62.2		
Api	1						110.0	109.9		
	2						77.9	77.9		
	3						80.3	80.3		
	4						75.4	75.3		
	5						65.5	65.5		
Glc	1								106.5	106.5
	2								76.2	76.2
	3								78.3	78.3
	4								71.6	71.6
	5								78.5	78.5
	6								62.2	62.6

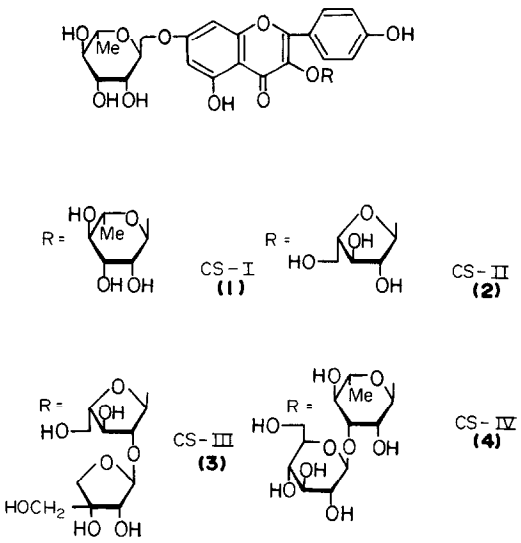
The signals marked *, †, ‡, §, ‖ may be reversed.

Extraction and isolation of compounds. Fresh leaves (300 g) of *C. sieboldii* collected at the Botanical Garden of Tokoshima University were refluxed with MeOH and evapd to give an extractive (87.7 g), which was then partitioned between BuOH and H_2O. Evapn of the organic solvent followed by refluxing with hexane gave an insoluble residue, which was subsequently subjected to CC on silica gel ($CHCl_3$–MeOH–H_2O; 4:1:0.1) and polyamide (MeOH) to afford **1**, R_f = 0.33, 96 mg; **2**, R_f = 0.40, 40 mg; **3**, R_f = 0.22, 500 mg; and **4**, R_f = 0.16, 500 mg. (TLC R_f values on silica gel in $CHCl_3$–MeOH–H_2O; 7:3:0.5.)

Kaempferol 3,7-O-bis-α-L-rhamnopyranoside (**1**). Pale yellow needles, mp 209–213°, $[\alpha]_D^{27}$ −190.0 (pyridine; *c* 1.0), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (CO), 1600 (double bond), 835, 805 (aromatic ring).

Acid hydrolysis of **1**. A soln of **1** (40 mg) in 2 N HCl–MeOH was refluxed for 2 hr, neutralized with 3% KOH–MeOH and concd. The deposited salts were filtered off and the soln was passed through a Sephadex LH-20 column to give kaempferol (**5**), yellow needles (8 mg), mp 275–277° and methyl α-L-rhamnopyranoside (TLC R_f value on silica gel 0.39 in $CHCl_3$–MeOH–H_2O; 4:1:0.1).

Octaacetate (**6**) *of* **1**. **1** (100 mg) was treated with Ac_2O (2 ml) and pyridine (2 ml) at room temp. overnight to give the octaacetate (**6**) (114 mg), colourless needles, mp 127–130°, $[\alpha]_D^{30}$ −44.0° ($CHCl_3$; *c* 1.0). MS (*m/z*): 558 (kaempferol + rha·3Ac), 286 (kaempferol), 273 (rha·3Ac), 170, 153. ^1H NMR ($CDCl_3$): δ0.88, 1.16 (each 3H, *d*, *J* = 7 Hz, 2 × rha 5-Me), 1.98–2.42 (24H, *m*, 8 × OAc), 6.76, 7.09 (each 1H, *d*, *J* = 2 Hz, 6 and 8-H), 7.28,



7.90 (each 2H, *d*, $J = 9$ Hz, 3′,5′-H and 2′,6′-H).

Enzymic hydrolysis of **1**. A mixture of **1** (171 mg) and crude hesperidinase (Tanabe Co. Ltd., 20 mg) in HOAc–NaOAc buffer soln (pH 4.5, 6 ml) was incubated at 40° for 3 min. MeOH was then added to the reaction mixture and evapd *in vacuo* to dryness to give a residue, the MeOH soluble part of which was subjected to silica gel CC eluting with $CHCl_3$–MeOH–H_2O (8:1:0.1) to yield a product (**7**), pale yellow powder (100 mg), $[\alpha]_D^{24}$ −143.9° (MeOH; *c* 5.08).

3-O-α-L-*Arabinofuranosyl kaempferol* 7-O-α-L-*rhamnopyranoside* (**2**). Pale yellow needles, mp 179–185°, $[\alpha]_D^{23}$ −172.0° (pyridine; *c* 0.44). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650, 1600, 835, 805. A small amount was treated with Ac_2O and pyridine to give the octaacetate; MS (*m/z*): 642 (kaempferol·2Ac + rha·3Ac), 600 (kaempferol·1Ac + rha·3Ac), 328 (kaempferol·1Ac), 286 (kaempferol), 273 (rha·3Ac), 259 (ara·3Ac).

Acid hydrolysis of **2**. A soln of **2** (30 mg) in 1 N HCl–dioxane was refluxed for 2 hr, the soln neutralized with 3% KOH and passed through a Sephadex LH-20 column to give **5** (5 mg), rhamnose and arabinose (TLC R_f values on silica gel 0.80, 0.33, 0.20, respectively, in $CHCl_3$–MeOH–H_2O; 7:3:0.5).

Enzymic hydrolysis of **2**. A mixture of **2** (235 mg), crude hesperidinase (20 mg) in HOAc–NaOAc buffer soln (pH 4.5, 6 ml) was incubated at 40° for 10 min. The soln treated in the same way as **1** gave a product (**8**), pale yellow needles (71 mg), mp 222–225°, $[\alpha]_D^{24}$ −161.5° (MeOH, *c* 1.09).

3-O-β-D-*Apiofuranosyl*-(1 → 2)-α-L-*arabinofuranosyl kaempferol* 7-O-α-L-*rhamnopyranoside* (**3**). Pale yellow powder, $[\alpha]_D^{27}$ −169.9° (pyridine; *c* 1.36). A small amount of **3** was acetylated with Ac_2O–pyridine to yield the decaacetate; MS (*m/z*): 600, 558, 475 (api-ara·5Ac), 328, 286, 273, 259. A small amount of **3** (5 mg) was also hydrolysed with 2 N H_2SO_4 in 50% EtOH giving **5**, rhamnose, apiose and arabinose (TLC R_f values on silica gel 0.80, 0.33, 0.31, 0.20, respectively, in $CHCl_3$–MeOH–H_2O; 7:3:0.5).

Enzymic hydrolysis of **3**. A mixture of **3** (237 mg), crude hesperidinase (20 mg) in HOAc–NaOAc buffer soln (6 ml) was incubated at 40° for 10 min. The reaction mixture treated in the same way as **1** afforded a product (**9**), pale yellow needles (120 mg), mp 167–170°, $[\alpha]_D^{27}$ −151.6° (pyridine; *c* 1.59).

3-O-β-D-*Glucopyranosyl*-(1 → 3)-α-L-*rhamnopyranosyl kaempferol* 7-O-α-L-*rhamnopyranoside* (**4**). Pale yellow crystals, mp 200–203°, $[\alpha]_D^{21}$ −100.0° (pyridine; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1600, 1490, 1460, 835. A trace of **4** was converted into the corresponding undecaacetate; MS (*m/z*): 600, 561 (glc-rha·6Ac), 331 (glc·4Ac), 286, 273, 169, 109. A small amount of **4** (4 mg) was hydrolysed with 2 N HCl–MeOH for 3 hr to yield **5**, methyl rhamnopyranoside and methyl glucopyranoside (TLC R_f values on silica gel 0.42, 0.38, 0.18, respectively, in $CHCl_3$–MeOH–H_2O; 4:1:0.1).

Enzymic hydrolysis of **4**. Incubation of **4** (64 mg) with crude hesperidinase (20 mg) in HOAc–NaOAc buffer soln (5 ml) at 40° for 10 min yielded a product (**10**), pale yellow powder (25 mg), $[\alpha]_D^{27}$ −141.2° (pyridine; *c* 0.80).

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