

## Structures of Two Acylated Flavonol Glucorhamnosides from *Ginkgo biloba* Leaves

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**Abstract** □ The position of interglycosidic linkages of two acylated flavonol glucorhamnosides from *Ginkgo biloba* leaves was unambiguously determined as 1 → 2 linkages rather than 1 → 4 ones on the basis of spectroscopic and chemical evidence.

**Keywords** □ *Ginkgo biloba*, Ginkgoaceae, acylated flavonol glucorhamnoside, interglycosidic linkage.

*Ginkgo biloba* extract obtained from the dried green leaves of *Ginkgo biloba* L. (Ginkgoaceae) is effective in treating various cardiovascular disorders<sup>1)</sup>. Up to now, thirteen flavonol glycosides were isolated and identified from *Ginkgo biloba* extract<sup>2-8)</sup>. Among these flavonol glycosides, two of them were acylated and characterized as kaempferol 3-O- $\alpha$ -(6''')-*p*-coumaroyl glucosyl- $\beta$ -1,4-rhamnoside (3) and the corresponding quercetin congener (4)<sup>3, 4)</sup>. As we reported in previous papers, seven flavonol glycosides were isolated from the leaves of *Ginkgo biloba*, two of which possess *p*-coumaroyl group in each structure<sup>9, 10)</sup>. This paper gives further evidence for the structures of these two acylated flavonol glucorhamnosides.

### RESULTS AND DISCUSSION

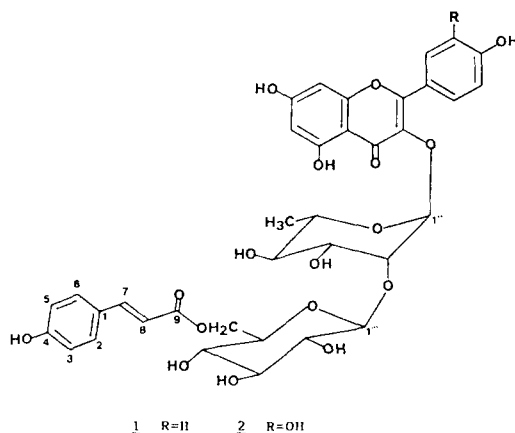
Two acylated flavonol glucorhamnosides were isolated as major components from the EtOAc fraction of the MeOH extract of *Ginkgo biloba* leaves and characterized as kaempferol 3-O- $\alpha$ -(6''')-*p*-coumaroylglucosyl- $\beta$ -1,2-rhamnoside (1) and quercetin 3-O- $\alpha$ -(6''')-*p*-coumaroylglucosyl- $\beta$ -1,2-rhamnoside (2) on the basis of <sup>13</sup>C-NMR spectral data<sup>9, 10)</sup>. On the other hand, Anton and his coworkers previously reported the isolation of two acylated congeners (3 and 4) from this plant<sup>3, 4)</sup>. Although the position of interglycosidic linkages is different in both compounds, spectral data of ours (1 and 2) are virtually identical to those of the reported (3 and 4) as shown in Table I. Therefore we conducted further experiments and unambiguously determined the position of interglycosidic linkages of the glucorhamnose moieties in 1 and 2.

Acetylation of 1 with Ac<sub>2</sub>O/pyridine gave a non-acetate (5) which showed four aromatic and five al-

iphatic acetyl signals in the <sup>1</sup>H-NMR spectrum. In the region of 3 to 6 ppm, the signals corresponding to methine and methylene protons of glucorhamnose moiety were observed as shown in Fig. 1a. To assign these protons, spin decoupling experiments were carried out. As shown in Fig. 1b, irradiation of the anomeric proton of rhamnose appeared at  $\delta$  5.64 simplified the broad triplet at  $\delta$  4.42 to a doublet, which led to assignment of the chemical shift of rhamnose H-2 proton. In a similar manner, the chemical shifts of the remaining protons of rhamnose were completely assigned as listed in Table II. As comparing the chemical shifts of rhamnose protons to those of peracetylated methyl  $\alpha$ -L-rhamnoside, the chemical shift of H-2 proton was upfield shifted by 0.78 ppm, indicating that the position of the attachment of glucose moiety was unambiguously C-2 hydroxyl group of rhamnose. Thus, we excluded the possibility that the terminal glucose moiety was bound to C-4 hydroxyl group of rhamnose unit. This result was further supported by the fact that permethylation of 1 followed by methanolysis afforded methyl glycosides of 2,3,4,6-tetra-O-methylglucose and 3,4-di-O-methylrhamnose. From the above findings, the structure of 1 was determined to be kaempferol 3-O- $\alpha$ -(6''')-*p*-coumaroylglucosyl- $\beta$ -1,2-rhamnoside). As shown in Table I, <sup>13</sup>C-NMR spectral data of the sugar moiety in 2 was superimposable to those of 1, indicating that the saccharide chain is identical in both compounds. In the light of the above findings, the structure of 2 was assigned to be quercetin 3-O- $\alpha$ -(6''')-*p*-coumaroylglucosyl- $\beta$ -1,2-rhamnoside). Matsumoto and Sei recently reported the isolation of two new flavone glycosides from *Ginkgo biloba* leaves and assigned their structures as 1 and 2 without any phy-

**Table I.**  $^{13}\text{C}$  nmr chemical shifts for acylated flavonol glucorhamnosides from *Ginkgo biloba* in  $\text{DMSO-}d_6$ 

Carbon	1 <sup>9)</sup>	3 <sup>3)</sup>	2 <sup>9)</sup>	4 <sup>4)</sup>
2	156.6	156.5	156.8	157.5
3	134.7	134.2	134.7	135.3
4	177.8	177.4	177.9	178.6
5	161.5	161.2	161.6	162.2
6	98.8	99.2	98.8	107.1
7	164.4	165.9	164.4	165.0
8	93.9	93.9	93.8	94.5
9	156.6	156.2	156.6	157.3
10	106.0	106.0	106.0	104.9
1'	120.6	120.3	121.1	121.5
2'	130.6	130.0	115.8	114.8
3'	115.6	115.4	145.5	145.5
4'	159.9	160.0	148.8	146.1
5'	115.6	115.4	115.9	116.5
6'	130.6	130.0	120.9	121.8
1''	100.8	100.6	100.8	99.6
2''	81.7	71.7	81.8	70.5
3''	70.4	70.4	70.5	72.7
4''	72.1	81.6	72.1	82.6
5''	70.1	69.8	70.0	70.5
6''	17.5	17.4	17.6	18.3
1'''	104.3	103.3	104.3	101.6
2'''	73.9	73.6	74.0	74.7
3'''	76.4	76.0	76.3	76.9
4'''	70.6	70.2	70.5	71.1
5'''	73.9	73.6	74.0	74.7
6'''	63.2	63.1	63.2	63.8
<i>p</i> -coumaroyl				
1	125.2	124.8	125.3	125.9
2	130.1	130.4	130.1	130.9
3	115.8	115.7	115.9	116.5
4	160.2	160.2	159.9	160.6
5	115.8	115.7	115.9	116.5
6	130.1	130.4	130.1	130.9
7	144.8	144.2	144.8	149.5
8	114.1	113.7	114.2	114.8
9	166.5	166.4	166.5	167.3



sico-chemical and spectral data<sup>5)</sup>.

## EXPERIMENTAL

Melting points are uncorrected.  $^1\text{H-NMR}$  spectra were obtained on either a Varian FT-80A (80 MHz) or a Jeol FX-100 (100 MHz) spectrometer using TMS as an internal standard. Eims were determined on a Hewlett-Packard 5985B GC/MS System equipped with direct inlet system.

### Isolation and purification

See previous paper<sup>9)</sup>.

### Acetylation of 1

A sample of **1** (100 mg) was treated with  $\text{Ac}_2\text{O}$ /pyridine (1:1) at room temperature overnight. Workup in the usual way afforded **5** as an amorphous white powder.

IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 1765, 1635, 1511, 1438, 1375, 1250, 1220, 1170, 1080, 1045, 969, 912, 855, 840;  $^1\text{H-NMR}$  (80 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.87 (3H, d,  $J=6.2$  Hz, Rha- $\text{CH}_3$ ), 1.96 (3H, s, OAc), 2.01 (6H, s,  $2\times$  OAc), 2.07 (3H, s, OAc), 2.10 (3H, s, OAc), 2.29 (3H, s, OAc), 2.32 (6H, s,  $2\times$  OAc), 2.39 (3H, s, OAc), 6.34 (1H, d,  $J=16$  Hz, cou C-8), 6.81 (1H, d,  $J=2$  Hz, H-6), 7.05 (2H, d,  $J=8.6$  Hz, cou C-3, 5), 7.24 (2H, d,  $J=8.7$  Hz, H-3', 5'), 7.28 (1H, d,  $J=2$  Hz, H-8), 7.48 (2H, d,  $J=8.6$  Hz, cou C-2, 6), 7.61 (1H, d,  $J=16$  Hz, cou C-7), 7.85 (2H, d,  $J=8.7$  Hz, H-2', 6') (cou = coumaroyl moiety).

### Permethylation of 1

**1** (100 mg) was permethylated with NaH (100 mg) and  $\text{CH}_3\text{I}$  (5 ml) by the Brimacombs's method<sup>11)</sup>. The product was purified by column chromatography with  $\text{CHCl}_3$  to afford nona-O-methylether of kaempferol 3-O- $\beta$ -glucosyl (1 $\rightarrow$ 2)- $\alpha$ -rhamnoside as amorphous solid.  $^1\text{H-NMR}$  (80 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.94 (3H, d,  $J=7.5$  Hz, Rha- $\text{CH}_3$ ), 3.20 (3H, s,  $\text{CH}_3$ ), 3.43 (6H,

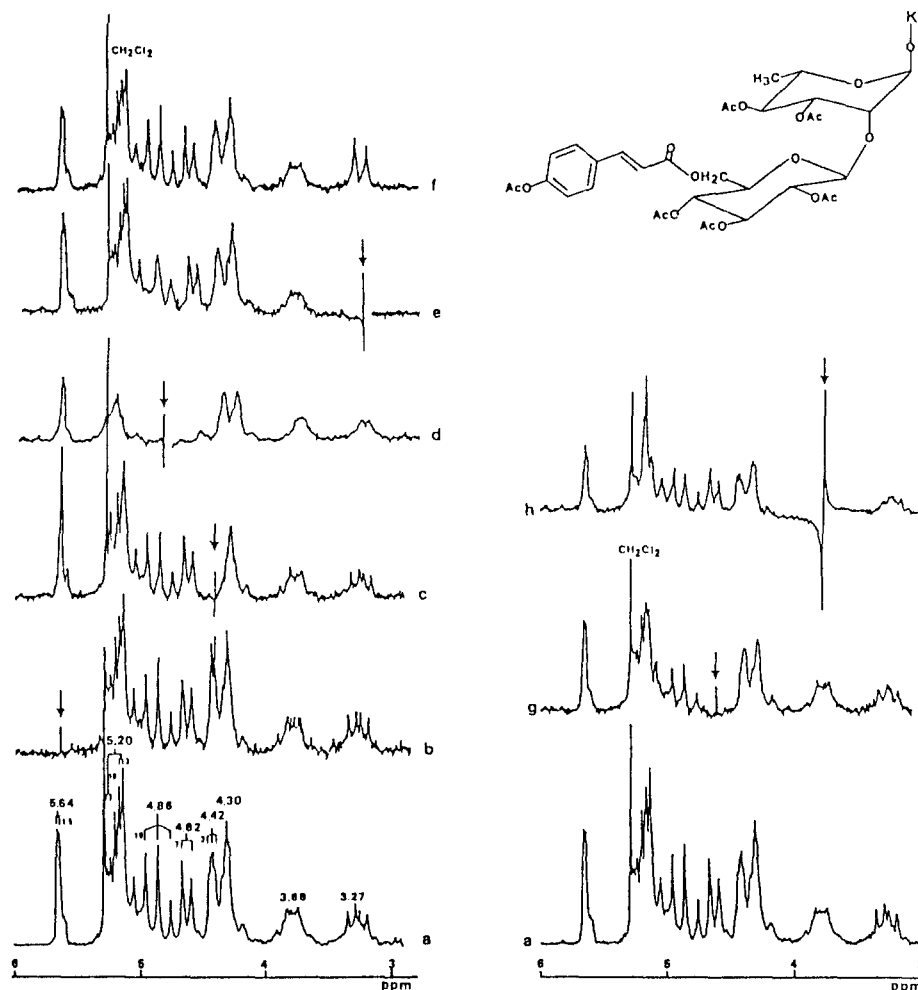


Fig. 1. Partial  $^1\text{H-NMR}$  spectra (3-6 ppm region) of kaempferol 3-O-[6''-O-*p*-coumaroyl- $\beta$ -D-glucopyranosyl (1  $\rightarrow$  2)- $\alpha$ -L-rhamnopyranoside] nonaacetate in  $\text{CDCl}_3$ .

a) The normal spectrum, b) Rhamnose H-1 ( $\delta$  5.64) decoupled, c) Rhamnose H-2 ( $\delta$  4.42) decoupled, d) Rhamnose H-4 ( $\delta$  4.86) decoupled, e) Rhamnose H-5 ( $\delta$  3.27) decoupled, f) Rhamnose methyl protons ( $\delta$  0.87) decoupled, g) Glucose H-1 ( $\delta$  4.62) decoupled, h) Glucose H-5 ( $\delta$  3.68) decoupled.

Table II.  $^1\text{H-NMR}$  chemical shifts for rhamnosyl protons of 5, peracetylated methyl  $\alpha$ -L-rhamnoside and rhamnobiases

Rhamnose proton	5	Peracetylated compounds of				
		methyl- $\alpha$ -L-rhamnoside	rhamnose <sup>12)</sup>	$\alpha$ -Rha (1 $\rightarrow$ 2)- $\alpha$ -Rha <sup>12)</sup>	$\alpha$ -Rha (1 $\rightarrow$ 3)- $\alpha$ -Rha <sup>12)</sup>	$\alpha$ -Rha (1 $\rightarrow$ 4)- $\alpha$ -Rha <sup>12)</sup>
H-1	5.64	4.61	6.01	6.05	6.02	5.92
H-2	4.42	5.20	5.20	4.01	5.25	5.22
H-3	5.20	5.33	5.33	5.20	4.16	5.22
H-4	4.86	5.04	5.03	5.08	5.14	3.70
H-5	3.27	3.84				
H-6	0.87	1.23	1.20		1.20	1.36

s,  $2 \times \text{CH}_3$ ), 3.50 (3H, s,  $\text{CH}_3$ ), 3.61 (6H, s,  $2 \times \text{CH}_3$ ), 3.86 (6H, s,  $2 \times \text{CH}_3$ ), 3.91 (3H, s,  $\text{CH}_3$ ), 4.53 (1H, d,  $J = 7.1$  Hz, Glc H-1), 5.56 (1H, d,  $J = 1.6$  Hz, Rha H-1), 6.32 (1H, d,  $J = 2.2$  Hz, H-6), 6.45 (1H, d,  $J = 2.2$  Hz, H-8), 6.98 (2H, d,  $J = 8.9$  Hz, H-3', 5'), 7.81 (2H, d,  $J = 8.9$  Hz, H-2', 6'); Ms (30 eV)  $m/z$  (rel. int.): 578 [ $\text{M}^+ - (3\text{CH}_3\text{OH} + \text{CH}_3\text{O} + \text{CH}_3)$ , 0.3], 550 (578-CO, 0.2), 547 (578- $\text{CH}_3\text{O}$ , 15.3), 501 [ $\text{M}^+ - \text{Glc}(\text{OCH}_3)_4$ , 0.3], 486 (501- $\text{CH}_3$ , 0.7), 485 [547-( $2\text{CH}_3 + \text{CH}_3\text{OH}$ ), 2.7], 453 (485- $\text{CH}_3\text{OH}$ , 1.3), 421 (485- $2\text{CH}_3\text{OH}$ , 0.3), 328 (genin, 100), 313 (328- $\text{CH}_3$ , 2.5), 310 (328- $\text{H}_2\text{O}$ , 13.0), 300 (328-CO, 4.5), 299 (328-CHO, 21.0), 285 (328- $\text{CH}_3\text{CO}$ , 3.1), 282 [328-(CO +  $\text{H}_2\text{O}$ ), 30.6], 219 [ $\text{Glc}(\text{OCH}_3)_4$ , 2.0], 187 (219- $\text{CH}_3\text{OH}$ , 52.8), 181, [( $\text{A}_1 + \text{H}$ ) $^+$ , 1.1], 155 (219- $2\text{CH}_3\text{OH}$ , 20.1), 135 ( $\text{B}_2^+$ , 10.6), 111 (56.5).

#### Methanolysis of nona-O-methylether

The above permethylether was refluxed with 2% methanolic HCl (20 ml) for 3 hr. The reaction mixture was concentrated to a half volume, then added to crushed ice and filtered. The filtrate was neutralized with  $\text{Ag}_2\text{CO}_3$ . The concentrated hydrolysate was examined by GLC and identified as methyl 2,3,4,6-tetra-O-methyl glucopyranoside (Rt: 5.88, 8.38) and methyl 3,4-di-O-methyl rhamnopyranoside (Rt: 6.35) [column: 10%DEGS on chromosorb WHP 100-120 mesh, 2.3 mm  $\times$  6 ft; column temp.: 170°C; flow rate ( $\text{N}_2$ ): 45.9 ml/min; chart speed: 1 cm/min]. The precipitate was recrystallized from MeOH to yield kaempferol 5,7,4'-tri-O-methylether as yellowish fine needles. mp 147-9°C; Ms (30 eV)  $m/z$  (rel. int.) 328 ( $\text{M}^+$ , 40.0), 313 ( $\text{M}^+ - \text{CH}_3$ , 2.0), 310 ( $\text{M}^+ - \text{H}_2\text{O}$ , 11.4), 300 ( $\text{M}^+ - \text{CO}$ , 3.8), 299 ( $\text{M}^+ - \text{CHO}$ , 17.3), 297 ( $\text{M}^+ - \text{OCH}_3$ , 15.9), 285 [ $\text{M}^+ - (\text{CO} + \text{CH}_3)$ , 18.1], 282 [ $\text{M}^+ - (\text{H}_2\text{O} + \text{CO})$ , 100], 267 (10.2), 255 (17.3), 242 (13.1), 239 (15.1), 227 (12.8), 211 (11), 135 (44.3).

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