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KAEMPFEROL TETRAGLUCOSIDES FROM CABBAGE LEAVES

JENS K. NIELSEN*, RIKKE NØRBÆK and CARL E. OLSEN

Chemistry Department, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

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Key Word Index—*Brassica oleracea*; cabbage; Cruciferae; flavonol glycosides; acylated; caffeoyl; feruloyl; sinapoyl.

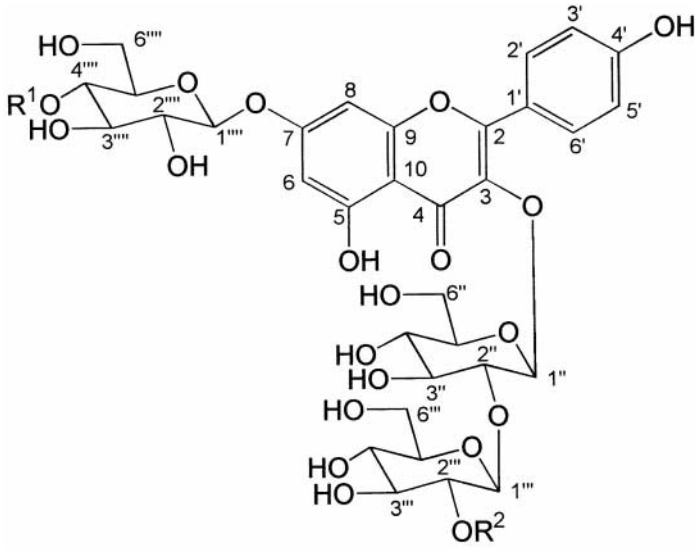
Abstract—Four flavonol glycosides were isolated from a leaf extract of cabbage and characterized by chemical and spectroscopic methods including ^{1}H and ^{13}C NMR and negative ion FAB-MS. The common structure of the four compounds was kaempferol–3-*O*-*β*-D-[*β*-D-glucopyranosyl(1 → 2)glucopyranoside]–7-*O*-*β*-D-[*β*-D-glucopyranosyl(1 → 4)glucopyranoside]. This compound was found unmodified or acylated at C-2‴ (outer glucose in sophorosyl moiety) with either sinapic acid, ferulic acid or caffeic acid. The possible role of diversity in glycosylation and acylation patterns of flavonol glycosides for plant defences against herbivores is discussed.

INTRODUCTION

Flavonol glycosides are of nearly ubiquitous occurrence in the plant kingdom. Kaempferol and quercetin are the most common flavonol aglycones and these two compounds are found in about half of all angiosperm species [1]. The similarity in aglycone structure is in contrast to a great diversity in the number, position and structures of carbohydrates and acyl groups attached to the flavonol aglycones [2]. Flavonol glycosides are often localized in epidermal cells and they are supposed to be involved in protection against UV-B radiation [3, 4]. The sugar moiety has no or very little effect on the UV absorption of these compounds, but acylation with hydroxycinnamic and other phenolic acids may increase the absorption at certain wavelengths. Although the effects of acylated flavonol glycosides as UV-B protectants seem not to have been evaluated, it is doubtful whether the great diversity in flavonol glycoside structures within the plant kingdom can be explained by their role as UV protectants.

Besides the function as light absorbants, many flavonol glycosides may have an effect on the interaction between plants and their associated herbivores. Several flavonoids have been identified as feeding or oviposition stimulants for various insects, while others are toxic or inhibitory to insect feeding [1, 5–8]. Flavonol glycosides from horseradish (*Armoracia rusticana* G., M. and Sch.) are feeding stimulants for the monophagous horseradish flea beetle, *Phyllotreta armoraciae* (Koch) [5]. The carbohydrate moiety is very important for feeding stimulation by these compounds [6] and plants might in some cases be able to escape from insect attack by changing the sugar moiety of their flavonol glycosides. Escape from herbivory could then be a driving force in the evolution of flavonol glycoside patterns in angiosperms. In order to study this hypothesis we want to compare the responses of horseradish flea beetles and other insects to a group of crucifers with different patterns of flavonol glycosides. Horseradish has a very simple pattern with only 2 major compounds, kaempferol- and quercetin–3-*O*-*β*-D-[*β*-D-xylopyranosyl(1 → 2)-galactopyranoside] [5, 9]. In contrast, cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC.) contains a complex mixture of more than 20 compounds of which seven have been identified previously as 3-*O*-*β*-D-sophoroside-7-*O*-*β*-D-glucosides of kaempferol and quercetin with and without further acylation with hydroxycinnamic acids [10]. We report here on the identification of four new kaempferol tetraglucosides which contribute to the complex mixture of flavonol glycosides found in cabbage leaves.

*Author to whom correspondence should be sent.



	R^1	R^2
1	H	H
2	H	sinapoyl
3	H	feruloyl
4	H	caffeoyl
5	β-D-glucosyl	H
6	β-D-glucosyl	sinapoyl
7	β-D-glucosyl	feruloyl
8	β-D-glucosyl	caffeoyl

RESULTS AND DISCUSSION

Four new kaempferol tetraglucosides (**5**–**8**) were isolated in pure form by repeated column chromatography and prep. paper chromatography. They occur in cabbage leaves together with similar triglucosides which have been identified previously [10]. The tetraglucosides could be distinguished from the corresponding triglucosides (**1**–**4**) by lower Rf-values on PC in BAW, while the UV-spectra in different shift reagents were almost identical to those published previously for the triglucosides [10]. ^{1}H NMR (Table 1) showed that four glucosyl units were present in the new compounds since all vicinal coupling constants were 8.4–9.6 Hz. All glucosyl units were β-linked since the coupling constants between the anomeric protons and H-2 were always 7–8 Hz [11]. The E configuration in the hydroxycinnamoyl part of the acylated tetraglucosides (**6**–**8**) was demonstrated by coupling constants of about 16 Hz in the ^{1}H NMR spectrum [10].

FAB-MS (Table 2) and ^{1}H and ^{13}C NMR (Table 3) suggested that the sugar part of the four tetraglucosides was identical and that pairs of tetra- and triglucosides were similar except for the presence in the tetraglucosides of an additional glucose attached to C4‴′ of the corresponding triglucoside. The presence of two diglucosyl units (as opposed to one mono- and one triglucosyl unit) in the tetraglucosides is suggested by the presence of fragments corresponding to [M–diglucosyl]$^-$ and the absence of a fragment at m/e = 447 [M–acyltriglucoside]$^-$ in the negative ion FAB mass spectra of the acylated tetraglucosides (**6**–**8**; Table 2). The [M–diglucosyl]$^-$ ion was not found in the mass spectra of the acylated triglucosides [10]. Linked scan confirmed that all fragment ions mentioned in Table 2 are daughter ions formed from the molecular ion in a single step.

The assignment of the four glucosyl units were carried out by 1D-HOHAHA and ^{1}H–^{1}H-COSY (Table 1). The positions of the glucosidic linkages of **5**–**8** were determined by NOE difference spectra. Strong negative NOEs were observed at the protons in the B-ring of kaempferol (H-5′, H-6′) by irradiation of H-1″ indicating that this sugar moiety is linked to C-3 of the aglycone. By irradiation of H-1‴, negative NOEs appeared to the same protons in the aglycone, but this time weaker. Furthermore, there was a strong negative NOE between H-1‴ and H-1″ indicating a 1 → 2 linkage between these two glucosyl units. In the same way, strong negative NOEs to the protons in the A-ring of kaempferol (H-6 and H-8) were observed by irradiation of H-1⁗. There was also a weak negative NOE between the two anomeric protons, H-1⁗ and H-1⁗′. This observation supports that a disaccharide moiety (with H-1⁗ and H-1⁗′) is attached to C-7 of the A-ring of kaempferol. The negative NOE

Table 1. ^{1}H chemical shifts of the carbohydrate moieties of **5**–**8** (DMSO-d_6 containing 10% TFA-*d*; 600 MHz instrument). Spin systems belonging to each glucose unit were identified by 1D HOHAHA

	5	**6**	**7**	**8**
Sugar at C-3:				
1″	5.69 d (7.2)	5.84 d (7.2)	5.80 d (7.2)	5.78 d (7.2)
2″	3.46 m, o	3.48 m, o	3.57 dd (8.4; 9.0)	3.58 t (7.8)
3″	3.45 m, o	3.45 m, o	3.46 m	3.45 m
4″	3.08 m, o	3.08 m, o	3.11 m, o	3.11 m, o
5″	3.10 m, o	3.07 m, o	3.11 m, o	3.11 m, o
6a″	3.47 m, o	3.48 m, o	3.50 m	3.51 m
6b″	3.25 m	3.22 m	3.27 m	3.28 m
1‴	4.60 d (8.4)	5.14 d (8.4)	5.13 d (8.4)	5.13 d (8.4)
2‴	3.05 dd (8.4; 9.0)	4.75 dd (8.4; 9.0)	4.73 dd (9.0; 9.6)	4.74 t (9.0)
3‴	3.18 dd (8.4; 9.0)	3.45 t (9.0)	3.48 m	3.48 dd (8.4; 9.0)
4‴	3.15 dd (9.0; 9.6), o	3.25 m, o	3.29 m, o	3.29 m, o
5‴	3.12 m, o	3.25 m, o	3.29 m, o	3.29 m, o
6a‴	3.56 d (4.2; 11.7)	3.69 m	3.74 m	3.74 m
6b‴	3.47 m	3.51 m	3.59 m	3.58 m
Sugar at C-7:				
1⁗	5.16 d (7.8)	5.18 d (8.4)	5.20 d (7.2)	5.20 d (7.8)
2⁗	3.32 t (8.4)	3.34 t (8.4)	3.32 t (9.0)	3.40 dd (7.8; 9.0)
3⁗	3.47 dd (8.4; 9.0)	3.50 t (9.0)	3.48 t (9.0)	3.56 t (9.0)
4⁗	3.42 dd (9.0; 9.6)	3.44 dd (9.0; 9.6)	3.41 m	3.49 dd (8.4; 9.0)
5⁗	3.62 m, o	3.63 m, o	3.63 m, o	3.70 m, o
6a⁗	3.75 d (4.2; 11.7)	3.76 m	3.75 m	3.82 m
6b⁗	3.63 m, o	3.63 m, o	3.63 m, o	3.69 m, o
1⁗′	4.29 d (8.4)	4.36 d (7.8)	4.35 d (8.4)	4.36 d (8.4)
2⁗′	3.02 dd (8.4; 9.0)	3.01 dd (8.4; 9.0)	3.08 dd (8.4; 9.0)	3.09 dd (7.8; 9.0)
3⁗′	3.16 t (9.0)	3.18 dd (8.4; 9.0)	3.22 dd (9.0; 9.6)	3.24 dd (8.4; 9.6)
4⁗′	3.06 t (9.6)	3.07 t (9.6)	3.12 dd (9.0; 9.6)	3.13 dd (8.4; 9.6)
5⁗′	3.21 m	3.23 m	3.25 m	3.27 m
6a⁗′	3.69 m	3.70 m	3.75 m	3.76 m
6b⁗′	3.41 m	3.41 m	3.47 m	3.47 m

Coupling constants *J* (in Hz) in parentheses; o: overlapping with other signals.

between H-1⁗ and H-1⁗′ (cellobiosyl moiety) was weaker than the negative NOE between H-1″ and H-1‴ (sophorosyl moiety). Instead, the negative NOE to H-3⁗ by irradiation of H-1⁗′ was much more intense indicating a $1 \rightarrow 4$ linkage between these two glucosyl units. This attachment was also confirmed by the downfield shift of *ca.* 0.3 ppm of H-4⁗ in the tetraglucosides compared to H-4 in other glucosyl units in the same molecules [11]. The site of acylation in **6**–**8** was confirmed by a downfield shift of *ca.* 1.3 ppm of H-2‴ of these compounds compared to the chemical shift value of protons in the same position in **5** [10, 12].

Compounds **5** and **6** were analysed by HSQC and HMBC and these investigations confirmed the assignments of all anomeric protons and carbons as well as a large number of other carbons in the ^{13}C NMR spectra (Table 3). All ^{13}C signals from the sophorosyl part of the triglucosides [10] could be found also in the spectra of the corresponding tetraglucosides, but the signals from the 7-*O*-*β*-D-glucosyl moiety had shifted according to the expectations if an additional monosaccharide had been attached to C-4⁗ [11]. An unsubstituted C-4 in glucose has a characteristic chemical shift value at *ca.* 70.0 ppm. The presence of only three signals in this region in the ^{13}C NMR spectra of the tetra- as well as the triglucosides suggests that the site of attachment of the new glucosyl unit was at C-4 of one of the glucosyl units present in the triglucosides. The downfield shift of C-4⁗ from *ca.* 70 to 79.6 ppm and the smaller upfield shift of neighbouring carbons (C-3⁗ and C-5⁗) is in agreement with spectra of glycosides with similar substitution patterns [11, 13, 14]. In the HMBC spectrum measured in DMSO-*d6* containing 10% TFA-*d*, signals at $\delta_H = 4.29$ ppm in **5** and $\delta_H = 4.36$ ppm in **6** (H-1⁗′) were shown to shift-correlate with a signal at $\delta_C = 79.6$ ppm (C-4⁗).

Table 2. Ions detected in the negative ion FAB mass spectra

Ion	**2**	**5**	**6**	**7**	**8**
$[M-H]^-$	977	933	1139	1109	1095
$[M\text{-glucosyl}]^-$	815	771	977	947	933*
$[M\text{-diglucosyl}]^-$	nd	609	815	785	771*
$[M\text{-acyl}]^-$	771	–	nd	nd	933*
$[M\text{-acylglucosyl}]^-$	609	–	771	771	771*
$[M\text{-acyldiglucosyl}]^-$	447	–	609	609	609*

*Same fragment ions are produced by release of a glucosyl and a caffeoyl moiety; nd: not detected.

The spectroscopic and chemical data presented above identify **5** as kaempferol–3-*O*-*β*-D-[*β*-D-glucopyranosyl($1 \rightarrow 2$)glucopyranoside]–7-*O*-*β*-D-[*β*-D-glucopyranosyl($1 \rightarrow 4$)glucopyranoside] and **6** as kaempferol–3-*O*-*β*-D-[2-*E*-sinapoyl-*β*-D-glucopyranosyl($1 \rightarrow 2$)glucopyranoside]–7-*O*-*β*-D-[*β*-D-glucopyranosyl($1 \rightarrow 4$)glucopyranoside]. The spectroscopic

Table 3. ^{13}C NMR chemical shifts of 5 glycosides (DMSO-d_6; 250 MHz instrument)

C	2	5	6	7	8
Aglycon					
2	156.1	156.2	156.2	156.2	156.6
3	133.0	132.9	133.0	132.9	133.5
4	177.3	177.4	177.3	177.3	177.8
5	160.6	160.8	160.7	160.7	160.8
6	99.2	99.1	99.2	99.2	99.2
7	162.6	162.5	162.5	162.4	162.8
8	94.2	94.3	94.2	94.2	94.8
9	155.7	155.8	155.7	155.7	156.2
10	105.5	105.5	105.5	105.5	106.0
1′	120.6	120.1	120.3	120.3	121.2
2′	130.8	131.0	130.8	130.8	131.3
3′	115.2	115.4	115.3	115.3	115.5
4′	160.1	160.8	160.5	160.6	160.2
5′	115.2	115.4	115.3	115.3	115.5
6′	130.8	131.0	131.0	130.8	131.3
Sugar at C-3					
1″	96.9	97.7*	97.0*	97.1	97.3*
2″	79.2	82.3*	79.2*	79.2	79.6*
3″	76.7	76.6	76.2	76.4	76.5
4″	70.1	69.6*	69.9*	69.9	70.1
5″	77.1	77.4*	77.2*	77.2	77.4
6″	60.8	60.7	60.8	60.8	60.8
1‴	98.8	104.0*	99.0*	99.0	99.1*
2‴	73.5	74.3*	73.6*	73.6	73.5*
3‴	74.3	76.5	74.3*	74.4	74.5*
4‴	70.1	70.0*	70.1*	69.9	70.2
5‴	76.0	76.9	77.1	76.7	76.9
6‴	60.5	60.4	60.5	60.5	60.1
Sugar at C-7					
1⁗	99.7	99.1*	99.2*	99.1	99.6*
2⁗	73.0	72.7*	72.7*	72.7	73.0*
3⁗	76.3	74.7*	74.7*	74.7	74.8
4⁗	69.5	79.6*	79.6*	79.6	79.8*
5⁗	77.0	75.0*	75.0*	75.0	75.3
6⁗	60.5	59.9	59.9	59.9	60.1
1⁗′		103.0*	103.0*	103.0	103.3*
2⁗′		73.2*	73.2*	73.2	73.5*
3⁗′		76.4	76.4	76.4	76.9
4⁗′		69.5*	69.9*	70.1	70.1
5⁗′		76.7	76.7	76.7	76.9
6⁗′		61.0	61.0	61.0	61.2
Acyl					
CH=CH*C*OO-	165.7		165.7	165.7	166.2
CH=*C*HCOO-	115.2		114.3	114.7	114.6
*C*H=CHCOO-	144.7		144.7	144.4	144.9
1	124.3		124.2	125.3	125.9
2	105.6		105.7	110.8	114.6
3	147.8		147.9	147.8	148.2
4	138.0		138.2	149.3	145.5
5	147.8		147.9	115.3	115.9
6	105.6		105.7	122.5	121.2
MeO-	56.0		55.9	55.4	

*Assignments have been confirmed by 2D techniques (^1H–^1H COSY, HSQC or HMBC).

data for **7** and **8** (Tables 1–3) show that these compounds have similar carbohydrate moieties as **6**, but they differ in the acyl moiety. By comparison with spectroscopical and chemical data published previously [10], **7** could be identified as kaempferol–3-*O*-*β*-D-[2-*E*-feruloyl-*β*-D-glucopyranosyl(1 → 2)glucopyranoside]–7-*O*-*β*-D-[*β*-D-glucopyranosyl(1 → 4)-glucopyranoside] and **8** as kaempferol–3-*O*-*β*-D-[2-*E*-caffeoyl-*β*-D-glucopyranosyl(1 → 2)glucopyranoside]-7-*O*-*β*-D-[*β*-D-glucopyranosyl(1 → 4)glucopyranoside]. The corresponding *p*-coumaroyl tetraglucoside of kaempferol as well as a number of acylated and unacylated quercetin tri- and tetraglucosides seemed to be present in smaller quantities in cabbage leaves but they have not been isolated in pure form.

The triglucosides **1**–**4** have previously been identified from cabbage leaves [10] and **1** and **2** have been identified from oilseed rape, *Brassica napus* L. [15]. No flavonol tetraglucosides have previously been identified from Cruciferae. The 1 → 4 linkage between glucosyl units is rare in flavonol glycosides compared to 1 → 2 and 1 → 6 linkages [2]. The disaccharide cellobiose (*β*-D-glucopyranosyl(1 → 4)-D-glucopyranose) has not previously been found in flavonol glycosides, but a *β* 1 → 4 linkage between glucosyl units has been found in a trisaccharide, sorborose (*O*-*β*-D-glucosyl(1 → 6)-*O*-*β*-D-glucosyl(1 → 4)glucose) [2]. A *β* 1 → 4 linkage between hexose units seems to be more common in saponin glycosides [13, 14].

The effect of the complex mixture of flavonol glycosides in cabbage leaves for phytophagous insects is currently being investigated. Flavonol glycosides have been recognized as important signal compounds especially in the interactions between plants and insects [5–8] and between plants and nitrogen fixing bacteria [16, 17]. The biological effects have in most cases been linked to a single or a few chemical structures, while careful structure activity relationships have rarely been established [6]. Luteolin 7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside is an oviposition stimulant for the black swallowtail butterfly *Papilio polyxenes* and this compound stimulates a contact chemoreceptor on the tarsi of the female butterfly [18]. The unacylated compound, luteolin 7-*O*-*β*-D-glucopyranoside is inactive in behavioural as well as electrophysiological assays performed on the same insect species [18]. *E*-chlorogenic acid acts as a behavioural synergist for luteolin 7-*O*-(6″-*O*-malonyl)-*β*-D-glucopyranoside and stimulates other chemosensory cells on the tarsi of the black swallowtail butterfly [18]. This investigation demonstrates that biological activity is closely related to chemical structure and to the presence of appropriate synergists. Synergists seem to be important for the ability of flavonol glycosides to stimulate oviposition in swallowtail butterflies, but not in monarch butterflies [7]. Feeding in the horseradish flea beetle is stimulated by glucosinolates as well as by flavonol glycosides from horseradish [5]. Therefore, the interaction between cruciferous plants and their associated insect fauna seems to be suitable for addressing questions on synergism as well as on structure activity relationships, since there are large variations in composition as well as in complexity of flavonol glycoside patterns in plant species attacked by these insects. These studies might contribute to a better understanding of the role of herbivores in the evolution of diversity of flavonol glycoside patterns in plants.

EXPERIMENTAL

Plant material

Cabbage (cv. Zefa Wädenswiler) was sown in the field in May. Leaves were harvested from July–October (several batches), lyophilized and stored dry at −20°C until extractions were made.

General techniques

UV-spectra were obtained in 20% EtOH and standard shift reagents were added [10]. The descending PC was run on Whatman No. 1 (analyt.) or Whatman No. 3 (prep.) chromatography paper in BuOH–HOAc–H_2O (12:3:5) (BAW). HPLC was performed on a reversed phase C18 column (5 μm particle size, 4.6 × 250 mm). Solvents were: 0.1% aq. TFA (A) and CH_3CN (B). The following gradient was used: 0–0.5 min: 5% B isocratic; 0.5–35 min: linear gradient 5–15% B and 35–45 min: linear gradient 15–30% B. The flow rate was 1.0 ml min^{-1}. Peaks were monitored at 260 and 330 nm. Negative ion FAB mass spectra were obtained in HEDS. B/E linked scan mass spectra were measured using the same conditions. Two NMR spectrometers were used. On a 250 MHz instrument (^{1}H, ^{1}H–^{1}H-COSY, ^{13}C and HSQC), spectra were recorded at ambient temperature in DMSO-d_6. In a few cases 10% D_2O was added in order to remove signals from interfering OH-groups. ^{1}H chemical shifts were relative to int. TMS; ^{13}C chemical shifts are relative to DMSO-d_6 at 39.3 ppm. Using a 600 MHz instrument (JNM alpha 600, JEOL) (^{1}H, ^{1}H–^{1}H-COSY, 1D-HOHAHA, difference NOE, ^{13}C, HSQC and HMBC), spectra were measured in DMSO-d_6 containing 10% TFA-*d* with internal standard CD_2HOD (3.326 ppm). 1D HOHAHA, difference NOE and 2D spectra were obtained using a pulse sequence supplied from JEOL.

Extraction and isolation

Lyophilized leaves (200 g $batch^{-1}$) were transferred to boiling 70% aq. EtOH (7.5 l) and homogenized for 5 min with an Ultra-Turrax homogenizer. After filtration, the residue was extracted × 2 with 70% aq. EtOH (5 l) at room temp. The filtrates were combined, evapd to a small vol (*ca.* 600 ml in H_2O) and extracted in a sepn funnel with equal amounts of $CHCl_3$ (×3) and later with EtOAc (×3). The aq. phase was further concentrated, centrifuged for 10 min at 15000 rpm before it was transferred to a column (10 × 23 cm) containing Polygosil C18 60-4063 (Macherey Nagel). The column was rinsed first with H_2O and later with increasing concentrations of aq. EtOH. Flavonol glycosides were eluted with 15–30% EtOH. Frs (200–500 ml) were collected according to the UV-absorption of the effluent. Frs from the Polygosil column were further sepd and purified on a PVP column (2.6 × 40 cm; Sigma). The column was rinsed first with 20% and later with 50% aq. EtOH. Frs which still contained more than one compound were sepd by prep. PC. After the solvent had evaporated from the paper sheets, the compounds were eluted with H_2O. All compounds were finally purified on Sephadex LH-20 (2.6 × 100 cm) rinsed with H_2O or on MCI GEL CHP20P (2.6 × 100 cm, Mitsubishi Chemical Co.) rinsed with 10–25% EtOH. The purity of the compounds and fractions was controlled by PC and HPLC.

Kaempferol–3-*O*-*β*-D-[*β*-D-*glucopyranosyl*(1 → 2)*glucopyranoside*]–7-*O*-*β*-D-[*β*-D-*glucopyranosyl*(1 → 4)-*glucopyranoside*] (**5**)

UV λ_{max} (nm): 265, 344; +NaOH: 274, 345sh, 382; +$AlCl_3$: 273, 295sh, 340, 389; +$AlCl_3$ + HCl: 272, 295sh, 340, 385; +NaOAc: 265, 385; +NaOAc + H_3BO_3: 266, 344. ^{1}H NMR (600 MHz): δ 8.11 (2H, *d*, *J* = 9.0 Hz, H-2′, H-6′), 6.97 (2H, *d*, *J* = 9.0 Hz, H-3′, H-5′), 6.84 (1H, *d*, *J* = 2.1 Hz, H-8), 6.49 (1H, *d*, *J* = 2.1 Hz, H-6). Assignments of sugar protons are shown in Table 1.

Kaempferol–3-*O*-*β*-D-[2-*E*-*sinapoyl*-*β*-D-*glucopyranosyl*(1 → 2)*glucopyranoside*]–7-*O*-*β*-D-[*β*-D-*glucopyranosyl*(1 → 4)*glucopyranoside*] (**6**)

UV λ_{max} (nm): 268, 334; +NaOH: 263, 385; +$AlCl_3$: 273, 300, 337, 400sh; +$AlCl_3$ + HCl: 271, 300, 339, 400sh; +NaOAc: 267, 345, 395sh; +NaOAc + H_3BO_3: 268, 334. ^{1}H NMR (600 MHz): δ 8.00 (2H, *d*, *J* = 9.0 Hz, H-2′, H-6′), 7.45 (1H, *d*, *J* = 15.6 Hz, sin: H-*β*), 6.96 (2H, *d*, *J* = 9.0 Hz, H-3′, H-5′), 6.77 (2H, *s*, sin: H-2, H-6), 6.70 (1H, *d*, *J* = 2.1 Hz, H-8), 6.46 (1H, *d*, *J* = 2.1 Hz, H-6), 6.40 (1H, *d*, *J* = 15.6 Hz, sin: H-α), 3.76 (6H, *s*, OCH_3).

Kaempferol–3-*O*-*β*-D-[2-*E*-*feruloyl*-*β*-D-*glucopyranosyl*(1 → 2)*glucopyranoside*]–7-*O*-*β*-D-[*β*-D-*glucopyranosyl*(1 → 4)*glucopyranoside*] (**7**)

UV λ_{max} (nm): 268, 331; +NaOH: 269, 295sh, 377; +$AlCl_3$: 276, 297, 335, 400sh; +$AlCl_3$ + HCl: 275, 297, 335, 400sh; +NaOAc: 266, 295sh, 345, 385; +NaOAc + H_3BO_3: 268, 295sh, 331. ^{1}H NMR (600 MHz): δ 8.02 (2H, *d*, *J* = 9.0 Hz, H-2′, H-6′), 7.49 (1H, *d*, *J* = 15.6 Hz, fer: H-*β*), 7.16 (1H, *d*, *J* = 1.6 Hz, fer: H-2), 6.95 (1H, *m*, fer: H-6), 6.95 (2H, *d*, *J* = 9.0 Hz, H-3′, H-5′), 6.76 (1H, *d*, *J* = 2.1 Hz, H-8), 6.74 (1H, *m*, fer: H-5), 6.39 (1H, *d*, *J* = 2.1 Hz, H-6), 6.39 (1H, *d*, *J* = 15.6 Hz, fer: H-α), 3.79 (3H, *s*, OCH_3).

Kaempferol–3-*O*-*β*-D-[2-*E*-*caffeoyl*-*β*-D-*glucopyranosyl*(1 → 2)*glucopyranoside*]–7-*O*-*β*-D-[*β*-D-*glucopyranosyl*(1 → 4)*glucopyranoside*] (**8**)

UV λ_{max} (nm): 267, 331; +NaOH: 273, 378; +$AlCl_3$: 273, 295, 341, 390sh; +$AlCl_3$ + HCl: 273, 295, 335, 400sh; +NaOAc: 266, 349;

+ NaOAc + H_3BO_3: 265, 346. ^{1}H NMR (600 MHz): δ 8.03 (2H, *d*, J = 9.0 Hz, H-2′, H-6′), 7.43 (1H, *d*, J = 15.6 Hz, caf: H-β), 6.98 (1H, broad *s*, caf: H-2), 6.96 (2H, *d*, J = 9.0 Hz, H-3′, H-5′), 6.87 (1H, *d*, J = 8.3 Hz., caf: H-6), 6.76 (1H, *d*, J = 2.1 Hz, H-8), 6.72 (1H, *d*, J = 8.3 Hz, caf: H-5), 6.48 (1H, *d*, J = 2.1 Hz, H-6), 6.26 (1H, *d*, J = 15.6 Hz, caf: H-α).

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