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Acylated flavonol glycosides from leaves of *Planchonia grandis*

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Three acylated flavonol glycosides have been identified from leaves of *Planchonia grandis* Ridley. They possess kaempferol as aglycone and two triglycosidic chains substituting hydroxyl groups at the 3- and 7-positions. The first glycosidic unit of each chain is esterified by a *cis* or *trans p*-coumaric acid. Structural elucidation was achieved by means of UV, NMR and mass spectrometry.

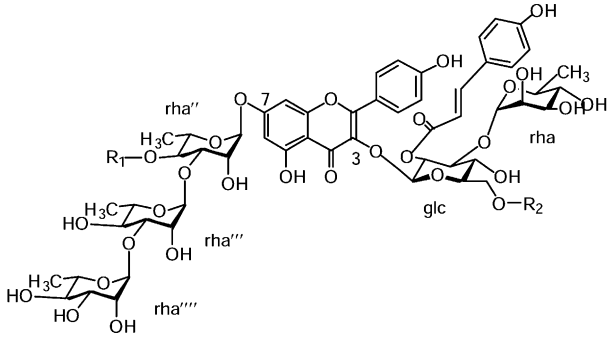
Keywords: *Planchonia grandis* Ridl.; Lecythidaceae; Flavonol glycosides; Acylated kaempferol glycosides

1. Introduction

During a systematic study on the chemistry of Lecythidaceae, we analysed the leaves of *Planchonia grandis* Ridley, collected in Malaysia. The genus *Planchonia* is distributed from the Andaman islands to the North of Australia. *P. valida* Bl., also from Malaysia, was shown to contain ellagic acid derivatives in the bark (Lowry, 1968). Several triterpenes and two acylated triterpenoid saponins were isolated from the stem-bark of *P. careya* (Khong and Lewis, 1977,1979). The ethanolic and ethyl acetate extracts from the leaves and stem bark of *P. grandis* were tested in a preliminary biological screening. No in vitro cytotoxic activity was revealed against P-388 cells and no activity was detected with the tubulin test and on topoisomerase I. Similarly, these extracts did not exhibit in vitro antibacterial activity. This paper reports on the isolation and structural elucidation of three new flavonol glycosides isolated from the leaves of this plant.

2. Results and discussion

Dried and powdered leaves of *P. grandis* were defatted with petroleum ether and then extracted with methanol-H_2O (4:1). Concentration of the solution yielded a residue which was dissolved in methanol and poured into acetone to form a precipitate, with the latter being dialysed against water. After removal of the tannin fraction by vacuum liquid chromatography (VLC), the purification of the extract was achieved by silica gel column chromatography and reversed-phase HPLC to afford three new flavonoid glycosides (**1**–**3**).



	R_1	R_2
1	*trans-p*-coumaroyl	β-D-glucosyl′
2	*cis -p*-coumaroyl	β-D-glucosyl′
3	*trans-p*-coumaroyl	α-L-rhamnosyl′

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Acid hydrolysis of the crude flavonoid extract yielded D-glucose and L-rhamnose identified by TLC and by measurement of their optical rotation after purification by prep. TLC. The UV spectral data of **1**–**3**, recorded in methanol, were similar with the characteristic maxima at 270 and 323 nm of kaempferol 3-*O*-glycosides (Markham and Mabry, 1975). Use of standard shift reagents showed that the C-5 and C-4′ hydroxyl groups of kaempferol were free and that the hydroxyl group at C-3 was substituted (Markham, 1993).

Compound **1** was a hexaglycoside as shown by the ^{1}H and ^{13}C NMR spectra with six anomeric proton signals at δ 5.67 (*d*, $J=8$ Hz), 5.56 (*brs*), 4.99 (*brd*, $J=1.5$ Hz), 4.90 (*brd*, $J=2$ Hz), 4.87 (*brs*), 4.18 (*d*, $J=7.8$ Hz) and carbons at δ 100.2, 99.7, 103.9, 104.4, 103.0 and 104.7, respectively. In the ESI-MS, the negative molecular ion $[M-H]^-$ detected at *m*/*z* 1485 indicated a $C_{69}H_{82}O_{36}$ molecular formula, which was confirmed by the observation of the $[M+Na]^+$ ion at *m*/*z* 1509 in the positive mode. This agreed with a kaempferol substituted by two glucose and four rhamnose residues, and acylated by two phenolic acids. The chemical shift and coupling constant data for the aromatic protons together with their corresponding ^{13}C NMR chemical shifts obtained from HSQC and HMBC experiments confirmed the identity of kaempferol as the aglycone (Mulinacci et al., 1995) (Table 1). The ^{1}H NMR spectrum of **1** exhibited other aromatic and ethylenic protons for two *p*-coumaroyl units (Carotenuto et al., 1996) (Table 1). The coupling constants (15.7 and 15.8 Hz) between the typical pair of doublets for olefinic protons (Table 1) indicated *trans* (*E*) configurations of the double bonds. In the ^{13}C NMR spectrum the relative upfield shifts of the C-3 and C-7 to δ 135.1 and 163.2 agreed with the glycosylation of kaempferol at the 3- and 7-positions (Mulinacci et al., 1995).

Starting from the anomeric protons at δ 5.67 and 4.18, the analysis of COSY and HOHAHA experiments allowed full identification of the spin systems of two β-D-glucoses, on the basis of their large coupling constants (Table 2). The first glucose (glc) had its H_{glc}-2 deshielded at δ 5.15 indicating that the hydroxyl group at C_{glc}-2 was acylated, and its C_{glc}-3 and C_{glc}-6 were deshielded (δ 83.0, 69.4) due to glycosylation at these two positions. The analysis of the HMBC spectrum showed $^{3}J_{C-H}$ correlations between the C_{glc}-6 and the anomeric proton of the second terminal glucose (glc′), and between H_{glc}-2 and the carbonyl (δ 168) of one *trans p*-coumaroyl unit. This disubstituted glucose is linked to the hydroxyl at C-3 of kaempferol since a $^{3}J_{C-H}$ correlation was observed between the H_{glc}-1 of glucose and C-3 of the aglycone. Despite the use of a 500 MHz NMR spectrometer, the presence of six sugar units in **1** resulted in considerable overlap of the other glycosidic protons. The identification of the four rhamnose units was realized from the combined analysis of COSY and HOHAHA spectra and using a 2D-*J* resolved proton experiment carried out to obtain unequivocal multiplicities (Table 2). The anomeric proton at δ 4.87 (*brs*) belongs to an α-L-rhamnose (rha) due to one characteristic methyl doublet at δ 1.22 ($J=6.1$ Hz) and one triplet for H_{rha}-4 at δ 3.36 ($J=8.9$ Hz). A $^{3}J_{C-H}$ correlation between the anomeric proton of this rhamnose and the C_{glc}-3 of the abovementioned disubstituted glucose was observed on the HMBC spectrum. Thus, a [β-D-glucopyranosyl (1→6) (α-L-rhamnopyranosyl (1→3))-(2-*O*-*trans para*-coumaroyl)-β-D-glucopyranosyl] chain was linked at position 3 of kaempferol. The MS/MS fragmentation of the $[M-H]^-$ peak yielded two product ions at *m*/*z* 869 due to the loss of this acylated triglycosidic chain at C-3, and at *m*/*z* 901 due to the loss of a second acylated glycosidic chain composed of three rhamnoses and one *para*-coumaric acid. From the three last broad signals of anomeric protons, the identification of rhamnosyl units in the second triglycosidic linear chain was performed on the basis of the same arguments as those described above. The first rhamnose (rha″; H-1 δ 5.56) of this chain, was acetylated by the second *trans p*-coumaroyl unit in position 4 and glycosylated in position 3, according to the deshielding of its $H_{rha''}$-4 (δ 5.30) and $C_{rha''}$-3 (δ 78.8). The sequencing was confirmed by a $^{3}J_{C-H}$ HMBC correlation between the carbonyl of the second *trans p*-coumaroyl unit (δ 168.1) and the $H_{rha''}$-4, and between the $C_{rha''}$-3 and the anomeric proton (δ 4.90) of the second rhamnosyl unit (rha‴) in the chain. According to a $^{3}J_{C-H}$ correlation between the $C_{rha''}$-3 (δ 79.4) of this intermediate rhamnose and the last rhamnosyl anomeric proton (rha⁗; H-1 δ 4.99), the second rhamnose in the chain was substituted in position 3 by the terminal rhamnose. The interglycosidic linkage of this acylated trirhamnosyl chain was confirmed in the ROESY spectrum by the observation of rotating frame Overhauser enhancements (ROEs) between the anomeric protons and the glycosidic protons of glycosylated positions. According to the observation of ROEs between $H_{rha''}$-1 of the first α-L-rhamnose in this chain and the H-6 and H-8 of the aglycone, the acylated trirhamnosyl chain was linked to the hydroxyl at the position 7 of kaempferol. Thus, the structure of the compound **1** is proposed to be kaempferol-3-*O*-[β-D-glucopyranosyl (1→6)-{α-L-rhamnopyranosyl(1→3)}-(2-*O*-*trans*-*p*-coumaroyl)]-β-D-glucopyranoside-7-*O*-[α-L-rhamnopyranosyl(1→3)-α-L-rhamnopyranosyl(1→3)-(4-*O*-*trans*-*p*-coumaroyl)]-α-L-rhamnopyranoside.

Comparison of the mass spectrum (ESI-MS) of compounds **2** and **1**, showed the presence of the same molecular ions in positive and negative modes ($[M+Na]^+$ at *m*/*z* 1509 and $[M-H]^-$ at *m*/*z* 1485). The MS/MS fragmentation of those molecular peaks were similar, indicating that flavonoids **1** and **2** were isomers. The analysis of NMR spectroscopic data indicated that

Table 1
^{1}H and ^{13}C spectral data of the kaempferol and *p*-coumaric acid parts of flavonoids **1**–**3** in CD_3OD

	1		2		3	
	C	H	C	H	C	H
2	159		159		159.3	
3	135.1		135.1		135.0	
4	179.3		179.3		179.2	
4a	107.7		107.7		107.6	
5	162.9		163		162.9	
6	100.2	6.47 (*d*, 2)	100.6	6.47 (*d*, 3)	100.6	6.48 (*d*, 1.9)
7	163.2		163.2		163.1	
8	95.8	6.74 (*d*, 2.5)	95.8	6.73 (*br s*)	95.7	6.74 (*d*, 1.6)
8a	157.9		157.9		157.9	
1′	122.4		122.4		122.5	
2′–6′	132.4	8.04 (*d*, 8.9)	132.4	8.05 (*d*, 8.7)	132.4	8.01 (*d*, 8.9)
3′–5′	116.4	6.90 (*d*, 8.7)	116.4	6.89 (*d*, 8.7)	116.4	6.91 (*d*, 8.8)
4′	161.8		161.8		161.7	
trans p-coum (at C_{glc} -2)						
1	127.0[a]		127.1		127.0[a]	
2–6	131.4	7.47[b] (*d*, 8.4)	131.5	7.46 (*d*, 8)	131.4	7.45[b] (*d*, 8.7)
3–5	116.8	6.79[c] (*d*, 8.3)	116.8	6.80 (*d*, 8)	116.8	6.80[c] (*d*, 8.8)
4	161.4		161.5		161.4	
7	147.6[d]	7.66[d] (*d*, 15.7)	147.7	7.68 (*d*, 16)	147.5[d]	7.70[d] (*d*, 16)
8	114.5	6.35 (*d*, 15.9)	114.5	6.35 (*d*, 16)	114.5	6.35 (*d*, 15.9)
9	168		168		168	
trans p-coum (at $C_{rha''}$-4)						
1	127.1[a]				127.1[a]	
2–6	131.4	7.46[b](*d*, 8.6)			131.4	7.46[b] (*d*, 8.6)
3–5	116.8	6.80[c] (*d*, 8.4)			116.8	6.79[c] (*d*, 8.7)
4	161.4				161.4	
7	147.7[d]	7.68[d] (*d*, 15.6)			147.7[d]	7.66[d] (*d*, 16.1)
8	114.5	6.36 (*d*, 15.9)			114.5	6.35 (*d*, 15.9)
9	168.1				168.1	
cis p-coum (at $C_{rha''}$-4)						
1			127.6			
2–6			134.2	7.70 (*d*, 8.5)		
3–5			115.8	6.74 (*d*, 8.4)		
4			160.4			
7			147.3	6.93 (*d*, 13.3)		
8			115.6	5.79 (*d*, 13.3)		
9			166.7			

[a], [b], [c], [d] Signals with the same superscript are interchangeable.

these two compounds only differed by the configuration of one *p*-coumaroyl unit (Table 1). At variance with **1**, the *p*-coumaroyl unit acylating the first α-L-rhamnose of the trirhamnosic chain linked on the 7-hydroxyl group of kaempferol was in a *cis* (*Z*) configuration, according to the lower value of the coupling constant ($J = 13.3$ Hz) of the olefinic protons (Fiorini et al., 1998). Finally, the proton and carbon data for the rest of **2** were similar to those of **1**. The structure of **2** is kaempferol-3-*O*-[β-D-glucopyranosyl (1→6)-{α-L-rhamnopyranosyl(1→3)}-(2-*O*-*trans*-*p*-coumaroyl)]-β-D-glucopyranoside-7-*O*-[α-L-rhamnopyranosyl(1→3)-α-L-rhamnopyranosyl(1→3)-(4-*O*-*cis*-*p*-coumaroyl)]-α-L-rhamnopyranoside.

The positive and negative ESI-mass spectra of flavonoid 3 gave molecular ions at *m*/*z* 1509 $[M+Na]^+$ and 1469 $[M-H]^-$, respectively, which differed with those of flavonoid **1** by 16 mass units. This result suggested the replacement of one glucose residue by a rhamnose unit. In the negative mode, the MS^2 fragment ion at *m*/*z* 869 also detected in the mass fragmentation of **1** and **2**, corresponded to the loss of the glycosidic chain linked to the C-3 of kaempferol. The chain on position 7 was common to the three flavonoid derivatives and produced the MS^3 fragment ion at *m*/*z* 583. In the NMR spectrum, the signals of three α-L-rhamnoses and one *trans p*-coumaroyl residue forming the common chain linked at position 7 were effectively recognized (Tables 1 and 2). The sequence was confirmed by the observation of the interglycosidic HMBC cross-peaks and ROE interactions. Thus, the modification in compound **3** took place in the chain linked in position 3 of kaempferol. As in flavonoid **1**, NMR showed signals for one β-D-glucose (H_{glc}-1 δ 5.63) acylated in position 2 by a second *trans p*-coumaroyl unit and glycosylated in

Table 2
^{1}H and ^{13}C spectral data of glycosidic part of flavonoids **1–3** in CD_3OD

	1		**2**		**3**	
	C	H	C	H	C	H
β-D-glucose (at C-3)						
1	100.2	5.67 (*d*, 8)	100.2	5.67 (*d*, 8.2)	100.3	5.63 (*d*, 8)
2	75.3	5.15 (*t*, 8.4)	75.3	5.15 (*t*, 8.4)	75.3	5.11 (*t*, 9)
3	83.0	3.80 (*t*, 9)	83.0	3.78 (*t*, 7.4)	83.0	3.78 (*t*, 9)
4	70.2	3.56	70.2	3.54	70.2	3.42 (*t*, 9)
5	78.2	3.56	78.0	3.54	78.2	3.49
6	69.4	4.04 (*br d*, 11.7)	69.3	4.04 (*d*, 12.6)	69.4	3.88 (*m*)
		3.70 (*dd*, 12–4)		3.68		3.47 (*dd*, 13–4.3)
α-L-rhamnose (at C_{glc}-3)						
1	103.0	4.87 (*brs*)	103.0	4.85 (*br s*)	103.0	4.87 (*brs*)
2	72.5	3.73 (*dd*, 3.5–2)	72.5	3.69 (*br d*, 2.5)	72.4	3.72 (*m*)
3	72.1	3.65 (*dd*, 9.1–3.5)	72.2	3.63 (*dd*, 9.5–3.1)	72.1	3.62 (*dd*, 9.8–3.2)
4	73.7	3.36 (*t*, 8.9)	73.7	3.34 (*t*, 9.5)	73.7	3.35 (*t*, 9.5)
5	70.3	3.98 (*dq*, 8.8–5.6)	70.3	3.96 (*dq*, 9.5–6.3)	70.3	3.96 (*dq*, 9.5–5.6)
6	17.8	1.22 (*d*, 6.1)	17.8	1.22 (*d*, 6)	17.8	1.22 (*d*, 6.2)
β-D-glucose′ (at C_{glc}-6)					α-L-rhamnose′ (at C_{glc}-6)	
1	104.7	4.18 (*d*, 7.8)	104.7	4.18 (*d*, 7.9)	102.3	4.55 (*brd*, 1.5)
2	75.0	3.07 (*t*, 8.4)	75.0	3.06 (*t*, 8.2)	72.1	3.63 (m)
3	77.8	3.18 (*t*, 8.5)	77.8	3.15 (*t*, 8.9)	72.3	3.52 (*dd*, 9.6–3.4)
4	71.4	3.21 (*t*, 8.9)	71.4	3.20 (*t*, 9.5)	73.8	3.27 (*t*, 9.6)
5	77.7	3.04	77.7	3.01	69.8	3.47
6	62.6	3.59 (*dd*, 12–5.6)	62.6	3.57 (*dd*, 12–5.6)	17.9	1.12 (*d*, 6.2)
		3.77 (*dd*, 11.9–2.5)		3.75 (*br d*, 11.8)		
α-L-rhamnose″ (at C-7)						
1	99.7	5.56 (*brs*)	99.6	5.55 (*brd*, 1.5)	99.7	5.56 (*brd*, 2)
2	71.5	4.19 (*m*)	70.3	4.15 (*br d*, 3)	71.5	4.19 (*dd*, 3.8–1.8)
3	78.8	4.17 (*m*)	78.2	4.07 (*dd*, 10.2–3.1)	78.8	4.14 (*dd*, 9.8–3.6)
4	73.2	5.30 (*t*, 9.5)	73.1	5.22 (*t*, 9.9)	73.1	5.30 (*t*, 9.8)
5	69.6	3.90 (*dq*, 9.5–6.2)	69.6	3.79	69.5	3.87
6	18.0	1.17 (*d*, 6.5)	18.0	1.15 (*d*, 6)	18.0	1.17 (*d*, 6.4)
α-L-rhamnose‴ (at $C_{rha''}$-3)						
1	104.4	4.90 (*brd*, 2)	104.3	4.85 (*br s*)	104.4	4.90 (*brd*, 1.5)
2	72.1	3.82 (*dd*, 3.4–2)	72.2	3.84 (*m*)	72.1	3.81 (*dd*, 2.8–1.5)
3	79.4	3.85 (*dd*, 9.4–3.5)	79.4	3.82 (*dd*, 9.5–3.1)	79.2	3.84 (*dd*, 9.5–2.8)
4	73.2	3.51 (*t*, 9.5)	73.1	3.49 (*t*, 9.1)	73.1	3.49 (*t*, 9.2)
5	70.7	3.89	70.7	3.86	70.7	3.88
6	18.0	1.29 (*d*, 6.5)	18.0	1.25 (*d*, 7)	18.0	1.28 (*d*, 6.2)
α-L-rhamnose⁗ (at $C_{rha'''}$-3)						
1	103.9	4.99 (*brd*, 1.5)	104.0	4.97 (*br s*)	103.9	4.99 (*brd*, 1.5)
2	72.1	3.95 (*dd*, 3.5–2)	72.2	3.93 (*br d*, 2.6)	72.1	3.94 (*dd*, 3–1.3)
3	72.1	3.70 (*dd*, 9.5–3.2)	72.2	3.71 (*dd*, 10–3)	72.1	3.69 (*dd*, 9.8–3.8)
4	74.0	3.33 (*t*, 9.3)	74.0	3.30 (*t*, 9.5)	74.0	3.34 (*t*, 9.5)
5	70.0	3.79	70.0	3.66	70.0	3.65
6	17.9	1.18 (*d*, 6.5)	17.9	1.07 (*d*, 6.1)	17.9	1.18 (*d*, 6.4)

position 3 by a terminal α-L-rhamnose (rha; H-1 δ 4.87) (Tables 1 and 2). From the last anomeric proton appearing as a broad doublet (δ 4.55, $J = 1.5$ Hz), a fifth α-L-rhamnose was actually identified. According to the $^{3}J_{C-H}$ HMBC correlation observed between this anomeric proton and the C_{glc}-6 (δ 68.2) of the glucose, the glucosyl unit was consequently disubstituted by rhamnoses in positions 3 and 6. Consequently, the structure of **3** is kaempferol-3-*O*-[α-L-rhamnopyranosyl (1→6)-{α-L-rhamnopyranosyl(1→3)}-(2-*O*-*trans*-*p*-coumaroyl)]-β-D-glucopyranoside-7-*O*-[α-L-rhamnopyranosyl(1→3)-α-L-rhamnopyranosyl(1→3)-(4-*O*-*trans*-*p*-coumaroyl)]-α-L-rhamnopyranoside.

Besides flavanol oligomers, anthocyanins and tannins containing a flavonoid moiety, the molecules described here are, to the best of our knowledge, the largest flavonoids derivatives described so far. The complexity of the mixtures and the difficulties associated with their separation and structural elucidation have left these substances untouched by the phytochemists and it is likely that more will be seen of them in the not too distant future.

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 500 NMR spectrometer at 500 and 125 MHz using CD_3OD as solvent. Two dimensional NMR experiments were performed using standard Bruker microprograms (Xwin-NMR version 2.6 software). The ESI-MS and ESI-MS^n were acquired with a Bruker Esquire LC–MS instrument. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Philips PU 8720 spectrometer. HPLC was performed on a Dionex apparatus equipped with a diode array detector.

3.2. Plant material

The leaves of *P. grandis* Ridley were collected in Kedah, Malaysia by C. Wiart. An herbarium specimen is kept at the Forest Research Institute of Malaysia at Kepong, under the reference KL 4484.

3.3. Extraction and isolation

Powdered leaves (520 g) were macerated at room temp. in 6 l of petroleum ether for 24 h and then lixiviated. These delipidated leaves were boiled in H_2O/MeOH (1:4, 6 l) for 15 h. After cooling and filtration, the aq. methanolic extract was evaporated to provide a residue which was dissolved in MeOH (1 l). The methanolic solution was added to Me_2CO (5 l) the ppt. (13.5 g) was filtered and dried over KOH in vacuo. A second precipitation was performed with Me_2CO (2.5 l) yielding a second ppt. (10.5 g) which was dried over KOH in vacuo. These precipitates were dissolved in H_2O and dialysed in cellulose tubing against H_2O for 48 h. After freeze-drying of the content of the tubes, 14 g of the extract was obtained, which was purified by VLC on silica gel using a gradient of $CHCl_3$–MeOH–H_2O (from 80:20:2 to 65:35:4) to remove tannins. The detannified extract (4 g) was purified by silica gel CC (160 g) using a gradient of CH_2Cl_2/MeOH (from 100:0 to 50:50). Frs 60–66 and 70–100 eluted with 80:20, containing a mixture of flavonoids **1**, **2** and **3** were purified by semi-prep. HPLC on a Dionex C-18 201 SP^{TM} column (5 μm particle size, 10×250 mm), kept at 25 °C, and using as solvent an isocratic mixt. H_2O/TFA 0.025%–CH_3CN (70:30) with a solvent flow rate of 3 ml min^{-1}. This purification afforded 1 (19.1 mg), 2 (8.4 mg) and 3 (8.8 mg).

3.4. Acid hydrolysis of flavonol glycosides

A portion of the crude extract (600 mg) was dissolved in a mixture (1:1, 40 ml) of H_2SO_4 (0.02 N) and 6.5% $HClO_4$ and refluxed for 3 h at 140 °C. After cooling and filtration, the acid aq. layer was neutralized with KOH (0.5 M) and freeze-dried. Two sugars were identified with authentic samples by TLC [MeCOEt/Me_2CH_2OH/Me_2CO/H_2O (20:10:7:6)] as rhamnose and glucose. After preparative TLC of the sugar mixture, the optical rotation of each purified sugar was measured: L-rhamnose $[\alpha]_D^{20}$ +3.5 ($c=0.01$, H_2O); D-glucose $[\alpha]_D^{20}$ +8.5 ($c=0.21$, H_2O).

3.5. Acylated flavonoid glycoside (1)

$[\alpha]_D^{20}$ +228.7 (MeOH, $c=0.167$); UV λ_{max}^{MeOH} nm: 228, 270, 318, 323; +$AlCl_3$: 230, 280, 323, 399; + $AlCl_3$/HCl: 230, 280, 323, 399; +NaOAc: 271, 318, 323. ESI-MS (positive) m/z: 1509.8 $[M+Na]^+$; ESI-MS^2 (1509.8) m/z: 893.5 [M+Na-chain at C-3]$^+$, 639.4 [chain at C-3+Na]$^+$; ESI-MS (negative) m/z: 1485.8 $[M-H]^-$, 1339.3 [M−H-146]$^-$, 901.3 [M−H-chain at C-7]$^-$, 869.3 [M−H-chain at C-3]$^-$; ESI-MS^2 (1339.4) m/z: 901.3; ESI-MS^2 (869.3) m/z: 583 [chain at C-7-H]$^-$. For ^1H NMR and ^{13}C NMR spectra: see Tables 1 and 2.

3.6. Acylated flavonoid glycoside (2)

$[\alpha]_D^{20}$ +342 (MeOH, $c=0.045$); UV λ_{max}^{MeOH} nm: 230 (*sh*), 271, 317; +$AlCl_3$: 230 (*sh*), 279, 320, 398; + $AlCl_3$/HCl: 230 (*sh*), 279, 320, 398; +NaOAc: 271, 317. ESI-MS (positive) m/z: 1509.3 $[M+Na]^+$, 1363 [M+Na-146]$^+$; ESI-MS^2 (1509.3) m/z: 893.5 [M+Na-chain at C-3]$^+$, 639.4 [chain at C-3+Na]$^+$; ESI-MS (negative) m/z: 1485.7 $[M-H]^-$, ESI-MS^2 (1485.7) m/z: 1339.3 [M−H-146]$^-$, 901.3 [M−H-chain at C-7]$^-$, 869.3 [M−H-chain at C-3]$^-$; ESI-MS^3 (1339.3) m/z: 901.3; ESI-MS^3 (901.3) m/z: 755.3 [M−H-chain at C-7-146]$^-$, 284.6 [kaempferol-2H]$^-$, 615 [chain at C-3-H]$^-$; ESI-MS^3 (869.3) m/z: 583.2 [chain at C-7-H]$^-$, 284.6. For ^1H NMR and ^{13}C NMR spectra: see Tables 1 and 2.

3.7. Acylated flavonoid glycoside (3)

$[\alpha]_D^{20}$ −122.8 (MeOH, $c=0.464$); UV λ_{max}^{MeOH} nm: 229, 270, 317, 323; +$AlCl_3$: 229, 279, 315, 400; + $AlCl_3$/HCl: 229, 279, 315, 400; +NaOAc: 270, 317, 323. ESI-MS (positive) m/z: 1509.5 $[M+K]^+$, 1493.7 $[M+Na]^+$; ESI-MS^2 (1493) m/z: 893.5 [M+Na-chain at C-3]$^+$, 623.4 [chain at C-3+Na]$^+$; ESI-MS (negative) m/z: 1469.9 $[M-H]^-$, 736 [M−H-chain at C-7-146-3H]$^-$; ESI-MS^2 (1469.9) m/z: 1323.4 [M−H-146]$^-$, 885.3 [M−H-chain at C-7]$^-$, 869.3 [M−H-chain at C-3]$^-$, 583.2 [chain at C-7-H]$^-$; ESI-MS^3 (1323.4) m/z: 885.3, 723.3 [M−H-chain at C-3-146]$^-$; ESI-MS^3 (885.3) m/z: 739.2 [M−H-chain at C-7-146]$^-$, 721.2 [M−H-chain at C-7-146-H_2O]$^-$, 599.2 [chain at C-3-H]$^-$, 284; ESI-MS^3 (869.3) m/z: 583.2, 284.2 [kaempferol-2H]$^-$. For ^1H NMR and ^{13}C NMR spectra: see Tables 1 and 2.

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