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POLYPHENOLIC GLYCOSIDES FROM DOUGLAS FIR INNER BARK

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Abstract—A chemical examination of the aqueous fraction of Douglas fir (*Pseudotsuga menziesii*) inner bark has led to the isolation and characterization of the novel compound epicatechin 7-*O*-β-D-glucopyranoside. Also isolated and identified were phloroglucinol 1-*O*-β-D-glucopyranoside, catechin 7-*O*-β-D-glucopyranoside, catechin 4′-*O*-β-D-glucopyranoside, 3′-*O*-methylepicatechin 7-*O*-β-D-glucopyranoside, dihydroquercetin 3′-*O*-β-D-glucopyranoside, dihydroquercetin 7-*O*-β-D-glucopyranoside and dihydrokaempferol 7-*O*-β-D-glucopyranoside.

INTRODUCTION

Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] is a valuable timber tree that is abundant in the Pacific Northwest of the United States. The economy of this region, and particularly of the state of Oregon, is largely derived from exploitation of this resource In Oregon alone, *ca* three million tons of Douglas fir bark are generated annually as waste, and this causes considerable disposal problems for the forest industries [1] As part of a program of evaluating this waste bark for commercial utilization, several chemical investigations have been conducted, particularly by Kurth and co-workers [2–5]. The present study is an extension of that evaluation, but it emphasizes the polyphenolic glycosides, some of the rarer, low M_r, water-soluble constituents that have often been neglected in other studies. Of particular interest are the flavan-3-ol glycosides, which have been almost unknown until recently, although their aglycones are widely distributed in the plant kingdom and are almost invariably accompanied by the proanthocyanidins, their higher oligomers

RESULTS AND DISCUSSION

The polyphenolic glycoside fraction was obtained by column chromatography with Sephadex LH-20 of the aqueous portion of the inner bark extract using methanol–water (1 1) as eluent. Initial separation of the glycosides was effected on an MCI-gel CHP-2OP column with methanol–water (3 7), and final purification into compounds **1**–**7** was achieved by repeated chromatography over Sephadex LH-20 using ethanol–water (3·17) as solvent

Compound **1** exhibited an orange to red colour on TLC when sprayed with vanillin–HCl solution Its ^{13}C NMR spectrum showed carbon signals characteristic of a flavan-3-ol with the hydroxylation pattern of the phloroglucinol A-ring and the catechol B-ring [6] The upfield position of the C-2 chemical shift (δ 80.1) suggested that the flavan possessed the 2,3-*cis* configuration This was supported by the ^{1}H NMR spectra, which showed minimal couplings (< 1 Hz) between the H-2 and H-3 protons In addition, the presence of six carbon signals in the aliphatic region indicated a hexose moiety. Mild hydrolysis with β-glucosidase yielded glucose and epicatechin, respectively. Thus the glucose was of the β-configuration, and this assignment was supported by the downfield position of the anomeric carbon (δ 102 4) of the sugar moiety. Comparing the flavan carbon resonances of **1** with those of epicatechin showed that all major differences in chemical shift values were restricted to the A-ring carbons. The largest of those differences was ascribed to the downfield shift (+Δ2.1 ppm) of the C-4a resonance and the associated smaller downfield shifts of the C-6 and C-8 resonances The fact that these '*para*' and '*ortho*' shift effects were caused by glycosidation [7] unambiguously defined the site of glycosidation as the 7-0. Fast atom bombardment mass spectrometry (FABMS) of **1** showed an $[M-H]^-$ ion peak at *m/z* 451, and this confirmed its chemical constitution as epicatechin 7-*O*-β-D-glucopyranoside, a new natural product among a limited class of epicatechin glycosides which have only recently been described [8, 9]

Compound **2** yielded glucose and a flavan on mild treatment with β-glucosidase. The close structural similarity of **2** and epicatechin 7-*O*-β-D-glucopyranoside was readily apparent from their NMR spectra. The presence of an additional methyl moiety in **2** was evident from a carbon chemical shift at δ 56.4, which indicated that one of the flavan hydroxyls was methylated This constitution was corroborated by FABMS, which showed an ion peak corresponding to $[M-H]^-$ at *m/z* 465. As the chemical shifts of the A-ring carbons were practically identical to those of **1**, it could be assumed that the sugar moiety in **2** was attached to the 7-position and that the methyl was at 3′- or 4′- A considerable upfield shift of the resonance (δ 111 9) of one of the methine carbons *ortho* to the oxygenated carbons also supported the assumption that one of the hydroxyl groups in the B-ring was methylated [9]. The structural assignment was resolved by consideration of the effect of methylation on the two carbons *para* to the possible methylation sites, namely the C-1′ and C-6′ Comparison of the B-ring carbon resonances with

Table 1 ^{13}C NMR chemical shifts (ppm) of dihydroflavonols and their glycosides in CD_3OD

	Aglycone moiety								
Compound*	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-4a	C-8a
Dihydroquercetin	84.8	73.5	198.1	164.3	97.3	168.4	96.3	101.7	165.0
6	84.9	73.5	198.4	164.4	97.4	168.7	96.4	101.9	165.3
7	85.2	73.7	199.2	164.6	98.3	167.2	97.0	103.4	164.1
Dihydrokaempferol	85.0	73.7	198.4	164.6	97.5	168.8	96.4	101.9	165.3
8	85.1	73.7	199.3	164.2	98.3	167.3	97.0	103.5	164.7

*See text for full names of numbered compounds

those of **1** and its aglycone showed that C-1′ was unaffected, whereas C-6′ had shifted downfield by 1.2 ppm. This indicated that methylation is present at C-3′ [7, 9]. The structural assignment was further supported by 2D NMR studies using correlation spectroscopy (COSY), which showed long-range couplings between the methoxyl protons and the only *meta*-coupled H-2′. Compound **2** was therefore identified as 3′-*O*-methylepicatechin 7-*O*-β-D-glucopyranoside (also known as simplocoside), a compound isolated once before from a Brazilian medicinal plant, *Symplocos uniflora* (Pohl) Benth [10]. Compounds **1** and **2** represent the only two examples of naturally occurring A-ring O-glycosides of epicatechin, although 3-*O*- and *C*-glycosides of epicatechin have been recently described [8].

Compounds **3** and **4** were also flavan glycosides with the same hydroxylation patterns as **1** and **2**. The downfield position of the C-2 chemical shifts observed in the ^{13}C NMR spectra of **3** and **4** indicated that the flavan moieties possessed the 2,3-*trans* configuration [6]. This was also apparent in their proton couplings ($J = 7.6$ Hz) between the H-2 and H-3. Treatment of both compounds with β-glucosidase yielded glucose and catechin, which indicated that the hexose was glucose with the β-configuration, as was also indicated by the downfield positions of the anomeric carbon chemical shifts. FABMS showed an $[M-H]^-$ ion peak at m/z 451 for both compounds, which confirmed their catechin glucoside constitution. Compounds **3** and **4** were therefore considered to be regioisomers that differ only in the position of glucosidation. Comparison of the flavan carbon chemical shift values of **3** with those of catechin showed that significant differences were confined to the A-ring carbon resonances. The largest of those differences was the C-4a, whose downfield shift to δ 103.6 was consistent with the occurrence of glycosidation at the *para* position to C-4a or at 7-O. This assignment was also supported by the associated, smaller downfield shifts of the C-6 and C-8, which were *ortho* to the site of glycosidation. Compound **3** is therefore catechin 7-*O*-β-D-glucopyranoside, recently isolated from *Schizandra nigra* Max. [11] and *Rheum* species [12]. In contrast, major differences between the chemical shift values of **4** and those of catechin were localized in the B-ring carbon resonances. The most pronounced difference was attributed to the C-1′, which had shifted downfield by 4.2 ppm from the corresponding carbon in catechin. Thus glucosidation has occurred in the *para* position to C-1′, and **4** is therefore catechin 4′-*O*-β-D-glucopyranoside, a metabolite also identified in the *Rheum* species [12].

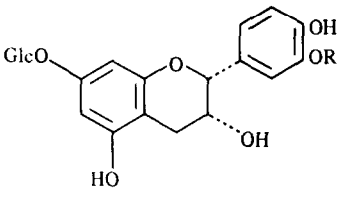
Compound **5**, which exhibited high mobility on cellulose TLC, gave an orange to red colour when the TLC was sprayed with vanillin–HCl solution. Both the ^{1}H and the ^{13}C NMR spectra of **5** were consistent with those of a phenolic glycoside. Two downfield carbon resonances (δ 160.9 and 2 × 160.1), attributed to three oxygenated carbons and three upfield methine carbon resonances (δ 98.0 and 2 × 96.7), were observed together with the hexose carbon resonances in the aliphatic region. In addition to the hexose protons, the ^{1}H NMR spectrum of **5** showed two sets of mutually *meta* coupled protons which integrated for one proton at δ 5.93 ($J = 2.1$ Hz) and for two protons at δ 6.06 ($J = 2.1$ Hz). This is consistent with the aglycone moiety being phloroglucinol. Mild hydrolysis of **5** with β-glucosidase yielded glucose and phloroglucinol, which confirmed that the compound was phloroglucinol 1-*O*-β-D-glucopyranoside. This chemical constitution was supported by FABMS studies, which showed an $[M-H]^-$ ion peak at m/z 287. Phloroglucinol 1-*O*-β-D-glucopyranoside (also known as phlorin) has also been reported in *Cornus capitata* Wall. (subgen. *Benthamia* Lindl.) [13] and *Cistus laurifolius* [14].

Compounds **6** and **7** were structurally similar and were readily identified as dihydroflavonol glycosides [15–17] from their ^{1}H and ^{13}C NMR spectra (Table 1). The large H-2 and H-3 coupling constants ($J = 11.7$ Hz) observed in their ^{1}H NMR spectra indicated that both compounds possessed the common 2,3-*trans* stereochemistry [16, 17]. Enzymic hydrolysis of **6** and **7** with β-glucosidase yielded glucose and dihydroquercetin, which indicated that both compounds are dihydroquercetin glucosides that differ only in the position of glucosidation.

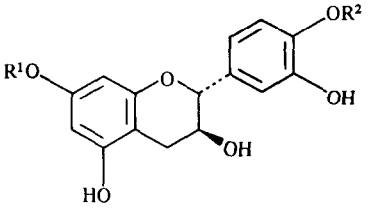
Comparison of the carbon chemical shifts of **6** with dihydroquercetin (Table 1) indicated that the major difference between **6** and its aglycone was in the B-ring, and this limited the site of glucosidation to either the 3′ or the 4′ positions. This was also supported by UV absorption studies in which the main band at 287 nm was shifted to 326 nm on addition of sodium acetate. The effect of glucosidation on the two carbons *para* to the possible glucosidation sites showed that C-6′ had shifted downfield to δ 124.7, whereas C-1′ was only marginally affected. Thus, **6** is dihydroquercetin 3′-*O*-β-D-glucopyranoside, which has also been isolated from the needles of *Pinus sylvestris* [18].

In contrast, the carbon resonances of the aglycone of **7** differed from those of dihydroquercetin only in the A-ring carbons, the most apparent difference being the C-4a resonance, which had shifted downfield to δ 103.4. The C-6 and C-8 resonances of **7** had also shifted downfield with respect to those of dihydroquercetin, a result of the *ortho* effects similarly observed in the ^{13}C NMR spectra of **1** and **3**. Compound **7** is therefore dihydroquercetin 7-*O*-β-D-glucopyranoside. The site of glucosidation was corroborated by UV spectroscopy as the main band at 286 nm was unaffected by the addition of sodium acetate.

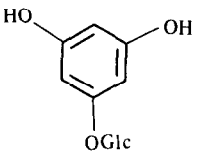
						Sugar moiety					
C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6
129 7	116 1	146 1	146 9	115 9	120 9						
130 0	118.3	146 5	149 0	116 9	124 7	104 1	74 9	78.4	71 5	77 6	62.6
129 6	116 0	146 2	147 1	116 1	121 0	101 2	74.6	78.1	71 1	77 7	62.3
129 4	130 4	116 3	159 2	116 3	130 4						
129 1	130 4	115 9	159 2	116 2	130 4	101 3	74.6	78.2	71 1	77 7	62.3



1 R = H
2 R = Me



3 R^1 = Glc, R^2 = H
4 R^1 = H, R^2 = Glc



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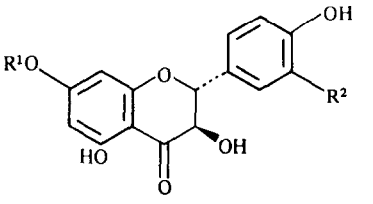

6 R^1 = H, R^2 = OGlc
7 R^1 = Glc, R^2 = OH
8 R^1 = Glc, R^2 = H

solution. Dihydroquercetin 7-*O*-*β*-D-glucopyranoside has recently been identified in *Podocarpus nivalis* [19] and in Hungarian sorghum [20].

The ^{1}H and ^{13}C NMR spectra of **8** indicated that it is another dihydroflavonol glycoside with the common 2,3-*trans* stereochemistry and the phloroglucinol A-ring oxidation pattern. The B-ring carbon chemical shifts showed two pairs of degenerate carbons at δ116.2 and 130.4 and an oxygenated aromatic carbon at δ159.2, characteristic of a *para*-substituted hydroxylated benzene ring. This was supported by the observation of a pair of two-proton, *ortho* coupled doublets (δ7.41, J=8.6 Hz; δ6.82, J =8.6 Hz) arising from two pairs of degenerate protons (H-2′, H-6′ and H-3′, H-5′, respectively) which were characteristic of a *para*-substituted benzene ring. Comparing the ^{13}C NMR of **8** with that of dihydrokaempferol showed the same type of downfield shifts that were observed for the C-4a and the associated C-6 and C-8 as encountered earlier in **1**, **2**, **3**, and **7** The site of glucosidation was further supported by UV evidence, since the main 283 nm band was unaffected by addition of base. Thus, **8** is dihydrokaempferol 7-*O*-*β*-D-glucopyranoside, reported previously in *Podocarpus hallii* and several other plants [19].

EXPERIMENTAL

IR spectra were recorded on KBr pellets Analytical TLC was performed on Schleicher and Schuell cellulose with *t*-BuOH–HOAc–H_2O (3.1 1, solvent A) and HOAc–H_2O (3:47, solvent B). FABMS was done with Kratos MS-5OTC mass spectrometer with samples dissolved in a matrix of a 5:1 mixture of dithiothreitol and dithioerythritol (Magic Bullet)

Extraction and isolation Fresh inner bark (1 kg) of a 120-year-old Douglas fir was extracted exhaustively with MeOH, and the combined extract concd on a rotary evaporator under red. pres The residual extract was diluted with H_2O, and the resulting aq. extract exhaustively washed, first with hexane and then with EtOAc, before freeze-drying, which yielded a fluffy brown solid (48 g) A portion of this solid (40 g) was applied to a Sephadex LH-20 column (5 × 25 cm), and the column washed with MeOH–H_2O (1 1) to yield a carbohydrate fraction (19 0 g), a flavanoid glycoside fraction (2 2 g), and an oligomeric procyanidin fraction (9 0 g) The flavanoid glycoside fraction was worked-up by repeated CC, first alternating between MCI CHP-20P 75-150 μ gel (MeOH–H_2O, 3·7) and Sephadex LH-20 (EtOH–H_2O, 1·1) and finally with Sephadex LH-20 (EtOH–H_2O, 3 17) to yield chromatographically homogeneous products

Enzymic hydrolysis was performed by dissolving a small sample of the glycoside in H_2O with a small amount of chromatographically purified *β*-glucosidase After 3 hr at ambient temp., the solution was freeze-dried and the products extracted with MeOH The MeOH-soluble portion was analysed by cellulose TLC, solvents A and B were used to detect aglycones, and *n*-BuOH–pyridine–H_2O (6 4 3) was used to detect glucose.

Epicatechin-7-O-β-D-glucopyranoside (1) was freeze-dried to an almost colourless solid (6 mg), $[\alpha]_{589}$ −61° (MeOH, *c* 0 1), R_f 0 25 (A) and 0.60 (B) ν_{max} (cm^{-1}): 3437 (b), 1625, 1521, 1443, 1360, 1164, and 1075 ^{13}C NMR (ppm, MeOH-d_4): 29.4 (C 4), 62.5 (C 6″), 67 3 (C 3), 71 4 (C 4″), 74.9 (C 2″), 78.1 (C 3″, C 5″), 80 1 (C 2), 97 4 (C 8), 97 6 (C 6), 102 4 (C 1″), 102 9 (C 4a), 115 4 (C

2'), 115.9 (C-5'), 119.4 (C-6'), 132.1 (C-1'), 145.8 (C-3'), 146.0 (C-4'), 158.0 (C-8a), 158.4 (C-7). ^{1}H NMR (MeOH-d_4) 2.7–3.0 (2H, *m*, H-4), 3.35–3.50 (4-H, *m*, H-2, H-3, H-4, H-5 of sugar), 3.70 (1H, *dd*, H-6a of sugar), 3.88 (1H, *dd*, H-6b of sugar), 4.20 (1H, *br t*, H-3), 4.7–5.0 (*br* – OH obscuring H-2 of aglycone and anomeric proton), 6.20 (2H, *br s*, H-6, and H-8), 6.70–6.83 (2H, *m*, H-5', H-6'), and 6.95 (1H, *d*, H-2')

3'-O-*Methylepicatechin*-7-O-*β*-D-*glucopyranoside* (**2**) was freeze-dried to a colourless solid (50 mg), $[\alpha]_{589}$ −66° (MeOH, *c* 0.2), R_f 0.48 (A) and 0.63 (B) ν_{max} (cm^{-1}) 3430 (b), 1625, 1609, 1520, 1463, 367, 1278, 1200, 1154, 1143, 1079, and 1031. ^{13}C NMR (ppm, MeOH-d_4) 29.4 (C-4), 56.4 (–OMe), 62.5 (C-6''), 67.3 (C-3), 71.3 (C-4''), 74.9 (C-2''), 78.1 (C-3''), 78.2 (C-5''), 80.0 (C-2), 97.0 (C-8), 98.5 (C-6), 102.5 (C-1''), 102.6 (C-4a), 111.9 (C-2'), 115.7 (C-5'), 120.6 (C-6'), 132.1 (C-1'), 147.0 (C-4'), 148.6 (C-3'), 157.1 (C-5), 157.8 (C-7), 158.4 (C-8a). ^{1}H NMR (MeOH-d_4) 2.8–3.0 (2H, *m*, H-4), 3.30–3.45 (4H, *m*, H-2, H-3, H-4, and H-5 of sugar), 3.72 (1H, *dd*, H-6 sugar), 3.80 (3H, *s*, –OMe), 3.88 (1H, *br d*, H-6 sugar), 4.15 (1H, *br t*, H-3), 4.80 (2H, *m*, H-2 and anomeric proton), 6.05 (1H, *d*, H-6), 6.20 (1H, *d*, H-8), 6.77 (1H, *d*, H-5'), 6.90 (1H, *dd*, H-6'), 7.10 (1H, *d*, H-2')

Catechin-7-O-*β*-D-*glucopyranoside* (**3**) was freeze-dried to a colourless solid (15 mg), $[\alpha]_{589}$ −40° (MeOH, *c* 0.1), R_F 0.45 (A) and 0.70 (B) ν_{max} (cm^{-1}) 3444, 1624, 1519, 1442, 1364, 1285, 1166, 1105, 1076, and 1047. ^{13}C NMR (ppm, MeOH-d_4) 28.5 (C-4), 62.5 (C-6''), 68.6 (C-3), 71.3 (C-4''), 74.8 (C-2''), 78.0 (C-3'', C-5''), 82.9 (C-2), 96.9 (C-8), 97.4 (C-6), 102.2 (C-1''), 103.6 (C-4a), 115.3 (C-2'), 116.1 (C-5'), 120.0 (C-6'), 132.1 (C-1'), 146.2 (C-3', C-4'), 156.8 (C-7), 157.5 (C-5), 158.6 (C-8a). ^{1}H NMR (MeOH-d_4) 2.5–2.9 (2H, *m*, H-4), 3.37–3.48 (4H, *m*, H-2, H-3, H-4, H-5 of sugar), 3.70 (1H, *dd*, H-6a of sugar), 3.80 (1H, *dd*, H-6b of sugar), 3.95 (H, *m*, H-3), 4.60 (1H, *d*, J = 7.4 Hz, H-2), 4.81 (1H, *d*, J = 7.3 Hz, anomeric H), 6.15 (1H, *d*, J = 2.2 Hz, H-6), 6.19 (1H, *d*, J = 2.2 Hz, H-8), 6.72 (1H, *dd*, H-6'), 6.76 (1H, *d*, J = 8.1 Hz, H-5'), 6.82 (1H, *d*, J = 1.94 Hz)

Catechin 4'-O-*β*-D-*glucopyranoside* (**4**) was freeze-dried to a colourless solid (35 mg), $[\alpha]_{589}$ −38° (MeOH, *c* 0.1), R_F 0.48 (A) and 0.50 (B) ν_{max} (cm^{-1}) 3420, 1624, 1513, 1448, 1277, 1143, 1103, 1071, and 1048. ^{13}C NMR (ppm, MeOH-d_4) 28.3 (C-4), 62.4 (C-6''), 68.8 (C-3), 71.3 (C-4''), 74.8 (C-2''), 77.6 (C-3''), 78.3 (C-5''), 82.4 (C-2), 95.5 (C-8), 96.4 (C-6), 100.8 (C-4a), 104.3 (C-1''), 115.8 (C-2'), 118.6 (C-5'), 119.9 (C-6'), 136.4 (C-1'), 146.5 (C-3'), 148.3 (C-4'), 157.6 (C-5, C-7), 157.8 (C-8a). ^{1}H NMR (MeOH-d_4) 2.40–2.90 (2H, *m*, H-4), 3.37–3.50 (4H, *m*, H-2, H-3, H-4, H-5 of sugar), 3.70 (1H, *dd*, H-6a of sugar), 3.87 (1H, *dd*, H-6b of sugar), 4.00 (1H, *m*, H-3), 4.62 (1H, *d*, J = 7.4 Hz, H-2), 4.70 (1H, *d*, J = 7.3 Hz, anomeric H), 5.86 (1H, *d*, J = 2.1 Hz, H-6), 5.92 (1H, *d*, J = 2.1 Hz, H-8), 6.82 (1H, *dd*, H-6'), 6.89 (1H, *d*, J = 2.0 Hz), 7.17 (1H, *d*, J = 8.5 Hz, H-5')

Phloroglucinol 1-O-*β*-D-*glucopyranoside* (**5**) was freeze-dried to a powder (6 mg), $[\alpha]_{589}$ −43° (MeOH, *c* 0.01), R_f 0.40 (A) and 0.80 (B) ν_{max} (cm^{-1}) 3430, 1613, 1522, 1452, 1366, 1284, 1161, 1075, and 828. ^{13}C NMR (ppm, MeOH-d_4) 62.5 (C-1''), 71.3 (C-4''), 74.9 (C-2''), 78.0 (C-3''), 78.1 (C-5''), 96.7 (C-2, C-6 of aglycone), 98.0 (C-4 of aglycone), 102.1 (C-1''), 160.1 (C-3, C-5 of aglycone), and 160.9 (C-1 of aglycone). ^{1}H NMR (MeOH-d_4) 3.37–3.48 (4H, m, H-2, H-3, H-4, H-5 of sugar), 3.70 (1H, *dd*, H-6a of sugar), 3.88 (1H, *dd*, H-6b of sugar), 4.70 (1H, *d*, J = 6.9 Hz, anomeric H), 5.96 (1H, *d*, H-4 of aglycone), and 6.07 (2H, *d*, H-2 and H-6 of aglycone)

Dihydroquercetin-3'-O-*β*-D-*glucopyranoside* (**6**) was freeze-dried to a solid (11 mg), $[\alpha]_{589}$ −26° (MeOH, *c* 0.3), R_f 0.67 (A) and 0.58 (B) λ_{max}^{MeOH} (nm) 287, 335 (sh), (NaOAc) 285, 326 (major band), ($AlCl_3$) 314, 378, ($AlCl_3$–HCl) 311, 378 ν_{max} (cm^{-1}) 3433, 1636, 1517, 1457, 1449, 1384, 1363, 1283, 1254, 1163, 1117, and 1077. ^{13}C NMR (ppm, MeOH-d_4) see Table 1. ^{1}H NMR (MeOH-d_4) 3.35–3.53 (4H, *m*, H-2, H-3, H-4, H-5 of sugar), 3.68 (1H, *dd*, H-6a of sugar), 3.88 (1H, *dd*, H-6b of sugar), 4.56 (1H, *d*, J = 11.5 Hz, H-3), 4.83 (1H, *d*, J = 7.3 Hz, anomeric H), 4.98 (1H, *d*, J = 11.5 Hz, H-2), 5.88 (1H, *d*, H-6), 5.9 (1H, *d*, H-8), 6.88 (1H, *d*, J = 7.6 Hz, H-5'), 7.09 (1H, *dd*, H-6'), 7.37 (1H, *d*, J = 1.9 Hz, H-2')

Dihydroquercetin 7-O-*β*-D-*glucopyranoside* (**7**) was freeze-dried to a colourless solid (33 mg), $[\alpha]_{589}$ −48° (MeOH, *c* 0.2), R_f 0.50 (A) and 0.50 (B) λ_{max}^{MeOH} (nm). 286, 320 (sh), (NaOAc) 286, 320 (sh), ($AlCl_3$) 310, ($AlCl_3$–HCl) 288, 308 ν_{max} (cm^{-1}) 3437, 1639, 1576, 1525, 1450, 1365, 1287, 1200, 1173, 1092, 1074, 1029, 986, and 779. ^{13}C NMR (ppm, MeOH-d_4) see Table 1. ^{1}H NMR (MeOH-d_4) 3.35–3.55 (4H, *m*, H-2, H-3, H-4, H-5 of sugar), 3.66 (1H, *dd*, H-6a of sugar), 3.87 (1H, *dd*, H-6b of sugar). 4.54 (1H, *d*, J = 11.7 Hz, H-3), 5.97 (1H, *d*, H-6), 6.00 (1H, *d*, H-8), 6.60 (*d*, J = 8.0 Hz, H-5'), 6.65 (1H, *dd*, H-6'), 6.77 (1H, *d*, H-2')

Dihydrokaempferol 7-O-*β*-D-*glucopyranoside* (**8**) was freeze-dried to a colourless solid (23 mg), $[\alpha]_{589}$ −33° (MeOH, *c* 0.2), R_f 0.60 (A) and 0.75 (B) λ_{max}^{MeOH} (nm) 283, (NaOAc) 283, ($AlCl_3$) 311, ($AlCl_3$–HCl) 288, 309. ν_{max} (cm^{-1}) 3423, 1636, 1520, 1450, 1286, 1247, 1204, 1169, 1076, and 834. ^{13}C NMR (ppm, MeOH-d_4) see Table 1. ^{1}H NMR (MeOH-d_4) 3.37–3.50 (4H, *m*, H-2, H-3, H-4, H-5 of sugar), 3.67 (1H, *dd*, H-6a of sugar), 3.85 (1H, *dd*, H-6b of sugar), 4.58 (1H, *d*, J = 11.8 Hz), 4.90–5.05 (2H, *m*, H-2 and anomeric H), 6.17 (1H, *d*, H-6), 6.20 (1H, *d*, H-8), 6.82 (2H, *d*, J = 8.6 Hz, H-3', H-5'), 7.35 (2H, *d*, J = 8.6 Hz, H-2', H-6')

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