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Flavonol glycosides of *Warburgia ugandensis* leaves

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Abstract

Four new flavonol glycosides: kaempferide 3-*O*-β-xylosyl (1→2)-β-glucoside, kaempferol 3-*O*-α-rhamnoside-7,4′-di-*O*-β-galactoside, kaempferol 3,7,4′-tri-*O*-β-glucoside and quercetin 3-*O*-[α-rhamnosyl (1→6)] [β-glucosyl (1→2)]-β-glucoside-7-*O*-α-rhamnoside, were characterized from a methanolic leaf extract of *Warburgia ugandensis*. The known flavonols: kaempferol, kaempferol 3-rhamnoside, kaempferol 3-rutinoside, myricetin, quercetin 3-rhamnoside, kaempferol 3-arabinoside, quercetin 3-glucoside, quercetin, kaempferol 3-rhamnoside-4′-galactoside, myricetin 3-galactoside and kaempferol 3-glucoside were also isolated. Structures were established by spectroscopic and chemical methods and by comparison with authentic samples.

Keywords: *Warburgia ugandensis*; Canellaceae; Flavonol glycosides

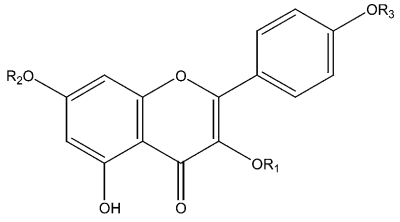
1. Introduction

The genus *Warburgia,* which belongs to the Canellaceae family, is widely used in traditional medicine within East Africa (Kokwaro, 1976). In Kenya, the family is represented by two species *W. ugandensis* Sprague and *W. stuhlmannii* Engl. and are distinguishable by the size of their leaves and color of the stem bark (Dale and Greenway, 1961; Beentje, 1994). Previous phytochemical studies on both taxa have led to the isolation of drimane sesquiterpenes, which have attracted interest because of their biological activities including; insect antifeedant (Nakanishi and Kubo, 1977), plant growth regulation (Jansen et al., 1988), molluscicidal and antifungal (Kubo et al., 1983).

In this communication, we report the isolation and characterization of four novel flavonol glycosides (**1**–**4**) together with eleven known flavonols. This is the first time that these compounds are reported from the plant.

2. Results and discussion

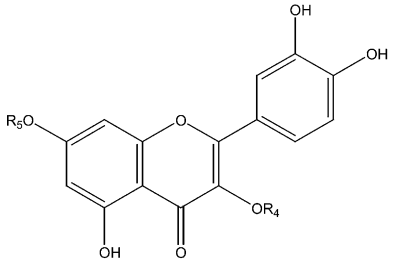
Compound **1** UV spectrum in MeOH and its changes after addition of shift reagents suggested the presence of free hydroxyl groups at C-5 and C-7 (Mabry et al., 1970). The assignment of the ^{1}H and ^{13}C NMR spectra supported by HMBC and NOESY correlations confirmed the aglycone as kaempferide (4′-*O*-methylkaempferol). Acid hydrolysis of the compound afforded glucose and xylose as the sugar residues confirmed by



1 R_1 = xylosyl (1→2) glucose, R_2 = H, R_3 = Me

2 R_1 = rhamnose, R_2 = R_3 = glucose

3 R_1 = R_2 = R_3 = glucose



4 R_4 = [rhamnosyl (1→6)] [glucosyl (1→2)]-glucoside, R_5 = rhamnose

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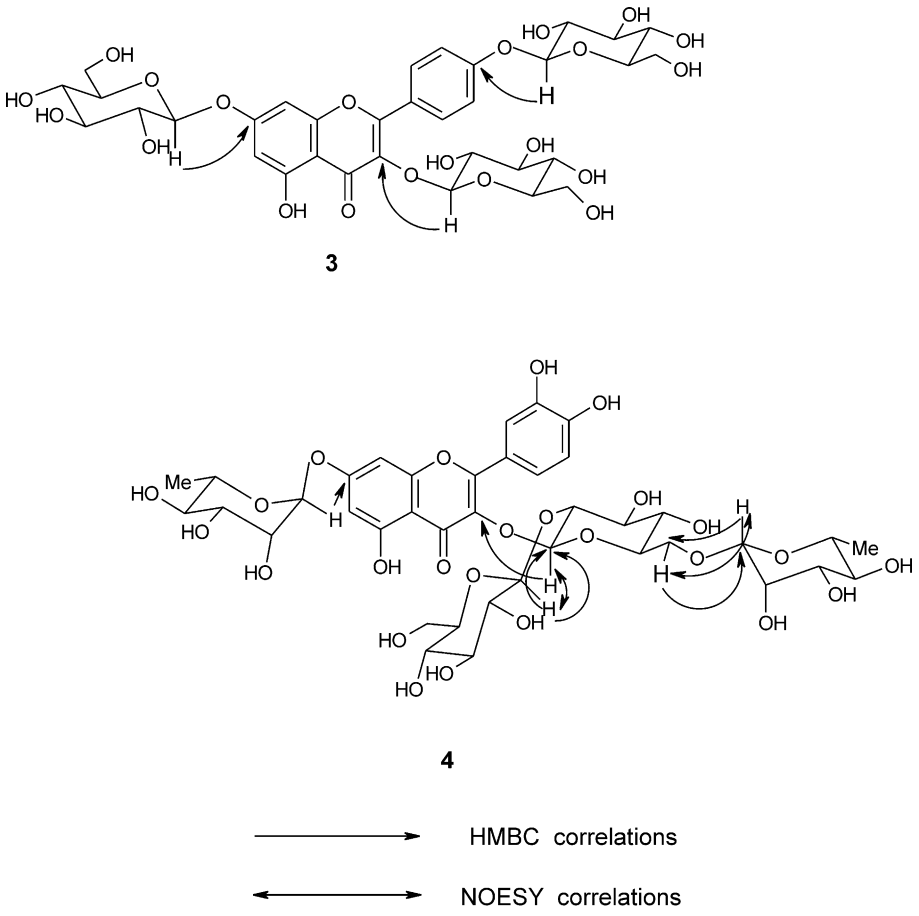

Fig. 1. Significant correlations in the HMBC and NOESY spectra for compounds **3** and **4**.

co-chromatography with authentic samples. The FAB mass spectrum peak at m/z 463 $[M\text{-}132+H]^+$ indicated that xylose is the terminal sugar. In the ^{13}C NMR spectrum, a signal due to C-2″ of glucose shifted downfield and appeared at δ 81.5 in comparison with kaempferol 3-glucoside (Manguro et al., 1997). This was further supported by the HMBC correlation peak observed between the anomeric proton (δ 4.52) of xylose and a carbon at δ 81.5 assigned to C-2″ of glucose, and also by the NOESY cross peaks between H-2″ (δ 3.40) of glucose and the anomeric proton (δ 4.52) of xylose. Therefore, **1** was characterized as kaempferide 3-*O*-β-xylosyl (1→2)-β-glucoside.

Compound **2** afforded rhamnose and galactose as the sugar residues on acid hydrolysis. Its positive ion FAB mass spectrum showed an $[M+H]^+$ ion at m/z 757, corresponding to $C_{33}H_{40}O_{20}$. The other prominent peak at m/z 287 $[M\text{-}146\text{-}2\times162+H]^+$ (loss of rhamnose and two galactose units) together with the chemical shift assignments of the ^1H and ^{13}C NMR spectral data confirmed the aglycone as kaempferol, and the upfield and downfield shifts were consistent with 3,7- and 4′-*O*-glycosylation (Agrawal, 1989). This was supported by the UV spectrum in methanol and its changes after addition of shift reagents, which were similar to those of kaempferol 3,4′-di-*O*-glucoside previously isolated from *Picea abies* (Slimestad et al., 1993) and kaempferol 3-(2″-*p*-coumaroylrhamnoside)-7-rhamnoside from *Cheilanthes fragrans* (Imperato, 1992).

In the HMBC spectrum, correlation peaks were observed between anomeric proton of galactose (δ 5.30) and C-4′ (δ 160.1), between the other galactose anomeric proton (δ 5.40) and C-7 (δ 164.8) and between the rhamnose anomeric proton at δ 5.15 and C-3 (δ 134.7). On this basis, the structure of compound **2** was established as kaempferol 3-*O*-α-rhamnoside-7,4′-di-*O*-β-galactoside.

Compound **3** showed a positive ion FAB mass at m/z 773 $[M+H]^+$, analyzing for $C_{33}H_{40}O_{21}$. Its UV spectrum in methanol and with addition of shift reagents indicated a free hydroxyl group at C-5, a feature also shown by ^1H NMR spectrum peak at δ 12.70, disappeared on D_2O addition. Acid hydrolysis afforded kaempferol and glucose, which together with other fragment peaks at m/z 611 $[M\text{-}162+H]^+$ (loss of glucose), 449 $[M\text{-}2\times162+H]^+$ (loss of 2 glucose units)

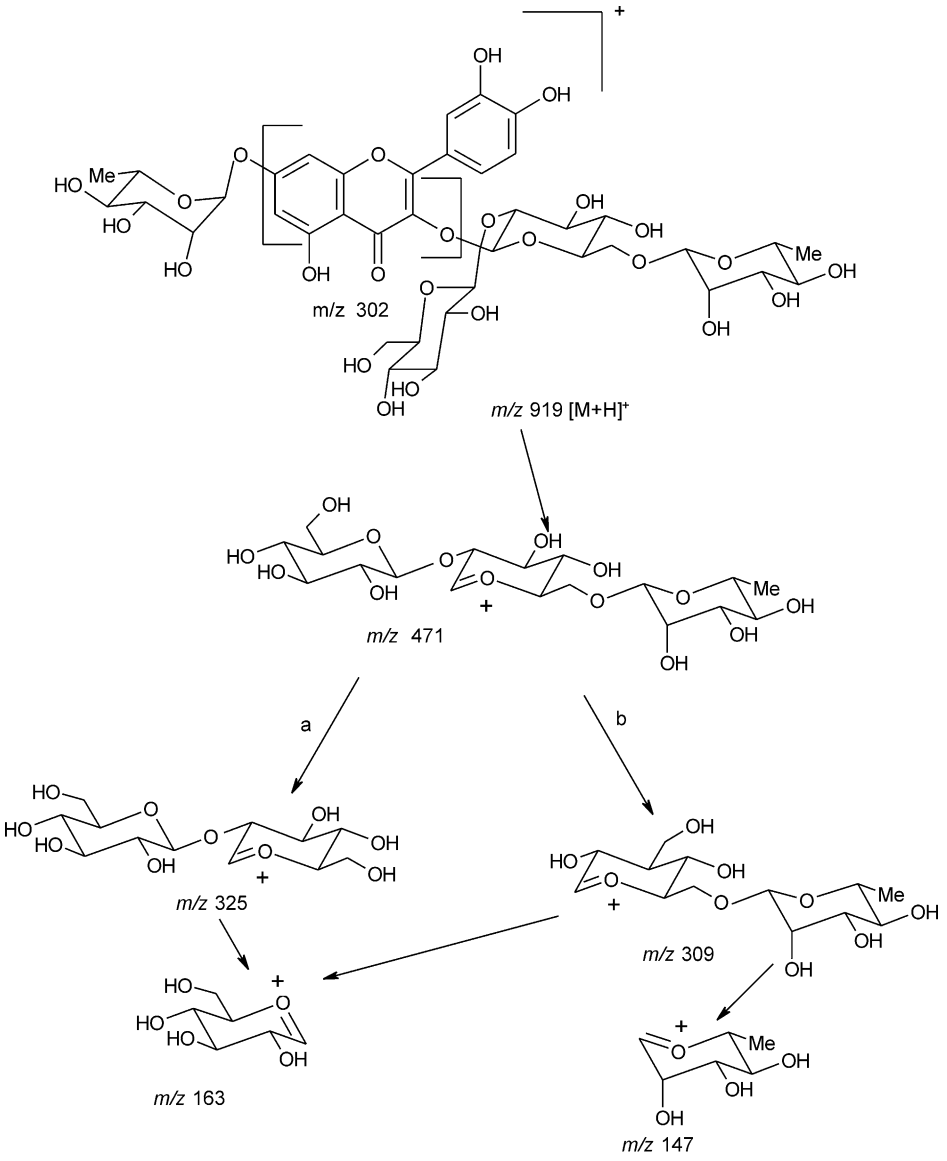

Fig. 2. Fragmentation of **4** during FAB.

and 287 $[M\text{-}3\times162+H]^+$ (loss of 3 glucose units) confirmed the triglycosidic nature of the compound.

Comparative analysis of ^{13}C NMR data of the aglycone carbons in **3** and kaempferol 3-rhamnoside-4′-galactoside (**13**) showed a δ 3.0 ppm downfield shift for C-7 in the former, suggesting glycosylation sites at 3,7,4′-positions. This was further supported by HMBC correlation (Fig. 1) peaks between the anomeric proton at δ 5.60, 5.40 and 5.25 with the corresponding aglycone carbons at δ 164.5 (C-7), 135.5 (C-3) and 162.5 (C-4′), respectively. Thus, **3** was identified as kaempferol 3,7,4′-tri-*O*-β-glucoside.

Compound **4** showed a molecular ion peak at *m*/*z* 919 $[M+H]^+$ in the positive ion FAB mass spectrum (corresponding to a formula of $C_{39}H_{50}O_{25}$). Acid hydrolysis gave quercetin, glucose and rhamnose, which were identified by direct comparison on TLC with authentic samples. The ^1H NMR spectrum revealed the presence of four anomeric protons at δ 5.50 (*d*, $J=1.1$ Hz), 5.30 (*d*, $J=7.8$ Hz), 4.70 (*d*, $J=7.7$ Hz) and 4.50 ($J=0.9$ Hz) indicating two glucose and two rhamnose units. Other fragments seen in the FAB-MS spectral data at *m*/*z* 773 $[M\text{-}146+H]^+$ (loss of rhamnose), 611 $[M\text{-}146\text{-}162+H]^+$ (loss of rhamnose and glucose), 465 $[M\text{-}2\times146\text{-}162+H]^+$ (loss of 2 rhamnoses and a glucose unit) and 303 $[M\text{-}2\times146\text{-}2\times162+H]^+$ (loss of 2 rhamnose and 2 glucose units), together with the characteristic cleavage behavior of the carbohydrate parts as summarized in Fig. 2 showed the presence of 2″,6″-disubstituted glucose unit bearing terminal glucose and rhamnose.

In the ^{13}C NMR spectrum, glycosylation shifts on the aglycone at C-3 and C-7, respectively relative to quercetin (**5**) (Manguro and Williams, 1996) together with the UV data indicated that the sugar units were attached at these positions. This was further corroborated by HMBC correlations shown in Fig. 1; correlation peaks were observed between the anomeric proton (δ 5.30) of one glucose and C-3 (δ 135.4), and the anomeric proton of rhamnose (δ 5.50) and C-7 (164.7) of the aglycone. Furthermore, a comparative analysis of the ^{13}C NMR data chemical shift of sugar units with those of quercetin 3-glucoside (**11**) showed glycosylation shift for C-2″ by ca 6.20 ppm and C-6″ by 6.00 ppm in the residual glucose unit. The signal at δ 81.3 attributed to C-2″ of the inner glucose suggested a glucosyl (1→2)-β-glucoside moiety (as in sophorosyl) while the signal at δ 67.7 attributed to C-6″ of the same glucose indicated a rhamnosyl (1→6)-β-glucoside moiety (as in rutinosyl) (Markham et al., 1992; Manguro and Williams, 1996).

The ^{13}C NMR data were in good agreement with [α-rhamnosyl (1→6)][β-glucosyl (1→2)]-β-glucosyl moiety as described for a 7-substituted quercetin derivative (Cimanga et al., 1997), and further supported by NOESY correlation peaks (Fig. 1). On this basis, **4** was established as quercetin 3-*O*-[β-glucosyl (1→2)][α-rhamnosyl (1→6)]-β-glucoside-7-*O*-α-rhamnoside.

The known flavonols also isolated from the same plant MeOH extract included quercetin (**5**), kaempferol (**6**), myricetin (**7**), kaempferol 3-rhamnoside (**8**), kaempferol 3-arabinoside (**9**), quercetin 3-rhamnoside (**10**), quercetin 3-glucoside (**11**), kaempferol 3-rutinoside (**12**), kaempferol 3-rhamnoside-4′-galactoside (**13**), myricetin 3-galactoside (**14**) and kaempferol 3-glucoside (**15**) (Mizuno et al., 1992a,b; Thomas-Lorentz et al., 1992; Manguro and Williams, 1996).

3. Experimental

3.1. General experimental procedures.

The UV and IR data were recorded on Hewlett Packard Array 8452A spectrophotometer and Perkin-Elmer FTIR 600 series, respectively. The FAB mass spectra were obtained on a VG ZABSPEC instrument. The NMR data were taken in DMSO-d_6 and $CDCl_3$–DMSO-d_6 on a 400 MHz Varian VXR-500 spectrometer. Semi-preparative high performance liquid chromatography (HPLC) was performed on a Bischoff instrument connected to 785 A programmable absorbance detector and a programmable monitor 8252 dual pen recorder. EIMS were measured using 70 eV MAT 8200 A Varian Bremen instrument. Silica gel for column chromatography and TLC plates was impregnated with 2% oxalic acid solution.

3.2. Plant material

The leaves of *W. ugandensis* were collected in Muguga around Kenya Forestry Research Institute in the month of March 1997. A voucher specimen (Olum WU/1997/KEFRI/3) has been deposited in the Kenya Forestry Research Institute Herbarium.

3.3. Extraction and isolation

The CH_2Cl_2 defatted leaves (approx. 5 kg) were further extracted in the cold with MeOH (7.5 l×3) for 2 weeks. The extracts were combined and concentrated under reduced pressure to afford a dark green material (165 g). A portion of the extract (160 g) was pre-adsorbed onto silica gel and applied to a 1/2 kg silica gel column and eluted with CH_2Cl_2–MeOH (5% increments of MeOH) and finally with MeOH. Three hundred fractions (each 100 ml) were collected and their composition monitored by TLC (solvents: CH_2Cl_2–MeOH, 9:1 and 1:1) and those showing similar TLC profiles were combined into four major fractions (I–IV).

Fraction I (fractions 30–120, 17.30 g) was further purified by repeated flash chromatography using CH_2Cl_2–MeOH (19:1) followed by the same solvent (9:1) to afford **5** (40 mg), **6** (31 mg), **7** (20 mg), **8** (42 g) and **9** (27 mg). Fraction II (fractions 121–187, 15.6 g) was similarly purified as in I with CH_2Cl_2–MeOH (9:1) followed by the same solvent in the ratio 4:1 to afford **10** (55 mg), **11** (35 mg), **15** (75 mg) and **14** (25 mg).

Fraction III (fractions 188–250, 13 g) upon further purification as described above using CH_2Cl_2–MeOH (4:1) followed by the same solvent (7:3) (collecting 10 ml each) gave **12** (17 mg), **13** (33.4 mg) and **1** (41 mg). Fraction IV (fractions 251–300, 25.6 g) mainly from methanol elution was rechromatographed over an open column using a CH_2Cl_2–MeOH mixture with increasing amounts of MeOH to give 150 fractions of 100 ml each. The eluates 20–90 were found to contain two components and were similarly purified as in fraction III to give **3** (76 mg) and **2** (25 mg). The remaining eluates (96–130) were combined and repeatedly purified by preparative reverse phase HPLC (solvent: MeOH–H_2O, 7:3) followed by recrystallization in aqueous MeOH to give **4** in 62.4 mg.

3.4. Kaempferide 3-O-β-xylosyl (12)-β-glucoside (*1*)

Amorphous yellow powder. UV λ_{max} (MeOH) nm: 268, 366; +$AlCl_3$: 267, 305, 345; +$AlCl_3$/HCl: 267, 304, 343; +NaOMe: 282, 396; NaOAc: 270, 351; +NaOAc/H_3BO_3: 270, 310, 314. IR ν_{max} (KBr) cm^{-1}: 3500 (O–H), 1665 (α,β-unsaturated C=O), 1625 (C=O, chelated), 1590, 1555, 1460, 1110, 1060. ^1H NMR ($CDCl_3$ + one drop DMSO-d_6) δ ppm: 12.45 (*s*, OH-5, D_2O exchang.), 10.50 (*s*, OH-7, D_2O exchang.), 8.0 (*d*, J=8.0 Hz, H-2′

and H-6′), 6.90 (*d*, *J* = 8.3 Hz, H-3′and H-5′), 6.28 (*d*, *J* = 1.9 Hz, H-8), 6.10 (*d*, *J* = 1.9 Hz, H-6), glucose: 5.40 (*d*, *J* = 7.7 Hz, H-1″), 3.76 (*d*, *J* = 6.8 Hz, H-6″$_B$), 3.60 (*d*, *J* = 6.5 Hz, H-6″$_A$), 3.55 (*m*, H-5″), 3.47 (dd, *J* = 5.5, 3.5 Hz, H-4″), 3.40 (dd, *J* = 8 Hz, H-2″), 3.36 (dd, *J* = 8, 5.4 Hz, H-3″), xylose: 4.52 (*d*, *J* = 7.7 Hz, H-1‴), 3.65 (*m*, H-5‴), 3.48 (*m*, H-4‴), 3.30 (*m*, H-3‴), 3.26 (*m*, H-2‴). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m*/*z* (%) 300 (100), 153, 137. FAB-MS (positive ion mode): *m*/*z* 595 $[M+H]^+$, 463 $[M-132+H]^+$, 301 $[M-132-162+H]^+$, 153, 137.

3.5. Kaempferol 3-O-α-rhamnoside-7,4′-di-O-β-galactoside (2)

Amorphous yellow powder. UV λ_{max} (MeOH) nm: 267, 310, 368; + $AlCl_3$: 266, 302, 344; + $AlCl_3$/HCl: 267, 304, 343; + NaOMe: 280, 358, 408; + NaOAc: 268, 310, 362; + NaOAc/H_3BO_3: 269, 312, 352. IR ν_{max} (KBr) cm^{-1}: 3550, 1650 (C=O, α,β-unsaturated), 1610 (C=O, chelated), 1590, 1450. ^{1}H NMR (DMSO-d_6) δ ppm: 12.50 (*s*, OH-5, D_2O exchang.), 8.40 (*s*, OH-4′, D_2O exchang.), 8.01 (*d*, *J* = 7.6 Hz, H-2′ and H-6′), 7.05 (*d*, *J* = 7.6 Hz, H-3′ and H-5′), 6.32 (*d*, *J* = 2.1 Hz, H-8), 6.20 (*d*, *J* = 2.1 Hz, H-6), 3-*O*-rhamnose: 5.15 (*d*, *J* = 0.8 Hz, H-1″), 3.53 (*m*, H-5″), 3.46 (*br s*, H-2″), 3.38 (dd, *J* = 9.2, 3.1 Hz, H-3″), 3.0 (*m*, H-4″), 1.08 (*d*, *J* = 6.5 Hz, Me-6″), 7-*O*-galactose: 5.40 (*d*, *J* = 7.6 Hz, H-1‴), 3.70 (*d*, *J* = 11.7, 3.4 Hz, H-6‴$_B$), 3.56 (*d*, *J* = 11.7, 5.2 Hz, H-6‴$_A$), 3.58 (*m*, H-5‴), 3.50 (*m*, H-4‴), 3.40 (*m*, H-2‴), 3.34 (*m*, H-3‴), 4′-*O*-galactose: 5.30 (*d*, *J* = 7.6 Hz, H-1⁗), 3.76 (*d*, *J* = 11.6, 3.4 Hz, H-6⁗$_B$), 3.65 (*d*, *J* = 11.6, 5.1 Hz, H-6⁗$_A$), 3.50 (*m*, H-5⁗), 3.47 (*m*, H-4⁗), 3.30 (*m*, H-3⁗), 3.24 (*m*, H-2⁗). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m*/*z* (%) 286 (100), 153 (10), 137 (14), 55 (30). FAB-MS (positive ion mode): *m*/*z* 757 $[M+H]^+$, 611 $[M-146+H]^+$, 449 $[M-146-162+H]^+$, 287 $[M-146-2\times162+H]^+$, 153, 137, 85, 55.

3.6. Kaempferol 3,7,4′-tri-O-β-glucoside (3)

Amorphous yellow powder. UV λ_{max} (MeOH) nm: 267, 367; + $AlCl_3$: 266, 305, 345; + $AlCl_3$/HCl: 265, 306, 345; + NaOMe: 281, 358, 408; + NaOAc: 268, 310, 362; NaOAc/H_3BO_3: 270, 312, 352. IR ν_{max} (KBr) cm^{-1}: 3550 (O–H), 1660, 1620, 1590, 1450, 1250, 1060. ^{1}H NMR (DMSO-d_6) δ ppm: 12.70 (*s*, OH-5, D_2O exchang.), 7.98 (*d*, *J* = 1.9 Hz, H-2′ and H-6′), 6.80 (*d*, *J* = 8.5 Hz, H-3′ and H-5′), 6.20 (*d*, *J* = 1.9 Hz, H-8), 6.10 (*d*, *J* = 1.9 Hz, H-6), 3-*O*-glucose: 5.40 (*d*, *J* = 7.8 Hz, H-1″), 7-*O*-glucose: 5.60 (*d*, *J* = 7.7 Hz, H-1‴), 4′-*O*-glucose: 5.25 (*d*, *J* = 7.6 Hz, H-1⁗), 4.0–3.15 region (*m*, 18H, overlapping signals). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m*/*z* (%) 286 (100), 153 (22), 137 (12) and 55 (30). FAB-MS (positive ion mode): *m*/*z* 773 $[M+H]^+$, 611 $[M-162+H]^+$, 449 $[M-2\times162+H]^+$, 287 $[M-3\times162+H]^+$, 153, 137, 43.

3.7. Quercetin 3-O-[β-glucosyl (1→2)][α-rhamnosy (16)]-β-glucoside-7-O-α-rhamnoside (4)

A yellow powder. UV λ_{max} (MeOH) nm: 257, 302, 358; + $AlCl_3$: 272, 304, 430; + $AlCl_3$/HCl: 272, 366, 404; + NaOMe: 272, 324, 412; + NaOAc: 260, 273, 394 430; + NaOAc/H_3BO_3: 256, 376. IR ν_{max} (KBr) cm^{-1}: 3520 (O–H), 1670, 1620, 1570, 1495, 1120. ^{1}H NMR (DMSO-d_6) δ ppm: 12.70 (*s*, OH-5, D_2O exchang.), 9.30 (*s*, OH-3′, D_2O exchang.), 8.50 (*s*, OH-4′, D_2O exchang.), 7.95 (*d*, *J* = 8.7 Hz, H-2′), 7.70 (*d*, *J* = 10.3, 2.0 Hz, H-6′), 6.80 (*d*, *J* = 8.5 Hz, H-5′), 6.40 (*d*, *J* = 2.0 Hz, H-8), 6.20 (*d*, *J* = 2.0 Hz, H-6), 3-*O*-glucose: 5.30 (*d*, *J* = 7.8 Hz, H-1″), 4.70 (*d*, *J* = 7.7 Hz, H-1‴), 4.50 (*d*, *J* = 0.9 Hz, H-1⁗), 7-*O*-rhamnose: 5.50 (*d*, *J* = 1.1 Hz, H-1⁗′), 4.10-3.0 (*m*, 20H, overlapping signals), 1.2 (*d*, *J* = 6.7 Hz, Me-6⁗′), 0.98 (*d*, *J* = 6.5 Hz, Me-6⁗). ^{13}C

Table 1
^{13}C NMR of compounds flavonol glycosides

Carbon	1	2	3	4	11	13
2	156.4	156.6	157.4	156.9	158.4	157.1
3	134.5	134.7	135.5	135.4	135.1	135.7
4	179.6	177.8	179.6	177.4	178.8	179.1
5	161.2	160.8	162.3	161.0	161.3	162.3
6	100.0	99.3	100.4	98.8	99.4	99.4
7	162.0	164.8	164.5	164.7	162.8	161.5
8	94.9	94.4	94.6	93.7	93.9	94.2
9	157.6	156.8	158.6	156.1	156.6	157.3
10	106.6	105.9	105.0	104.8	104.2	105.6
1′	121.6	120.5	122.6	122.3	122.7	122.4
2′	132.0	131.0	132.3	116.5	117.4	133.0
3′	116.6	115.7	116.6	144.5	144.8	117.1
4′	161.5	160.1	162.5	148.0	147.7	162.7
5′	116.6	115.7	116.6	115.7	116.3	117.1
6′	132.0	131.4	132.0	121.6	122.2	133.0
4′-OMe	56.7					
1″	105.1	100.9	105.3	104.4	105.7	101.7
2″	81.5	70.2	74.4	81.3	75.1	70.7
3″	77.9	68.7	76.8	74.6	76.3	72.3
4″	71.0	73.2	71.0	69.5	71.4	71.8
5″	78.0	71.1	75.4	77.8	77.3	69.8
6″	62.0	17.8	61.4	67.7	61.7	18.0
1‴	102.6	105.2	106.2	101.3		105.3
2‴	71.8	73.2	73.5	73.4		74.6
3‴	71.2	75.4	77.3	76.8		75.7
4‴	67.5	77.3	71.9	71.5		70.7
5‴	62.8	76.1	77.2	76.5		76.9
6‴		61.8	61.0	62.2		62.4
1⁗		104.1	104.5	98.2		
2⁗		74.5	72.9	70.2		
3⁗		76.9	76.4	70.8		
4⁗		71	69.2	72.4		
5⁗		77.0	76.3	70.1		
6⁗		62.4	60.3	17.9		
1⁗′				105.5		
2⁗′				70.1		
3⁗′				71.6		
4⁗′				71.5		
5⁗′				70.2		
6⁗′				17.8		

NMR data: see Table 1. EIMS (70 eV): *m/z* (%) 302 (100), 273 (25), 153 (21), 137 (15), 69 (18), 43 (30). FAB-MS (positive ion mode): *m/z* 919 $[M+H]^+$, 773 $[M-146+H]^+$, 611 $[M-146-162+H]^+$, 465 $[M-2\times146-162+H]^+$, 303 $[M-2\times146-2\times162+H]^+$, 151, 137.

3.8. Acid hydrolysis

Compounds (**1–4**), 10 mg each in a mixture of 8% HCl (2 ml) and MeOH (20 ml) were separately refluxed for 2 h. The reaction mixtures were reduced in vacuo to dryness, dissolved in H_2O (3 ml) and neutralized with NaOH. The neutralized products were subjected to TLC analysis (eluent: EtOAc–MeOH–H_2O–HOAc, 6:2:1:1). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 100 °C. The sugars were identified after comparison with authentic samples.

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