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FURTHER FLAVONOL GLYCOSIDES OF *EMBELIA SCHIMPERI* LEAVES

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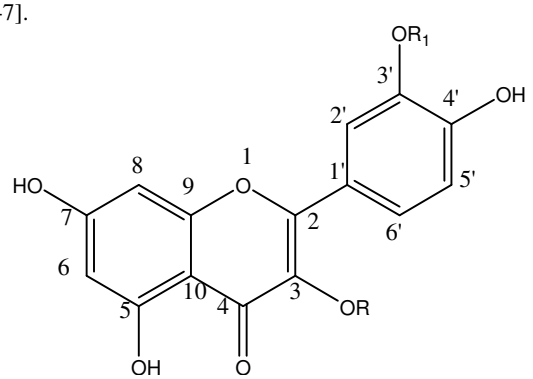
ABSTRACT. Fractionation of the methanolic extract of *Embelia schimperi* leaves has led to the isolation of two novel flavonol glycosides. The compounds were characterized as isorhamnetin 3-*O*-β-galactoysyl (1→ 4)-β-galactoside and quercetin 3-*O*-[α-rhamnosyl (1→2)] [α-rhamnosyl (1→ 4)]-α-rhamnoside. Also reported from the same extracts were known compounds quercetin, myricetin, quercetin 3-*O*-α-rhamnoside, quercetin 3-*O*-β-glucoside, quercetin 3-*O*-rutinoside, myricetin 3-*O*-β-xyloside, isorhamentin 3-*O*-β-glucoside and myricetin 3-*O*-β-glucoside. Their structural elucidation was accomplished using spectral measurements and chemical methods.

KEY WORDS: *Embelia schimperi*, Flavonol glycosides, Isorhamnetin 3-*O*-β-galactoysyl (1→ 4)-β-galactoside, Quercetin 3-*O*-[α-rhamnosyl (1→2)] [α-rhamnosyl (1→ 4)]-α-rhamnoside

INTRODUCTION

Embelia schimperi Vatke is one of the five Myrsinaceae species endemic to Kenya and is widely used in traditional medicine as an anthelmintic and anti-microbial [1]. Phytochemically, the plant is typified by the presence of benzoquinone derivatives; 2,5-dihydroxy-3-undecyl-1,4-benzoquinone (trivial name: embelin) and 2,5-dihydroxy-3-tridecyl-1,4-benzoquinone (trivial name: rapanone) [2, 3]. In a recent study [4], a novel flavonol glycoside along with four known ones has been isolated from this source. In continuing investigation of a methanolic extract of the plant leaves, we now report the isolation and characterization of two new flavonol glycosides (**1** and **2**). Also being reported are eight known ones identified as isorhamnetin 3-*O*-β-glucoside (**3**), quercetin 3-*O*-α-rhamnoside (**4**), quercetin (**5**), myricetin (**6**), quercetin 3-*O*-β-glucoside (**7**), myricetin 3-*O*-β-glucoside (**8**), myricetin 3-*O*-β-xyloside (**9**) and quercetin 3-rutinoside (**10**) [5-7].



1 R = galactosyl (1→ 4)-galactoside, R_1 = Me; **2** R = [rhamnosyl (1→ 2)][rhamnosyl (1→ 4)]-rhamnoside, R_1 = H; **3** R = glucose, R_1 = Me; **4** R = rhamnose R_1 = H.

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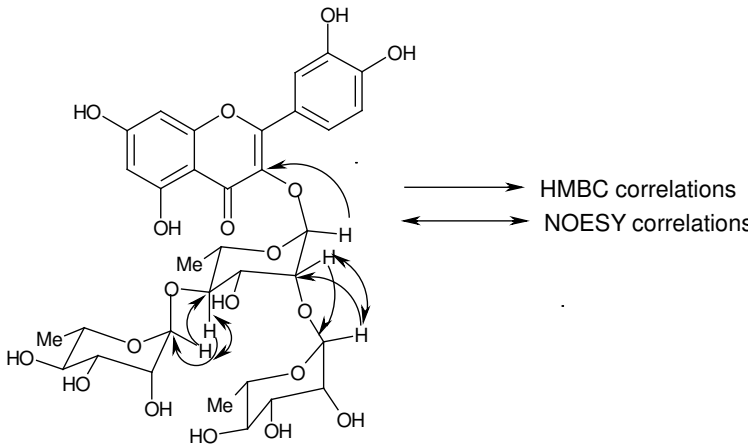
RESULTS AND DISCUSSION

Compound **1** afforded UV data, which suggested free hydroxyls at C-5, C-7, and C-4 [8-10]. The assignment of the ^{1}H and ^{13}C NMR data supported by NOESY and HMBC correlations confirmed the aglycone as isorhamnetin (3'-*O*-methylquercetin) substituted at C-3. Acid hydrolysis gave galactose as the sugar residue. The FAB mass spectrum results indicated galactosylgalactoside bios as evidenced by the cleavage behaviour (see experimental section). In the ^{13}C NMR spectrum a signal due to C-4" of residual galactose shifted downfield at δ 82.40 in comparison to isorhamnetin 3-*O*-β-glucoside (**3**), suggesting interglycosidic linkage at this position. This was corroborated by HMBC correlation between the anomeric proton (δ 4.65) of terminal galactose and a carbon (δ 82.40) assigned to C-4" of the residual galactose and further confirmed by NOESY cross peaks between the terminal galactose anomeric proton (δ 4.65) and the C-4" proton (δ 3.68). Therefore compound **1** was concluded as isorhamnetin 3-*O*-β-galactosyl (1→ 4)-β-galactoside.



2

Figure 1. Pertinent HMBC and NOESY correlations observed with compound **2**.

Compound **2** showed a molecular ion peak at *m/z* 741 $[M+H]^+$, analyzing for C_{33} $H_{40}O_{19}$. Acid hydrolysis afforded rhamnose as the sugar residue. Its ^{1}H NMR data showed a 2H AX and a 3H ABX system characteristic of quercetin moiety [11] while the UV data recorded in MeOH and with addition of common shift reagents [8, 9] suggested that the residual sugar was attached at C-3 position of the aglycone, a fact further supported by HMBC correlations as outlined in Figure 1. From the mass spectrum data, fragments at *m/z* 595 $[M-146 + H]^+$ (loss of rhamnose), 449 $[M-2 \times 146 + H]^+$ (loss of two rhamnoses), 303 $[M-3 \times 146 + H]^+$ (loss of three rhamnoses), 339 $[3 \times \text{rhamnose}]^+$, 293 $[2 \times \text{rhamnose}]^+$ (Figure 2) together with three anomeric protons of rhamnose units at δ 5.10 (d, J = 1.1 Hz), 4.75 (d, J = 1.2 Hz) and 4.50 (d, J = 1.0 Hz) indicated the presence of three sugar residues in the molecule. Furthermore, a comparative analysis of the ^{13}C NMR chemical shift of the sugar unit with those of quercetin 3-*O*-α-rhamnoside (**4**) showed glycosylation shift for C-2" (11.80 ppm) and C-4" (10.30 ppm) in the residual ramnose unit, thus suggesting the presence of 2",4"-substituted rhamnose unit bearing two other terminal ones. The signal at δ 82.30 attributed to C-2" of the residual rhamnose suggested rhamnosyl (1→2)-rhamnoside moiety [12] while the signal at δ 81.60 attributed to C-4" of the same rhamnose

indicated a rhamnosyl (1→ 4)-rhamnoside moiety [13], a fact further supported by NOESY and HMBC spectra correlations (Figure 1). On this basis compound **2** was established as quercetin 3-*O*-(α-rhamnosyl (1→2)] [α-rhamnosyl (1→ 4)]-α-rhamnoside.

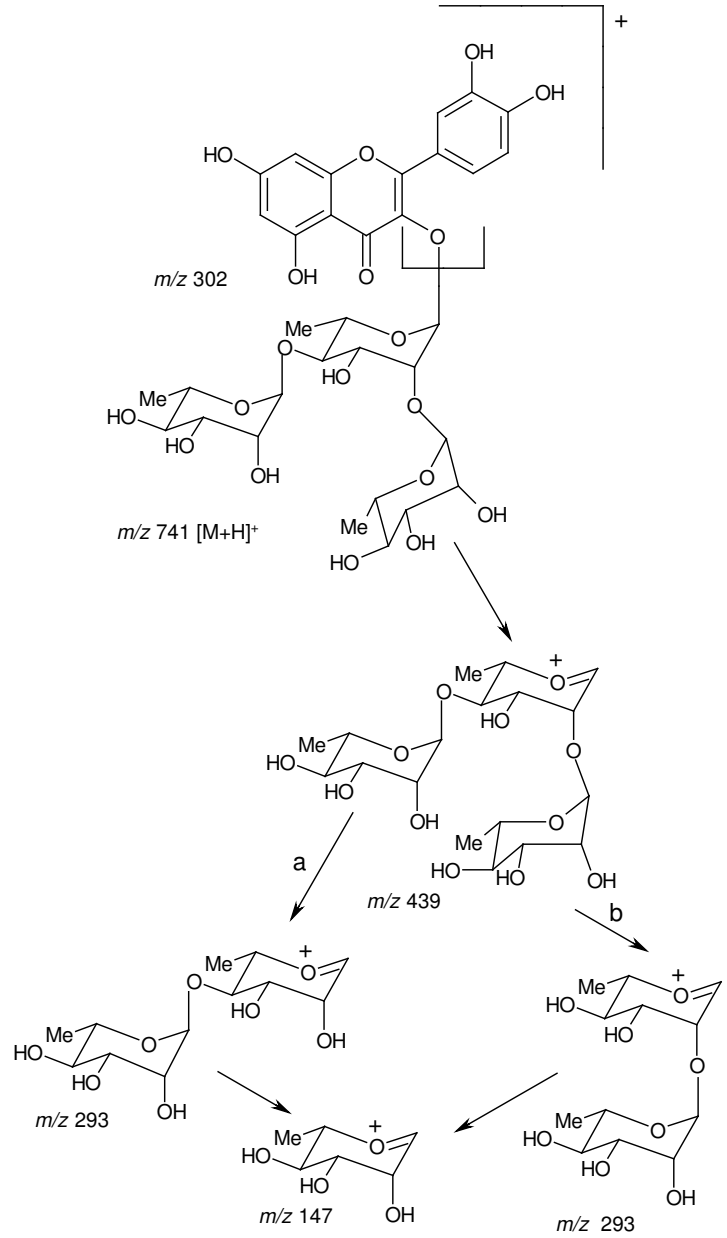


Figure 2. Fragmentation of compound 2 during FAB.

Table 1. ^{13}C NMR data of compounds **1-4**.

Carbon No.	**1**	**2**	**3**	**4**
1				
2	156.80	157.30	158.70	158.30
3	135.75	134.20	135.40	135.60
4	177.40	177.70	179.40	178.65
5	161.50	161.30	163.04	162.30
6	99.01	98.70	98.86	98.80
7	164.45	164.20	165.91	163.70
8	94.00	93.80	94.73	94.02
9	156.10	156.80	158.20	157.40
10	104.10	104.00	105.80	104.50
1'	120.75	120.70	123.10	122.35
2'	113.90	115.60	116.00	116.60
3'	148.35	147.80	148.40	149.10
4'	147.16	145.20	150.80	148.20
5'	115.65	115.40	114.40	116.00
6'	121.90	121.10	123.80	123.50
1''	103.80	101.20	104.71	102.10
2''	74.30	82.30	75.90	70.50
3''	76.50	78.01	77.08	71.90
4''	82.40	81.60	71.50	71.30
5''	73.00	76.80	76.50	69.70
6''	61.90	18.80	62.60	17.80
1'''	104.80	99.80		
2'''	72.45	70.40		
3'''	74.85	72.90		
4'''	68.70	71.00		
5'''	75.30	70.10		
6'''	60.50	18.30		
1''''		101.10		
2''''		71.40		
3''''		73.50		
4''''		72.00		
5''''		69.80		
6''''		17.70		
3'-OMe	57.60		58.20	

EXPERIMENTAL

General. UV spectra were recorded on a 8452 A Hewlett Packard Array spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$-DMSO-d_6 mixture and DMSO-d_6 on a Bucker WM instrument operating at 360 and 90 MHz, respectively. EIMS were measured using 70 eV MAT 8200 A Varian Bremen instrument. Polish Academy of Sciences provided FAB-MS spectra. Silica gel for column and TLC plates were impregnated with 2% oxalic acid solution. Semi-preparative high performance liquid chromatography (HPLC) was performed on a Bischoff instrument connected to a 785 programmable absorbance detector and a programmable monitor 8252 dual pen recorder.

Plant material. *Embelia schimperi* leaves were collected from Ngong hills in June 1997 and voucher specimens were identified after comparison with authentic samples at Botany Department Herbarium, Nairobi University.

Extraction and isolation. The CH_2Cl_2 defatted powdered leaves (approx. 1 kg) was further extracted with MeOH (2.5 L x 3) for one week. The extracts were combined and freed from the solvent under reduced pressure to give a dark green residue (105 g). A portion of the extract (100 g) was subjected to column chromatography with CH_2Cl_2-MeOH gradient to pure MeOH affording 160 fractions of 100 mL each. The composition of the fractions were determined by TLC using solvent system CH_2Cl_2-MeOH (9:1, 3:2) and those showing similar profiles were combined to give three major pools. The first pool (fractions 20-80, 25 g) was subsequently subjected to repeated flash chromatography with CH_2Cl_2-MeOH (95:5) followed by the same solvent system in the ratio 9:1 to give **5** (29 mg), **6** (55 mg) and **4** (24 mg). Fractions 81-105 (Pool II) was evaporated to give 17 g which upon repeated flash chromatography with CH_2Cl_2-MeOH (4:1) and collecting 10 mL each, afforded **7** (66 mg), **8** (34 mg), **3** (65 mg) and **9** (35 mg). Pool III (fractions 106-160, 35 g) mainly from methanol elution was further purified by column chromatography over sephadex LH-20 using different solvent systems. Final purification of the eluates by preparative HPLC on reverse phase (RP-18) using MeOH-H_2O (7:3) gave **10**, **1** and **2** in 75 mg, 48 mg, and 55 mg, respectively.

*Isorhamnetin 3-O-β-galactosyl (1→4)-β-galactoside (**1**)*. Amorphous yellow powder. UV λ_{max} (MeOH) nm: 257, 268, 358; (+$AlCl_3$) 268, 301, 360, 398; (+$AlCl_3$/HCl) 262, 302, 402, 424; (+NaOMe) 272, 324, 420; (+NaOAc) 270, 322, 408; (NaOAc/H_3BO_3) 256, 266, 362. IR ν_{max} (KBr) cm^{-1}: 3510, 3450, 1680, 1650, 1580, 1460, 1410, 1390, 1365, 1250, 1200, 1180, 1050, 900. ^{1}H NMR ($CDCl_3$+one drop DMSO-d_6) δ: 12.64 (s, OH-5, D_2O exchang.), 10.20 (br s, OH-7, D_2O exchang.), 8.30 (br s, OH-4', D_2O exchang.), 7.90 (d, *J* = 2.3 Hz, H-2'), 7.80 (dd, *J* = 8.2, 2.3 Hz, H-6'), 6.98 (d, *J* = 8.2 Hz, H-5'), 6.50 (d, *J* = 1.8 Hz, H-8), 6.35 (d, *J* = 1.8 Hz, H-6), 3.85 (s, OMe); residual galactose: 5.30 (d, *J* = 7.7 Hz, H-1"), 3.78 (d, *J* = 12.1, 3.2 Hz, H-6"$_B$), 3.68 (m, H-4"), 3.60 (dd, *J* = 12.1, 5.4 Hz, H-6"$_A$), 3.40 (m, H-3"), 3.25 (m, H-5"), 3.16 (m, H-2"); terminal galactose: 4.65 (d, *J* = 7.60 Hz, H-1'"), 3.97 (m, H-6'"$_B$), 3.73 (m, H-6'"$_A$), 3.55 (m, H-4'"), 3.45 (m, H-3'"), 3.30 (m, H-5'"), 3.27 (m, H-2'"). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m/z* (%) 302 (100), 153 (27), 137 (18), 136 (9). FAB-MS (positive ion mode): 641 $[M+H]^+$, 479 $[M-162+H]^+$, 317 $[M-2 \times 162+H]^+$, 153, 137.

*Quercetin 3-O-[α-rhamnosyl(1→2)] [α-rhamnosyl[1→4]-α-rhamnoside (**2**)*. A yellow powder. UV λ_{max} (MeOH) nm: 258, 301, 358; (+$AlCl_3$) 274, 300, 430; (+$AlCl_3$/HCl) 270, 300, 402; (+NaOMe) 272, 328, 414; (+NaOAc) 273, 324, 380, 418; (NaOAc/H_3BO_3) 260, 379. IR ν_{max} (KBr) cm^{-1}: 3500, 1665, 1570, 1510, 1430, 1250, 950. ^{1}H NMR (99% DMSO-d_6) δ: 12.80 (s, OH-5, D_2O exchang.), 10.30 (br s, OH-7, D_2O exchang.), 9.20-8.50 (br s, OH-3' and 4', D_2O exchang.), 7.80 (d, *J* = 2 Hz, H-2'), 7.64 (dd, *J* = 8.4, 2 Hz, H-6'), 6.80 (d, *J* = 8.3 Hz, H-5'), 6.40 (d, *J* = 2.2 Hz, H-8), 6.24 (d, *J* = 2.2 Hz, H-6); residual rhamnose: 5.10 (d, *J* = 1.1 Hz, H-1"), 3.70 (d, *J* = 9.3, 7.6 Hz, H-2"), 3.47 (t, *J* = 9.2 Hz, H-3"), 3.36 (t, *J* = 9.1 Hz, H-4"), 3.20 (ddd, *J* = 9.1, 5.3, 2.3Hz, H-5"), 1.20 (d, *J* = 6.5 Hz, Me-6"); 2"-*O*-rhamnose: 4.50 (d, *J* = 1.0 Hz, H-1'"), 3.45 (dd, *J* = 9.3, 7.5 Hz, H-2'"), 3.39 (t, *J* = 9 Hz, H-3'"), 3.34 (t, *J* = 9.1 Hz, H-4'"), 3.24 (dd, *J* = 9.1, 5.2, 2.5 Hz, H-5'"), 1.0 (d, *J* = 6.6 Hz, Me-6'"); 4"-*O*-rhamnose: 4.75 (d, *J* = 1.2 Hz, H-1""), 3.85 (d, *J* = 3.3 Hz, H-2""), 3.60 (dd, *J* = 8.5, 3.3 Hz, H-3""), 3.30 (d, *J* = 11, 6.2 Hz, H-5""), 3.20 (dd, *J* = 9.5, 7.8 Hz, H-4""), 0.80 (d, *J* = 6.7 Hz, Me-6""). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m/z* (%) 302 (100), 162 (4), 153 (20), 147 (7), 137 (9). FAB-MS (positive ion mode): 741 $[M+H]^+$, 595 $[M-146+H]^+$, 449 $[M-2 \times 146+H]^+$, 303 $[M-3 \times 146+H]^+$, 339, 293, 180, 151, 146, 137.

*Isorhamnetin 3-O-β-glucoside (**3**)*. A pale yellow powder. UV λ_{max} (MeOH) nm: 260, 304, 360; (+$AlCl_3$) 268, 302, 402; (+$AlCl_3$/HCl) 272, 302, 398; (+NaOMe) 272, 396; (+NaOAc) 270, 320, 360, 406; (NaOAc/H_3BO_3) 260, 370. IR ν_{max} (KBr) cm^{-1}: 3450, 1650, 1600, 1580, 1440, 1360, 1290, 1140, 1050, 990. ^{1}H NMR ($CDCl_3$+one drop DMSO-d_6) δ: 12.50 (s, OH-5, D_2O exchang.), 10.25 (br s, OH-7, D_2O exchang.), 8.60 (br s, OH-4', D_2O exchang.), 7.82 (d, *J* = 1.9 Hz, H-2'), 7.46 (dd, *J* = 8.4, 2.0 Hz, H-6'), 6.85 (d, *J* = 8.4, 2.0 Hz, H-5'), 6.31 (d, *J* = 2.1 Hz, H-8), 6.16 (d, *J* = 2.1 Hz, H-6), 3.85 (s, OMe); residual glucose: 5.10 (d, *J* = 7.6 Hz, H-1"), 3.80 (dd, *J* = 11.6, 3.6 Hz, H-6"$_B$), 3.66 (dd, *J* = 11.6, 5.8 Hz, H-6"$_A$), 3.50 (t, *J* = 8.4 Hz, H-4"), 3.45 (m, H-3"), 3.37 (dd, *J* = 10.2,7.7 Hz, H-2"), 3.28 (m, H-4"). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m/z* (%) 316 (100), 245 (10), 150 (5), 128 (10), 83 (57). FAB-MS (positive ion mode): 449 $[M+H]^+$, 317 $[M-162+H]^+$, 151, 137.

*Quercetin 3-O-α-rhamnoside (**4**)*. A yellow amorphous powder. UV λ_{max} (MeOH) nm: 260, 300, 357; (+$AlCl_3$) 272, 301, 428; (+$AlCl_3$/HCl) 272, 301, 401; (+NaOMe) 271, 330, 414; (+NaOAc) 274, 320, 384; (NaOAc/H_3BO_3) 260, 382. IR ν_{max} (KBr) cm^{-1}: 3500, 1665, 1580, 1520, 1480, 1450, 1120, and 1020. ^{1}H NMR ($CDCl_3$+ DMSO-d_6) δ: 12.45 (s, OH-5, D_2O exchang.), 10.50 (br s, OH-7, D_2O exchang.), 9.20-8.50 (br s, OH-3' and 4', D_2O exchang.), 7.86 (d, *J* = 2.2 Hz, H-2'), 7.70 (dd, *J* = 8.4, 2.3 Hz, H-6'), 6.90 (d, *J* = 8.4 Hz, H-5'), 6.40 (d, *J* = 2 Hz, H-8), 6.20 (d, *J* = 2 Hz, H-6); rhamnose: 5.15 (d, *J* = 0.9 Hz, H-1"), 3.65 (br d, *J* = 3.5 Hz, H-2"), 3.55 (dd, *J* = 9.1, 3.1Hz, H-3"), 3.44 (m, H-4"), 3.35 (m, H-5"), 1.00 (d, *J* = 6.5 Hz, Me-6"). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m/z* (%) 302 (100), 153 (21), 137 (30).

Acid hydrolysis. Compounds (**1**-**4**), 15 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 under pressure 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 ^{0}C. The sugars were identified after comparison with authentic samples.

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