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A FLAVONOL GLYCOSIDE FROM *EMBELIA SCHIMPERI* LEAVES

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Key Word Index—*Embelia schimperi*; Myrsinaceae; quercetin 3-galactosyl (1 → 2) rhamnoside; structural elucidation.

Abstract—A new flavonol glycoside, quercetin 3-galactosyl (1 → 2) rhamnoside, has been isolated from the leaves of *Embelia schimperi*. The known compounds quercetin 3-rutinoside, quercetin 3-rhamnoside, quercetin 3-galactoside, myricetin and quercetin were also identified from this plant. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Embelia schimperi Vatke (Myrsinaceae) is therapeutically important in herbal medicine; its fruits, root and stem barks and leaves are widely used [1]. Previous phytochemical reports on this plant revealed the existence of long-chain alkyl-1,4-benzoquinones as major constituents [2, 3]. In this paper, we report the isolation and characterization of a new flavonol glycoside (**1**) from a methanol extract of the plant leaves.

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow powder. Its UV spectral data and their changes after addition of common shift reagents [4, 5] suggested that **1** is a flavonol with a substituted hydroxyl group at C-3. This result was confirmed by ^{1}H NMR data, which evidenced A-ring hydroxyl resonances at δ 12.70 (5-hydroxyl) and 10.90 (7-hydroxyl) and the *ortho*-dihydroxyl groups in the B-ring at δ 9.50 (3′-OH) and 8.90 (4′-hydroxyl); all are D_2O exchangeable. Besides the substitution pattern, the spectrum confirmed the absence of any other hydrogen bearing substituents such as prenyl and *C*-methyl, a fact which was supported by the characteristic aglycone pattern of a quercetin derivative: a 2H AX and a 3H ABX system. Acid hydrolysis showed the presence of galactose and rhamnose, identified by TLC after comparison with authentic sugars. The positive FAB mass spectrum exhibited a molecular ion peak at *m/z* 611 $[M + H]^+$, which is in accord with the formula $C_{27}H_{30}O_{16}$ and was further corroborated by ^{13}C NMR (Table 1) spectrum (27 carbon atoms). In the ^{1}H NMR spectrum, two anomeric protons at δ 5.50 (J = 0.9 Hz) and 4.80 (J = 7.7 Hz) were observed in each doublet, indicating an inner rhamnose and a terminal galactose. The position of attachment of galactose to rhamnose was deduced from the ^{13}C NMR data. The C-2″ of rhamnose shifted downfield by about 12.3 ppm in comparison with **3** and appeared at δ 82.4, while the anomeric carbon of galactose underwent an upfield shift at

Table 1. ^{13}C NMR (CD_3OD) spectra of compounds **1** and **3**

C	1	3
2	159.20	158.8
3	135.50	134.2
4	178.90	179.70
5	162.80	163.30
6	99.70	98.80
7	165.80	166.30
8	94.40	94.00
9	159.00	158.40
10	105.20	104.70
1′	122.10	121.30
2′	116.60	115.60
3′	145.70	144.90
4′	148.90	148.60
5′	115.80	115.40
6′	112.80	122.00
1″	102.90	102.00
2″	82.4	70.10
3″	72.00	70.30
4″	74.10	74.70
5″	71.50	71.00
6″-Me	17.90	17.60
1‴	103.20	—
2‴	74.20	—
3‴	76.90	—
4‴	71.3	—
5‴	75.60	—
6‴	62.30	—

δ 103.2. The chemical shift values of the sugar moieties were in good agreement with the reported data for quercetin 3-glucosyl (1 → 2) rhamnoside and kaempferol 3-glucosyl (1 → 2) rhamnoside [6, 7], a fact which was supported by ^1H–^{13}C long-range correlation 2D spectral data. Thus, **1** is quercetin 3-galactosyl (1 → 2) rhamnoside. Finally, the known compounds **2**–**6** were identified by comparison of their spectroscopic data with those in the literature [8–11].

EXPERIMENTAL

General. TLC of glycosides and aglycones was carried out on 3% oxalic acid treated precoated silica gel 60FG 254 aluminium sheets (Merck). Silica gel (70–230 mesh) was used for open CC while for flash CC silica gel 60 FG (02–0.7 mm) was used. UV spectra were recorded on a 8452A Hewlett Packard array spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Brucker WM instrument at 250 and 62.5 MHz, respectively. Prep. HPLC was performed on a Bischoff instrument using a model pump connected to 785 A programmable absorbance detector and a programmable monitor 8252 dual pen recorder.

Plant material. Leaves of *E. schimperi* were collected in March 1990 in the Ngong hills and voucher specimens (Manguro and Mathenge 90/3) are deposited at the University of Nairobi, Botany Department Herbarium.

Extraction and isolation. Defatted powdered dry leaves (1 kg) were extracted with MeOH (2 l × 3) at room temp. for 1 week. The combined extracts were evapd to dryness under red. pres., yielding a dark green residue (165 g). A portion of the extract (150 g) was subjected to open CC, eluting with increasing amounts of MeOH 5–90% in CH_2Cl_2 and lastly the column was washed with MeOH. Frs of 250 ml each (130 frs) were collected and those showing similar TLC profiles were combined into pools (I–III). Pool I (frs 1–90, 15.0 g) was purified by flash CC (400 g, 3.0 × 60 cm) using CH_2Cl_2–MeOH-(19:1) as eluent and collecting 10 ml each, to give myricetin (**6**) (25 mg), quercetin (**5**) (45 mg) and quercetin 3-galactoside (**4**) (50 mg). Pool II (frs 91–110, 5.70 g) was found to contain 2 compounds and was further purified as already described for pool I to afford **4** (14 mg) and quercetin 3-rhamnoside (**3**) (26 mg). Pool III (frs 111–130, 7.5 g) was rechromatographed on an open column (500 g, 3.0 × 60 cm) with CH_2Cl_2–MeOH mixts of increasing polarity and lastly MeOH giving 40 frs of 100 ml each. The eluates (1–30) afforded quercetin 3-rutinoside (**2**) in 15 mg. The remaining frs were finally purified by semi-prep. HPLC on a reverse phase column (RP-18) using MeOH-H_2O (7:3) to give 10.5 mg pure **1**.

Quercetin 3-galactosyl (1 → 2) rhamnoside (**1**). UV λ_{max}^{MeOH} nm: 358, 300, 258; (+ $AlCl_3$/HCl) 400, 302, 272; (+ NaOMe) 410, 331, 272; (+ NaOAc) 386, 324, 270; (+NaOAc/H_3BO_3) 378, 262. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ: 12.70 (*br s*, 5′-OH), 10.90 (*br s*, 7-OH), 9.50 (*br s*, 3′-OH), 8.90 (*br s*, 4′-OH), 7.60 (*d*, J = 2 Hz, H-2′), 7.55 (*dd*, J = 11, 2.2 Hz, H-6′), 6.80 (*d*, J = 8.4 Hz, H-5′), 6.40 (*d*, J = 1.9 Hz, H-8), 6.25 (*d*, J = 1.9 Hz, H-6), 5.50 (*d*, J = 0.9 Hz, H-1″), 4.80 (*d*, J = 7.7 Hz, H-1‴), 3.80 (*dd*, J = 10.2, 3 Hz, H-6‴$_A$), 3.73 (*m*, H-2″), 3.65 (*m*, H-5″), 3.56 (*dd*, J = 9.5, 4.6 Hz, H-6‴$_B$), 3.50–3.0 (H-3′, H-4″, H-2‴, H-4‴, H-5‴, overlapping signals), 0.90 (*d*, J = 6.7 Hz, 6″-Me). EIMS (70 eV): *m/z* (%) 302 (100), 153 (14), 137 (29). FABMS: *m/z* 611 $[M + H]^+$, 303 [quercetin + H]$^+$, 153, 136.

Acid hydrolysis. Compound **1** (10 mg) in a mixt. of 8% HCl (2 ml) and MeOH (20 ml) was refluxed at 100° for 2 hr. The reaction mixt. was evapd *in vacuo* to dryness, dissolved in H_2O (2 ml) and neutralized with NaOH. The neutralized product was subjected to TLC (EtOAc–MeOH–H_2O–HOAc, 6:2:1:1) and PC (80% PhOH). Chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 110° for 5 min. The sugar were identified after comparison with authentic samples.

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