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FLAVONOL GLYCOSIDES FROM *VERNONIA GALAMENSIS* SSP. *NAIROBIENSIS*

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Key Word Index—*Vernonia galamensis* ssp. *nairobiensis*; Asteraceae; flavonol glycosides; isorhamnetin 3-*O*-apiosylgalactoside; quercetin 3-*O*-apiosylgalactoside; quercetin 3-*O*-robinobioside.

Abstract—A new flavonol glycoside has been isolated from the leaves of *Vernonia galamensis* ssp. *nairobiensis*. Its structure has been established as isorhamnetin 3-*O*-β-D-apio-D-furanosyl(1 → 2)-β-D-galactopyranoside on the basis of spectral data (UV, ^{1}H and ^{13}C NMR, D/CIMS) and by acid hydrolysis. Characterization of the peracetate derivative confirmed the configuration of the apiosyl moiety. In addition, the known flavonol glycosides; quercetin 3-galactoside, quercetin 3-apiosyl(1 → 2)galactoside and quercetin 3-rhamnosyl(1 → 6)galactoside have been isolated and identified. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The genus *Vernonia* (Asteraceae) contains more than 500 species distributed in the tropical and subtropical areas of the world, especially Africa and South America [1]. *V. galamensis* (Cass.) Less., earlier named *V. pauciflora* (Willd.) Less., is now divided into several subspecies and varieties [2]. *V. galamensis* ssp. *nairobiensis* is an herbaceous plant growing in Kenya and in Tanzania. A decoction of the leaves of *V. galamensis* is used in traditional medicine against stomach aches [3]. Sesquiterpene lactones [4, 5], fatty acids [6, 7] and sterols [8] have been reported from *V. galamensis*, but nothing is known about its phenolic constituents.

RESULTS AND DISCUSSION

HPLC analyses with diode-array detection revealed the presence of several flavonoids in the methanolic leaf extract of *V. galamensis* ssp. *nairobiensis*. The UV spectra of these flavonoids were characteristic of 3-*O*-glycosylated flavonols [9]. Fractionation of the methanol extract by a combination of gel chromatography on Sephadex LH 20, low pressure liquid chromatography (LPLC) on RP-18 and centrifugal partition chromatography (CPC) afforded the flavonol glycosides **1**–**4** (see Experimental).

Compounds **1**–**3** were shown to be quercetin 3-*O*-glycosides, and identified as quercetin 3-*O*-β-D-galactopyranoside (hyperoside) [10], quercetin 3-*O*-β-D-apio-D-furanosyl(1 → 2)galactopyranoside [11] and quercetin 3-*O*-α-L-rhamnopyranosyl(1 → 6)-β-D-galactopyranoside (quercetin 3-robinobioside) [12], respectively, from their spectroscopic data (UV, D/CIMS, ^{1}H and ^{13}C NMR). Acid hydrolysis followed by TLC analysis of aglycones and sugars further confirmed the identities of these compounds.

The structure of **4** was elucidated as follows. The D/Cl mass spectrum showed a quasimolecular ion at *m/z* 611 ($[M + H]^+$). Fragment ions were detected at *m/z* 479 ($[M + H - 132]^+$) and 317 ($[M + H - 294]^+$), corresponding to the successive loss of pentosyl and hexosyl moieties. While hydrolysis of **4** with 2M HCl gave only galactose and isorhamnetin, a milder hydrolysis with 0.1 N H_2SO_4 afforded galactose, apiose and isorhamnetin. The sugars and aglycone were identified by TLC comparison with authentic samples. UV spectral analysis of **4** with the usual shift reagents before and after hydrolysis [9] showed that the sugars were attached to the 3-hydroxyl and that there was a methoxy group at the 3′-position. The ^{1}H NMR spectrum of **4** confirmed the aglycone to be isorhamnetin. In addition, two doublets at 5.62 ($J = 7.5$ Hz, H-1″) and 5.32 ($J = 1.0$ Hz, H-1‴) demonstrated the β-configuration of both galactosyl and apiosyl residues. In the ^{13}C NMR spectrum of **4**, compared with **1**, C-2″ was shifted downfield of 3.5 ppm, while C-1″ was observed at higher field (3.0 ppm). These data demonstrated unambiguously the position of attachment of the apiosyl moiety as the 2″ hydroxyl. The ^{13}C NMR spectral data of the sugar moiety of **4** were in complete agreement with the values reported for quercetin 3-*O*-β

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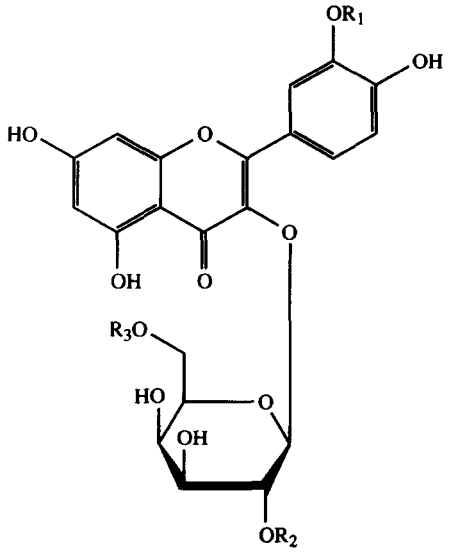
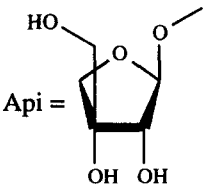


	R_1	R_2	R_3
1	H	H	H
2	H	Api	H
3	H	H	Rha
4	Me	Api	H



- D - apio - D - furanosyl(1 → 2) - β - D - galactopyranoside [11] and kaempferol 3-*O*-β-D-apio-D-furanosyl(1 → 2) - β - D - galactopyranoside [13]. Acetylation of **4** gave peracetate **4a**. The ^{1}H NMR data of **4a** could be completely assigned and were in agreement with the values reported for kaempferol 3-*O*-β-D-apio-D-furanosyl(1 → 2)-β-D-galactopyranoside peracetate [13]. NOE experiments on **4a** confirmed the erythrofuranosyl structure of the apiosyl moiety: presaturation of H_a-4‴ (δ 4.78) resulted in an enhancement of the signal at δ 5.30 (H-2‴), which indicated both protons to be on the same face of the ring and consequently the erythro stereochemical relation of HO-2‴ and HO-3‴. Thus, **4** is identified as isorhamnetin 3-*O*-β-D-apio-D-furanosyl(1 → 2)-β-D-galactopyranoside, a new natural flavonol.

Compounds **1**–**4** are the first flavonoids to be isolated from *V. galamensis* ssp. *nairobiensis*. Isorhamnetin has already been isolated from *V. nitidula* [14] but no glycosylated derivatives have been identified in the genus *Veronia* previously. Apiose is very rare in the Asteraceae and in the genus *Vernonia* it has neither been found as a free sugar nor as a glycosyl moiety. Compound **2** has been isolated previously from *Securidaca diversifolia* (Polygalaceae) [11], while **3** has been synthesized [15] and found in several plant species including *Strychnos variabilis* (Loganiaceae) [12], *Taverniera aegyptica* (Leguminosae) [16], *Crataegus pinnatifida* (Rosaceae) [17], *Chenopodium pallidicaule* (Chenopodiaceae) [18] and four species of *Solidago* (Asteraceae) [19].

EXPERIMENTAL

General. Mps are uncorr. UV: shift reagents prepared according to [9]. TLC: pre-coated silica gel 60 F_{254} aluminium sheets (Merck) and Diol HPTLC plates (Merck). CPC: Quattro countercurrent chromatograph (AECS, Bridgend, Wales, UK); 250 ml coil; 800 rpm; 2 ml min^{-1}. D/CIMS: positive ion mode, NH_3 as reactant gas. Thermospray MS (TSPMS): 0.5 M NH_4OAc (0.2 ml min^{-1}). ^{1}H and ^{13}C NMR: 200.06 and 50.3 MHz, respectively; δ relative to TMS.

Plant material. Veronia galamensis ssp. *nairobienssi* was collected on 26 November 1991 in Kinale Forest, near Nairobi, Kenya. A voucher specimen has been deposited at the East African Herbarium in Nairobi, Kenya.

Extraction and isolation. Air-dried leaves (107 g) were successively extracted at room temp. with CH_2Cl_2 (6.4 g) and MeOH (12.4 g). MeOH extract (10 g) separated by CC on Sephadex LH 20 (MeOH) to give eight frs (1–8). Only frs 5 and 6 contained flavonol glycosides and were further sepd. Fr. 6 (300 mg) was submitted to LPLC using a Lobar® RP-18 column (40–63 μm; 310 × 25 mm, i.d.) with MeOH–H_2O (4:6) to afford **1** (72 mg). Fr. 5 (600 mg) was sepd using the same procedure with MeOH–H_2O (3:7) and yielded eight frs (5a–5h). Frs 5c (40 mg), 5e (62 mg) and 5 g (270 mg) were purified by CPC ($CHCl_3$–MeOH–*n*-BuOH–H_2O, 7:6:3:4, lower phase as mobile phase) and yielded **2** (4 mg), **3** (7 mg) and **4** (59 mg), respectively.

Hydrolysis of **2–4** *with* 2M HCl. Each compound (1 mg) was refluxed in 2M HCl (10 ml) for 4 hr. The aglycones were extracted with EtOAc and identified by TLC comparison with authentic samples of isorhamnetin and quercetin (silica gel, toluene–ethyl formate–HCOOH, 5:4:1; Diol, EtOAc–petrol, 1:1). The aq. layer was neutralized by addition of $NaHCO_3$. After freeze drying, the sugars were extracted with pyridine, the solvent evapd and the residue dissolved in a small amount of MeOH. Analysis of the sugars by TLC on silica gel with EtOAc–MeOH–H_2O–HOAc (57:13:13:17); detection with *p*-anisidine phthalate.

Hydrolysis of **4** *with* 0.1 N H_2SO_4. Compound **4** (1 mg) was refluxed in 0.1 N H_2SO_4 (5 ml) for 30 min. Extraction, TLC and detection of the sugars were carried out as above.

Quercetin 3-O-β-D-*galactopyranoside* (*hyperoside*, **1**). Amorphous, yellow powder; mp 235–239°; TLC [SiO_2, EtOAc–MeCOEt–HCOOH–H_2O, 5:3:1:1 (system A)]: R_f 0.44; D/CIMS: *m/z* 465 $[M+H]^+$, 303 $[M+H-162]^+$.

Quercetin 3-O-β-D-*apio*-D-*furanosyl*(1→2)-β-D-*galactopyranoside* (**2**). Amorphous, yellow powder; mp 186–192°; TLC (system A): R_f 0.26; D/CIMS: *m/z* 597 $[M+H]^+$, 465 $[M+H-132]^+$, 303 $[M+H-294]^+$; UV, ^1H and ^{13}C NMR as in [11].

Quercetin 3-O-α-L-*rhamnopyranosyl*(1→6)-β-D-*galactopyranoside* (**3**). Amorphous, yellow powder; mp 205–209°; TLC (system A): R_f 0.27; D/CIMS: *m/z* 611 $[M+H]^+$, 465 $[M+H-146]^+$, 303 $[M+H-308]^+$; UV, ^1H and ^{13}C NMR as in [12].

Isorhamnetin 3-O-β-D-*apio*-D-*furanosyl*(1→2)-β-D-*galactopyranoside* (**4**). Amorphous, yellow powder; mp 166–168°; TLC (system A): R_f 0.25; $[\alpha]_D$ −78° (MeOH; *c* 0.1); UV λ_{max} (MeOH) nm (log ε): 260 (4.20), 271 sh (4.15), 358 (4.12); ($AlCl_3$) 273, 305, 367, 409; ($AlCl_3$ + HCl) 274, 300 sh, 364, 411; (NaOMe) 277, 388; (NaOAc) 279, 325, 375; (NaOAc + H_3BO_3) identical with MeOH; D/CIMS: *m/z* 611 $[M+H]^+$, 479 $[M+H-132]^+$, 317 $[M+H-294]^+$; ^1H NMR (DMSO-d_6): δ 3.20–3.90 (unresolved sugar protons), 3.87 (3H, *s*, OCH_3), 5.32 (1H, *d*, *J* = 1.0 Hz, H-1‴), 5.62 (1H, *d*, *J* = 7.5 Hz, H-1″), 6.20 (1H, *d*, *J* = 2.0 Hz, H-6), 6.41 (1H, *d*, *J* = 2.0 Hz, H-8), 6.98 (1H, *d*, *J* = 8.8 Hz, H-5′), 7.53 (1H, *d*, *J* = 2.2 Hz, H-2′), 7.91 (1H, *dd*, *J* = 2.2, 8.8 Hz, H-6′); ^{13}C NMR (DMSO-d_6): δ 55.6 (OCH_3), 60.1 (C-6″), 64.4 (C-5‴), 68.3 (C-4″), 73.8 (C-3″), 73.9 (C-4‴), 74.8 (C-2″), 75.7 (C-5″), 76.1 (C-2‴), 79.3 (C-3‴), 93.5 (C-8), 98.8 (C-6)*, 99.0 (C-1″)*, 103.9 (C-10), 108.8 (C-1‴), 111.2 (C-2′), 115.2 (C-5′), 122.1 (C-6′), 133.6 (C-3), 146.0 (C-3′), 150.0 (C-4′), 155.2 (C-2)†, 156.3 (C-9)†, 161.3 (C-5), 164.4 (C-7), 177.4 (C-4).

Acetylation of **4**. A solution of **4** (12 mg) in Ac_2O–pyridine (1:1) (2 ml) was kept at room temp. for 16 hr. After addition of ice, the aq. layer was extracted with EtOAc. The aq. phase was discarded and the organic layer was extracted with a 2.5% aq. solution of $CuSO_4$ to remove pyridine, washed and evaporated to dryness. The crude product was purified by LPLC on a Lobar® Diol column (40–63 μm; 310 × 25 mm, i.d.) with petrol–EtOAc (3:2) to afford 12 mg of isorhamnetin 3-*O*-β-D-apio-D-furanosyl(1→2)-β-D-galactopyranoside nonacetate (**4a**). Amorphous powder; mp 87–90°; $[\alpha]_D$ −38° ($CHCl_3$; *c* 0.1); TSPMS: *m/z* 989 weak $[M+H]^+$, 731 $[M+H-ApiAc_3]^+$, 547 $[M+H-442]^+$, 443 $[M+H-(Api\text{-}Gal)Ac_6]^+$, 259 $[M+H-730]^+$; ^1H NMR ($CDCl_3$): δ 1.90, 2.00, 2.01, 2.06, 2.07, 2.11 (3H, each *s*, aliphatic OAc), 2.34, 2.35, 2.40 (3H, each, *s*, aromatic OAc), 3.80 (3H, *m*, H-5″, H_2-6″), 3.92 (3H, *s*, OCH_3), 4.02 (1H, *dd*, *J* = 7.8, 10.3 Hz, H-2″), 4.09 (1H, *d*, *J* = 10.4 Hz, H_a-5‴), 4.34 (1H, *d*, *J* = 10.4 Hz, H_b-5‴), 4.78 (1H, *d*, *J* = 12.5 Hz, H_a-4‴), 4.96 (1H, *d*, *J* = 12.5 Hz, H_b-4‴), 5.05 (1H, *d*, *J* = 3.1, 10.3 Hz, H-3″), 5.24 (1H, *s*, H-1‴), 5.30 (1H, *s*, H-2‴), 5.31 (1H, *d*, *J* = 3.1 Hz, H-4″), 5.54 (1H, *d*, *J* = 7.8 Hz, H-1″), 6.81 (1H, *d*, *J* = 2.2 Hz, H-8), 7.04 (1H, *d*, *J* = 8.8 Hz, H-5′), 7.32 (1H, *d*, *J* = 2.2 Hz, H-6), 7.88 (1H, *d*, *J* = 2.2 Hz, H-2′), 8.04 (1H, *dd*, *J* = 2.2, 8.8 Hz, H-6′).

*,†Assignments with same superscripts interchangeable.

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