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KAEMPFEROL GLYCOSIDES FROM *ALBIZIA VERSICOLOR*

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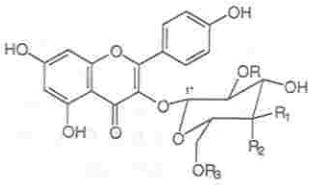
ABSTRACT. Three kaempferol glycosides were isolated from the methanolic extract of the stem bark of *Albizia versicolor* and identified as kaempferol-3-neohesperidoside, kaempferol-3-*O*-(2^G-α-rhamnopyranosyl)-rutinoside and mauritianin on the basis of NMR studies. These glycosides are all new to the genus.

INTRODUCTION

The root and stem barks of *Albizia versicolor* Welw. ex Oliv. (Leguminosae) are used in Kenyan herbal medicine as an anthelmintic, purgative and analgesic [1]. In some parts of the country the same plant parts are used for making medications for venereal diseases [2]. No previous work had been done on A. versicolor, but other *Albizia* species have yielded a number of triterpenes [3-6], alkaloids [7-11] and flavonoids [12-16]. Most reports on the flavonoids of *Albizia* has focused on the aglycone moieties; with little attention to the structures of intact glycosides. From the methanolic extract of the stem bark of A. *versicolor*, we have isolated three kaempferol glycosides and identified them as kaempferol-3-neohesperidoside (**1**), kaempferol-3-*O*-(2^G-α-rhamnopyranosyl)-rutinoside (**2**) and mauritianin (**3**). These compounds are new to the genus and their structures were established by spectroscopic analysis.



	R	R_1	R_2	R_3
1	α-rhamnosyl	OH	H	H
2	α-rhamnosyl	OH	H	α-rhamnosyl
3	α-rhamnosyl	H	OH	α-rhamnosyl

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow solid with UV and IR characteristics typical of a flavone (see Experimental). The ^{1}H NMR spectral data (Table 1) showed signals in the aromatic region for the para-substituted B-ring of a flavonoid, and two *mata* coupled protons for H-6 and H-8

of the A-ring. A highly deshielded resonance at δ13.40 was attributable to the hydrogen bonded hydroxyl proton 5-OH. These resonances were suggestive of kaempferol skeleton. The spectrum showed other signals suggestive of pyranose monosaccharides, among them two anomeric protons at δ6.67 (doublet) and 6.46 (broad singlet). A *J*-modulated ^{13}C NMR spectrum likewise showed resonances that were attributable to kaempferol and two hexose sugars (Table 1). The COSY-45 spectrum, starting from the anomeric proton at δ6.67 showed all the axial coupling system of β-glucopyranose. The other anomeric proton at δ6.46 exhibited a spin system terminating in a secondary methyl which was typical of α-rhamnose.

Table 1. ^{1}H and ^{13}C NMR shifts for compounds **1-3**.

C/H	1	2	3	1	2	3
2	-	-	-	157.9	156.8	157.5
3	-	-	-	134.7	137.1	137.7
4	-	-	-	179.1	172.7	172.7
4a	-	-	-	106.0	115.9	115.9
6	6.68 *d* (2.2)	6.98 *d* (8.8)	6.98 *d* (2.2)	100.1	114.8	114.8
7	-	-	-	166.1	157.8	157.9
8	6.69 *d* (2.2)	7.46 *d* (2.2)	7.46 *d* (2.2)	94.9	110.2	110.3
8a	-	-	-	157.5	155.5	155.4
1'	-	-	-	122.8	128.9	129.3
2'/6'	8.52 *d* (8.8)	8.21 *d* (8.8)	8.24 *d* (8.8)	132.2	131.5	131.6
3'/5'	7.29 *d* (8.8)	7.34 *d* (8.8)	7.32 *d* (8.8)	116.7	122.9	122.7
4'	-	-	-	162.0	153.9	153.8
1''	6.67 *d* (7.8)	5.82 *d* (7.8)	5.62 *d* (7.6)	103.0	99.7	100.4
2''	4.61 *t* (8.9)	3.89 *dd* (9.6, 7.8)	4.03 *dd* (10.2, 7.6)	79.8	79.3	76.3
3''	4.40 *t* (8.9)	5.43 *t* (9.5)	5.23 *dd* (10.1, 3.3)	79.2	75.2	73.9
4''	4.18 *t* (8.8)	4.97 *t* (9.9)	5.38 *s*	72.3	69.8	69.1
5''	3.95 *m*	3.94 *m*	4.14 *t* (5.5)	79.3	73.4	72.3
6''	4.34 *dd* (11.0, 2.1)	3.71 *dd* (11.4, 2.4)	3.60 *dd* (10.6, 5.0)	62.8	66.5	67.1
6''	4.20 *dd* (11.0, 5.2)	3.35 *dd* (11.4, 4.9)	3.33 *dd* (10.5, 6.0)	-	-	-
1'''	6.46 *s*	4.93 *d* (1.6)	4.97 *d* (1.6)	100.9	99.5	99.5
2'''	4.84 *dd* (3.4, 1.3)	5.08 *dd* (3.5, 1.6)	5.13 *dd* (3.5, 1.6)	73.2	71.0	71.2
3'''	4.82 *dd* ((9.3, 3.4)	5.32 *dd* (10.2, 3.5)	5.38 *dd* (10.3, 3.5)	73.3	69.6	69.8
4'''	4.30 *t* (9.4)	4.96 *t* (10.0)	5.01 *t* (10.1)	74.7	71.5	71.5
5'''	5.00 *m*	4.35 *m*	4.53 *m*	70.5	67.5	67.5
6'''	1.59 *d* (6.2)	0.73 *d* (6.2)	0.84 *d* (6.2)	18.9	17.1	17.2
1''''	-	4.57 *d* (1.6)	4.58 *d* (1.6)		98.5	98.5
2''''	-	5.12 *dd* (3.57, 1.6)	5.10 *dd* (3.5, 1.7)		70.3	70.2
3''''	-	5.04 *dd* (10.2, 3.6)	5.04 *dd* (10.0, 3.1)		70.0	70.0
4''''	-	4.84 *t* (10.0)	4.84 *t* (10.1)		71.2	71.1
5''''	-	3.61 *m*	3.63 *m*		67.2	67.2
6''''	-	0.85 *d* (6.2)	0.94 *d* (6.3)		17.4	17.6

Compound **1** run in C_5D_5N, compounds **2** and **3** run as the peracetates in Me_2CO-d_6.

The UV spectrum in methanol and on addition of shift reagents [20] showed absorption bands consistent with the kaempferol skeleton with substitution at the 3-OH and free hydroxyls at positions 5, 7 and 4', thus leaving C-3 as the site of attachment of a disaccharide.

The positions of linkages between the sugars were revealed by application of the HMBC technique [21]. The proton H-2" of glucose, resonating at δ4.61, showed ^2J and ^3J correlations with the anomeric carbons at δ103.0 and 100.9, respectively, indicating that the rhamnose was linked to C-2 of glucose, giving the disaccharide neohesperidoside. Conclusive support for the structure of **1** was obtained from the FABMS which revealed a pseudo-molecular ion peak at *m/z* 595 $[M+H]^+$ corresponding to the molecular formula $C_{27}H_{30}O_{15}$. Other significant peaks were noted at *m/z* 449 $[M+H-146]^+$ and *m/z* 287 $[M + H - 308]^+$ (kaempferol ion), corresponding to the successive loss of rhamnose and glucose from the molecular ion. The spectral data for **1** was found consistent with the structure of kaempferol-3-neohesperidoside, a known compound [17].

The ^1H NMR data (Table 1) of the peracetate of compound **2** again revealed signals in the aromatic region attributable to the kaempferol skeleton and the UV spectra was in agreement with a kaempferol skeleton with 3-substitution, as in **1**. The remainder of the ^1H NMR spectrum showed signals attributable to a trisaccharide, with three anomeric protons resonating at δ5.82 (*d*, J = 7.8 Hz), 4,57 (*d*, J = 1.6 Hz) and 4.93 (*d*, J = 1.6 Hz). The appearance of two methyl doublets in the highfield region strongly suggested that two of the sugars were rhamnose and the COSY-45 spectrum confirmed the presence of spin systems for a β-glucose and two α-rhamnose units. The ^{13}C NMR (*J*-modulated) revealed resonances typical of kaempferol, glucose and two rhamnose units (Table 1).

The HMBC spectrum resolved the problem of inter-sugar linkages and confirmed the site of attachment of the trisaccharide to the aglycone. The anomeric proton of glucose showed a ^3J correlation with the carbon resonances at δ137.1, which is attributable to C-3 of the kaempferol while the H-2 signal of the glucose showed a comparable correlation with the rhamnose anomeric carbon at δ99.5, requiring that one rhamnose was linked to glucose through the C-2, as in **1**. The second rhamnose anomeric exhibited a ^3J correlation with the methylene carbon (C-6) of the glucose, thus placing this rhamnose at C-6 of the glucose. Substitution at C-6 of glucose was also indicated by the low shield of *ca.* 4 ppm in the ^{13}C resonance for that carbon in comparison with **1** [22].

The FABMS of **2** showed the anticipated pseudo-molecular ion at *m/z* 741 $[M+H]^+$ corresponding to the molecular formula $C_{33}H_{40}O_{19}$. Other significant ions were at *m/z* 597 due to the loss of one rhamnose and *m/z* 287 for the kaempferol ion after loss of the entire trisaccharide. The spectral data for **2** was consistent with the structure of kaempferol-3-*O*-(2^G-α-rhamnopyranosyl)-rutinoside, a known natural product [20].

The UV spectrum of **3** and ^1H NMR spectrum (Table 1) of the peracetate were once more indicative of the existence of a kaempferol skeleton substitute at C-3 with a trisaccharide in which two of the hexose units were rhamnose. Tracking of the spin system for the third hexose by COSY-45 revealed that while H-1 (anomeric), H-2 and H-3 were all axial (as in β-glucose) H-4 was equatorial and that consequently it was β-galactose rather than β-glucose. The ^{13}C NMR (*J*-modulated) spectrum displayed values that were assignable to those of kaempferol, galactose and two rhamnose units. The HMBC spectrum was again valuable confirming the connectivity between the anomeric proton of galactose and C-3 of kaempferol and between H-2 and H-6 of galactose and the anomeric carbons of the two rhamnose units. The FABMS data was identical to that of **2** implying that the difference between the two compounds was only the stereochemistry of the inner sugar. Thus **3** was identified as kaempferol-3-*O*-[2,6-di-*O*-(α-rhamnopyranosyl)]-β-galactopyranoside (mauritianin), a known natural product [19].

EXPERIMENTAL

Mps, Uncorr; UV, MeOH; IR, KBr; FABMS, VG ZAB-E with a nitrobenzyl alcohol (NOBA) matrix; NMR, Bruker AMX-400 in Me_2CO-d_6 (δH 2.05, δC 29.92), C_5D_5N (δC 135.91)

Plant material. The stem bark of *Albizia versicolor* was collected in August 1992 from Kwale District, Kenya. The plant was identified by the staff of the Botany Department, University of Nairobi, and the voucher specimen is on deposit at the Herbarium of the same department.

Extraction and isolation of the compounds. The powdered bark (500 g) was successively extracted using petrol, CH_2Cl_2 and MeOH and the extracts dried *in vacuo*. The MeOH extract (5.0 g) was introduced in to a column packed with Sephadex LH-20. Elution was carried out isocratically using MeOH at a flow rate of 0.2 mL min^{-1}, collecting fractions of 5-10 mL. Purity of compounds was monitored on TLC with solvent, MeOH/$CHCl_3$/H_2O/AcOH (50:34:6:1), visualization being by a combination of UV activity and spraying with vanillin-H_2SO_4 reagent. Two fractions Fl (50 mg) and F2 (500 mg) containing mixtures of compounds **1-3** were obtained. Fraction Fl was subjected to HPLC separation using the solvent MeOH/H_2O (35:65) at a flow rate of 4 mL min^{-1} yielding 15 mg of **1**. Fraction F2 was treated similarly, the HPLC solvent being MeCN/H_2O (15:85) at a flow rate of 3 mL min^{-1}, affording 11 mg of **2** and 12 mg of **3**.

Kaempferol-3-neohesperidoside.(**1**). Yellow needles from MeOH, mp 200-201° (lit. [17] 201-202°). Found: M^+ 594.0231, $C_{27}H_{30}O_{15}$, requires 594.0230; UV λ_{max} nm: 262, 288 sh, 345; (+NaOMe) 269, 320, 393; (+$AlCl_3$) 268, 301 sh, 347 sh, 394; (+$AlCl_3$ + HCl) 268, 298 sh, 346 sh, 393; (+NaOAc) 270, 295 sh, 378; (+NaOAc+H_3BO_3) 262, 290 sh, 346; IR ν_{max} cm^{-1}: 3400 br, 2910, 1665, 1600; FABMS *m/z*: 595.2 $[M+H]^+$, 449 $[M+H\text{-}146$ (rhamnosyl)$]^+$, 287 $[M+H$-(rhamnosyl)-162 (glucosyl)$]^+$.

Kaempferol-3-O-2^G-α-rhamnopyranosyl)-rutinoside (**2**). Yellow needles from MeOH/MeCN, mp 204-206° (lit. [18] 204-205°) Found: M^+ 740.0029, $C_{33}H_{40}O_{19}$, requires 740.0027; UV λ_{max} nm: 262, 287 sh, 345; (+ NaOMe) 270, 322, 397; (+ $AlCl_3$) 269, 300 sh, 347 sh, 392; (+ $AlCl_3$ + HCl) 269, 298 sh, 346 sh, 393; (+ NaOAc) 270, 303 sh, 373; (+ NaOAc + H_3BO_3) 262, 290 sh, 344; IR ν_{max} cm^{-1}: 3400 br, 2910, 1665, 1600. FABMS *m/z*: 741.0 $[M + H]^+$, 595 $[M + H - 146$ (rhamnosyl)$]^+$, 449 $[M + H - 292$ (rhamnosyl)$^2]^+$, 287 $[M + H -$ (rhamnosyl)^2-162 (glucosyl)$]^+$.

Mauritianin (**3**). Yellow needles from MeOH/MeCN, mp 201-203° (lit. [19] 202-204°). Found: M^+ 740.0029, $C_{33}H_{40}O_{19}$, requires 740.0027; UV λ_{max} nm: 260, 289 sh, 347; (+ NaOMe) 270, 320, 395; (+ $AlCl_3$) 270, 301 sh, 350 sh, 395; (+ $AlCl_3$ + HCl) 269, 299 sh, 345 sh, 392; (+ NaOAc) 270, 303 sh, 375; (NaOAc + H_3BO_3) 262, 292 sh, 348; IR ν_{max} cm^{-1}: 3400 br, 2910, 1665, 1600, 1600; FABMS *m/z*: 741.0 $[M+H]^+$, 595 $[M+H$-146 (rhamnosyl)$]^+$, 449 $[M+H$-292 (rhamnosyl)$^2]^+$, 287 $[M+H$-(rhamnosyl)^2-162(galactosyl)$]^+$.

Acetylation of compounds 1 and 2. About 10 mg of the sample was dissolved in pyridine (1 mL) and acetic anhydride (1 mL) added. The reaction was allowed to proceed in the cold for 18 h. The reaction mixture was dried under N_2, dissolved in $CHCl_3$ and the peracetate purified through a small column of Sephadex LH-20.

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