Natural Product Sciences
8(3) : 77-82 (2002)

Flavonol Glycosides of *Maesa Lanceolata* Leaves

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Abstract – An investigation of the methanolic extract of *Maesa lanceolata* leaves has led to the isolation of four novel flavonol glycosides characterised as myricetin 3-*O*-2", 3", 4"-triacetylxylopyranoside (**1**), quercetin 3-*O*-β-3"', 6"'-diacetylglucopyranosyl-(1→4)-α-2", 3"-diacetylrhamnopyranoside (**2**), myricetin 3-*O*-xylopyranosyl-(1→3)-α-rhamnopyranoside (**3**) and quercetin 3-*O*-β-galactopyranosyl-(1→4)-α-rhamnopyranoside-7-*O*-β-galactopyranoside (**4**). Also isolated from the same extract were known flavonols; quercetin (**5**), myricetin (**6**), quercetin 3-*O*-xylopyranoside (**7**), quercetin 3-*O*-α-rhamnopyranoside (**8**), myricetin 3-*O*-α-rhamnopyranoside (**9**), myricetin 3-*O*-β-galactopyranoside (**10**) and quercetin 3-*O*-rutinoside (**11**).

Key words – *Maesa lanceolata*; Myrsinaceae; flavonol glycosides; leaves

Introduction

Maesa lanceolata forsk is one of the five Myrsinaceae species endemic to Kenya and is highly valued in herbal medicine as anthelminitics and antimicrobiotics (Kokwaro, 1976). Phytochemically, the species is typified by the 19-carbon side chain benzoquinones; 2, 5-dihydroxy-6-methyl-3-(nonadec-14-enyl)-1, 4-benzoquinone (maesaquinone) and 5-acetoxy-2-hydroxy-6-methyl-3-(nonadec-14-enyl)-1, 4-benzoquinone (acetylmaesaquinone) (Midiwo *et al.*, 1990). In a recent paper (Midiwo and Manguro, 1996), we reported the isolation of 2-*O*-methylembelin (muketanin) from this source.

As part of our continuing phytochemical study of the plant, we now report the isolation of four novel flavonol derivatives (**1-4**) along with seven other known ones from the methanolic extract of the powdered plant leaves (Manguro *et al.*, 1995; Manguro and Williams, 1996; Manguro *et al.*, 1997).

Experimental

General experimental procedures – UV spectra were recorded on a 8452 A Hewlett Packard Array spectrophotometer. ^{1}H and ^{13}C NMR spectral data were obtained on Brucker WM instrument operating at 400 and 100 MHz, respectively. IR data were recorded on Perkin-Elmer - FTIR 600 series as KBr pellet. The FAB mass spectral data was provided by Polish Academy of Sciences while EIMS were measured at 70 eV using MAT 8200 A Varian Bremen instrument. Silica gel for both column and TLC were impregnated with 2% oxalic acid solution.

Plant material – *M. lanceolata* leaves were collected around Nakuru town along the Nakuru-Eldoret Highway in May 1997, and the voucher specimens (Manguro 97/5/ML/L) were identified after comparison with authentic samples at the University of Nairobi, Botany Department Herbarium.

Extraction and isolation – The CH_2Cl_2-defatted leaves (approx. 5 kg) were further extracted with MeOH (7.5 l×3) for two weeks at room temperature. The combined extract (approx. 20 *l*) was evaporated under reduced pressure to yield a dark green residue (375 g). A portion of the extract (300 g) was chromatographed over an open column with a gradient elution of CH_2Cl_2-MeOH (95:5) to MeOH, collecting 100 ml each (total 450 fractions). The corresponding eluates were combined into four major pools (I-IV) depending on TLC profiles.

Pool I (fractions 50-160) was evaporated to give 17 g which was subsequently subjected to repeated low pressure chromatography using CH_2Cl_2-MeOH (95:5) followed by the same solvent system in the ratio 9:1, collecting every 10 ml to afford **1** (86 mg), **5** (155 mg) and **6** (96 mg). Fractions 161-263 constituted pool II (16.5 g) and was

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similarly purified as in pool I using the same solvent system in the ratio 85:15 to afford a futher **6** in 57 mg, **7** (120 mg), **2** (70 mg) and **8** (77 mg). Pool III (fractions 265-375, 32 g) yielded a gummy material after evaporation *in vacuo* and was rechromatographed over an open column with CH_2Cl_2-MeOH of increasing polarity and finally with MeOH, affording 160 fractions of 100 ml each. The eluates 10-155 were found to contain the desired compounds, thus they were combined and further purified by flash chromatography with CH_2Cl_2-MeOH (4:1) followed by the same solvent in the ratio 3:2 to give **9** (60 mg), **10** (49 mg), **11** (130 mg) and **3** (69 mg). The remaining fractions (fractions 377- 445, 45 g), mainly from methanol elution which constituted pool IV, were combined, evaporated and rechromatographed as in pool III to afford eluates which contained four major components. These were combined and further repeatedly purified by flash chromatography using MeOH-CH_2Cl_2 (3:2), followed by crystallization in MeOH-H_2O (9:1) afforded an additional **10** in 38 mg, **11** in 59 mg, **3** in 30 mg and **4** (44 mg).

Myricetin 3-*O*-β -2", 3", 4"-triacetylxylopyranoside (**1**). Pale yellow amorphous powder, mp 181-183°C. UV λ_{max} (MeOH) nm: 258, 302, 352; (+$AlCl_3$) 270, 316, 440; (+$AlCl_3$/HCl) 272, 310, 400; (+NaOMe) 266, 398, degenerate with time; (+NaOAc) 274, 320, 378; (+NaOAc/H_3BO_3) 260, 300, 376. IR ν_{max} (KBr) cm^{-1}: 3500 (OH), 1725 (C=O, ester), 1655 (C=O, α, β-unsaturated), 1495, 1370, 1190, 1020, 900, 860, 770. ^1H NMR ($CDCl_3$ + one drop DMSO-d_6) δ ppm: 12.67 (1H, s, OH-5, D_2O exchange), 10.24 (1H, s, OH-7, D_2O exchang.), 8.60 (2H, s, OH-3'and 5', D_2O exchange), 8.20 (1H, s, OH-4', D_2O exchange), 6.80 (1H, s, H-2' and 6'), 6.25 (1H, d, J = 1.8 Hz, H-8), 6.10 (1H, d, J = 1.8 Hz, H-6); xylose: 5.25 (1H, d, J = 8.3 Hz, H-4"), 5.15 (1H, d, J = 7.6 Hz, H-1"), 4.90 (1H, m, H-3"), 4.70 (1H, m, H-2"), 3.80 (1H, m, H-$5"_B$), 3.60 (1H, m, H-$5"_A$), 2.25, 2.15, 2.05, (9 H, s, 3 x OAc). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m/z* (%) 318 (100), 289 (4), 243 (11), 215 (2), 153 (10), 114 (6), 73 (14), 43 (65). Positive ion FAB-MS: *m/z* 577 $[M + H]^+$, 319 $[M-132+H]^+$, 153, 43.

Quercetin 3-*O*-β-3"', 6"'-diacetylglucopyranosyl-(1→4)-α-2", 3"-diacetylrhamnopyranoside (**2**). Amorphous pale yellow powder, mp 215-218°C. UV λ_{max} (MeOH) nm: 256, 300, 360; (+$AlCl_3$) 270, 302, 430; (+$AlCl_3$/HCl) 272, 300, 402; (+NaOMe) 272, 326,408; (+NaOAc) 274, 322, 378; (+NaOAc/H_3BO_3) 264, 380. IR ν_{max} (KBr) cm^{-1}: 3500 (OH), 1665 (C=O), 1560, 1520, 1445, 1370. ^1H NMR ($CDCl_3$ + one drop DMSO-d_6) δ ppm: 12.60 (1H, br s, OH-5, D_2O exchange), 10.40 (1H, br s, OH-7, D_2O exchange), 9.30-8.50 (2H, br s, OH-3' and 4'), 7.70 (1H, d, J = 2 Hz, H-2'), 7.58 (1H, dd, J = 8.2, 2.1 Hz, H-6'), 6.84 (1H, d, J = 2 Hz, H-5'), 6.40 (1H, d, J = 2.1 Hz, H-8), 6.20 (1H, d, J = 2.1 Hz, H-6), inner rhamnose: 5.40 (1H, d, J = 1.1 Hz, H-1"), 5.02 (1H, dd, J = 8.7, 3.2 Hz, H-3"), 4.90 (1H, d, J = 8.3 Hz, H-2"), 3.50 (1H, dd, J = 5.5 Hz, H-4"), 3.35 (1H, ddd, J = 8.7, 5.0, 2.3 Hz, H-5"), 0.80 (3H, d, J = 6.4 Hz, Me-6"); terminal glucose: 4.75 (1H, d, J = 7.7 Hz, H-1"'), 5.20 (1H, dd, J = 11.6, 2.5 Hz, H-6_B"'), 4.95 (1H, dd, J = 11.6, 5.2 Hz, H-6_A"'), 4.70 (1H, t, J = 8.8 Hz, H-3"'), 3.60 (1H, dd, J = 9.6, 7.5 Hz, H-2"'), 3.38 (1H, t, J = 9 Hz, H-4"'), 3.22 (1H, ddd, J = 9.0, 5.3, 2.2 Hz, H-5"'), 2.20, 2.15, 2.03, 1.97 (12H, all s, 4 x OAc). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m/z* (%) 302 (100), 153 (20), 137 (6), 55 (60). FAB-MS (positive ion mode) : *m/z* 779 $[M+ H]^+$, 533 [M-246 +H]$^+$, 303 [M- 246-230 +H]$^+$, 476 [glucose + rhamnose + 4 acetoxys]$^+$, 163 [glucose]$^+$, 147 [rhamnose]$^+$, 153, 137, 55.

Myricetin 3-*O*-β-xylopyranosyl-(1→3)-α-rhamnopyranoside (**3**). Yellow amorphous powder. UV λ_{max} (MeOH) nm: 258, 304, 368; (+ $AlCl_3$) 272, 312, 446; (+ $AlCl_3$/HCl) 272, 310, 414; (+ NaOMe) 270, 324, 414, 384; (+ NaOAc) 274, 320; (+ NaOAc/ H_3BO_3) 260, 300, 386. IR ν_{max} (KBr) cm^{-1}: 3550 (OH), 1650, 1570, 1500, 1400, 1250, 970, 800. ^1H NMR ($CDCl_3$ + one drop DMSO-d_6) δ ppm. 12.70 (1H, s, OH-5, D_2O exchange), 10.20 (1H, s, OH-7, D_2O exchange), 9.30-8.10 (2H, br s, OH-3', 4' and 5', D_2O exchange), 7.30 (1H, s, H-2' and H-6'), 6.50 (1H, d, J = 2.0 Hz, H-8), 6.35 (1H, s, J = 2.0 Hz, H-6), rhamnose: 5.20 (1H, d, J = 0.9 Hz, H-1"), 3.65 (1H, J= 3.1 Hz, H-2"), 3.52 (1H, dd, J = 8.7, 3.1 Hz, H-3"), 3.46 (1H, dq, J = 10.7, Hz, H-5"), 3.34 (1H, dd, J = 9.5, 8.1 Hz, H-4"), 0.97 (3H, d, J = 6.5 Hz, Me-6"), xylose: 4.50 (1H, d, J = 7.6 Hz, H-1"'), 3.75 (1H, m, H-$5"'_B$), 3.50 (1H, m, H-4"'), 3.40 (1H, m, H-$5"'_A$), 3.30 (1H, m, H-3"'), 3.10 (1H, m, H-2"'). ^{13}C NMR data (DMSO-d_6) δ ppm: see Table 1. EIMS (70 eV): *m/z* (%) 318 (100), 289 (1), 243 (2), 215 (5), 153 (40), 136 (25), 109 (8). FAB-MS (positive ion mode): *m/z* 597 $[M + H]^+$, 465 [M - 132 + H]$^+$, 319 [M - 132 - 146 +H]$^+$, 153, 137, 110, 80.

Quercetin 3-*O*-β-galactopyranosyl (1→4)-α-rhamnopyranoside-7-*O*-β -galactopyranoside (**4**). A yellow powder. UV λ_{max} (MeOH) nm: 256, 302, 360; (+$AlCl_3$) 272, 300, 414; (+ $AlCl_3$/HCl) 272, 350, 410; (+ NaOMe) 272, 308, 364; (+ NaOAc) 272, 320, 380; (+ NaOAc/H_3BO_3) 270, 330. IR ν_{max} (KBr) cm^{-1}: 3550 (OH), 1660, 1575, 1515, 1485, 1430, 1150, 970. ^1H NMR (DMSO-d_6) δ ppm: 12.40 (1H, s, OH-5, D_2O exchange), 9.30 (1H, s, OH-4', D_2O exchange), 8.30 (1H, s, OH-3', D_2O exchange), 7.76 (1H, d, J = 2.1 Hz, H-2'), 7.65 (1H, d, J = 8.4, 2.1 Hz, H-6'), 6.90 (1H, d, J = 8.4 Hz, H-5'), 6.34 (1H, d, J = 2.2, H-8), 6.12 (1H, d, J = 2.2 Hz, H-6); residual rhamnose: 5.20 (1H, d, J = 0.9 Hz, H-1"), 4.0 (1H, br d, J = 3.1 Hz, H-2"), 3.52 (1H, dd, J = 8.8,

3.1 Hz, H-3"), 3.40 (1H, dd, J = 9.4, 8.8 Hz, H-4"), 3.26 (1H, dq, J = 9.6, 5.8 Hz, H-5"), 0.92 (3H, d, J = 6.1 Hz, Me-6"); terminal galactose: 4.40 (1H, d, J = 7.8 Hz, H-1'''), 3.90 (1H, dd, J = 11.4, 2.3 Hz, H-6'''$_B$), 3.80 (1H, dd, J = 11.4, 4.9 Hz, H-6'''$_A$), 3.34 (1H, m, H-5'''), 3.30 (1H, m, H-3'''), 3.25 (1H, m, H-4'''), 3.10 (1H, m, H-2'''); 7-*O*-galactose: 5.60 (1H, d, J = 7.7 Hz, H-1''''), 3.87 (1H, dd, J = 11.80, 2.6 Hz, H-6''''$_B$), 3.71 (1H, dd, J = 11.80, 5.1 Hz, H-6''''$_A$), 3.60 (1H, m, H-5''''), 3.42 (1H, m, H-3''''), 3.33 (1H, m, H-4''''), 3.18 (1H, m, H-2''''). ^{13}C NMR data: see Table 1. EIMS (70 eV): *m/z* (%) 302 (100), 180 (1), 162 (3), 146 (5), 153 (22), 137 (33). FAB-MS (positve ion mode) : *m/z* 773 [M + H]$^+$, 611 [M - 162 + H]$^+$, 465 [M - 162 - 146 + H]$^+$, 449 [M - 2 x 162 + H]$^+$, 309, 303 [M- 2 x 162 -146 + H]$^+$, 163, 180, 153, 137.

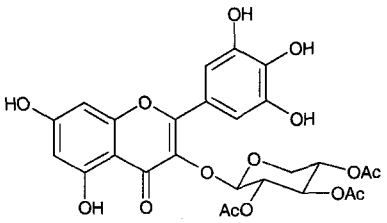
Myricetin 3- *O*-xylopyranoside (**7**). Yellow powder. UV λ_{max} (MeOH) nm: 260, 304, 366; (+$AlCl_3$) 271, 308, 450; (+$AlCl_3$/HCl) 272, 310, 420; (+NaOMe) 270, 324, 410; (+NaOAc)

274, 318, 378; (+NaOAc/H_3BO_3) 260, 300, 386. IR ν_{max} (KBr) cm^{-1}: 3500 (OH), 1680 (C=O, α, β-unsaturated), 1625, 1580, 1520, 1490, 1405. ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ ppm: 12.80 (1H, s, OH-5, D_2O exchange), 10.30 (1H, s, OH-7, D_2O exchange), 8.60 (1H, s, OH-3' and OH-5', D_2O exchange), 8.10 (1H, br s, OH-4', D_2O exchange), 7.34 (1H, s, H-2' and H-6'), 6.40 (1H, d, J = 1.9 Hz, H-8), 6.25 (1H, d, J = 1.9 Hz, H-6); xylose: 5.20 (1H, d, J = 7.7 Hz, H-1"), 4.0 (1H, m, H-5"$_B$), 3.70 (1H, m, H-5"$_A$), 3.50 (1H, m, H-4"), 3.37 (1H, m, H-3"), 3.21 (1H, m, H-2"). ^{13}C NMR data: See Table 1. EIMS (70 eV): *m/z* (%) 318 (100), 289 (1), 245 (4), 216 (8), 153 (10), 136 (5), 108 (39. FAB-MS (positive ion mode) : *m/z* 451 [M +H]$^+$, 319 [M-132 +H]$^+$, 153, 150.

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

Results and Discussion

Compound **1** displayed a molecular ion peak at *m/z* 577 in the FAB mass spectrum, analyzing for $C_{26}H_{24}O_{15}$ formula. Acid hydrolysis released xylose as the sugar residue identified by TLC after comparison with an authentic sample. The UV



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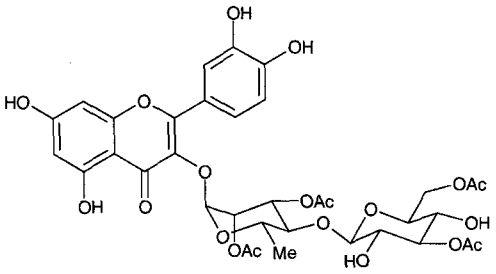

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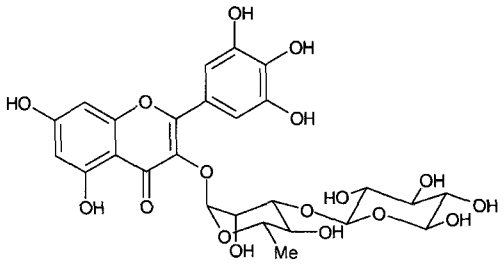

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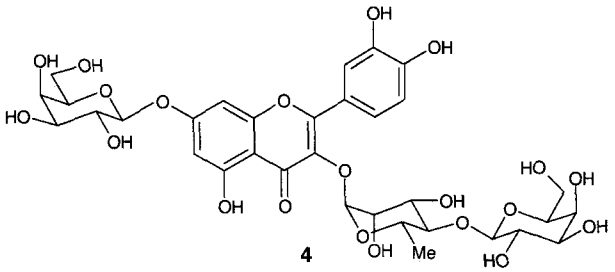

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spectrum in MeOH and its changes after addition of shift reagents (Mabry *et al.*, 1970; Markham, 1982) suggested the aglycone as myricetin with a substituted 3-hydroxy group (Arya *et al.*, 1992; Slimsted *et al.*, 1993). The aglycone was confirmed by ^{1}H NMR, in which two proton singlet at δ 6.80 ascribed to H-2' and H-6', and the characteristic *meta*-coupled doublets were detected at δ 6.25 (J =1.8 Hz) and 6.10 (J =1.8 Hz), respectively.

A comparative analysis of shift values (^{1}H NMR data) of compound **1** and myricetin 3-*O*-xylopyranoside (**7**) indicated certain correlations which were of diagnostic value. In the case of **1**, the effect of acetoxy substituents on C-2", C-3" and C-4" are noteworthy by producing downfield shifts at δ 4.70 (H-2"), 4.90 (H-3") and 5.25 (H-4"), respectively, a

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

Carbon	1	2	3	**4**	7
2	155.90	156.80	156.40	156.50	156.40
3	133.40	135.60	133.90	135.60	134.10
4	178.01	178.40	177.40	177.70	177.90
5	161.50	161.70	161.20	161.50	160.90
6	98.10	98.90	98.60	98.80	98.50
7	163.80	164.40	164.10	164.70	163.60
8	93.80	93.70	93.30	93.40	92.80
9	156.40	156.50	156.20	156.30	155.70
10	103.90	104.20	103.80	104.01	103.40
1'	120.20	121.20	119.80	121.20	119.30
2'	109.70	116.00	108.40	116.10	107.90
3'	145.50	144.70	146.50	145.01	145.00
4'	137.10	148.30	136.70	148.60	136.20
5'	146.10	115.80	146.50	115.30	146.10
6'	109.70	121.80	108.40	122.10	107.80
1"	103.00	102.40	102.60	101.90	104.60
2"	73.50	70.20	71.90	70.30	74.10
3"	76.80	70.80	81.60	70.40	76.20
4"	70.50	82.30	73.10	81.50	70.50
5"	62.50	70.10	72.70	70.60	64.80
6"		17.80	17.60	17.30	
1'''		104.90	101.50	103.90	
2'''		74.50	75.40	71.30	
3'''		76.30	76.20	73.20	
4'''		71.40	72.10	68.90	
5'''		73.40	65.40	75.90	
6'''		64.10		61.70	
1''''				105.60	
2''''				73.20	
3''''				74.40	
4''''				69.80	
5''''				76.20	
6''''				61.60	
OAc	171.01 170.35 170.70	169.90 170.20 171.40 171.60			
Me-OAc	25.40 25.80	25.10 25.60 25.80 26.20			

fact supported by multiplicity and decoupling experiments. Attempted irradiation of the peaks at δ 4.90 (H-3") and 4.70 (H-2") caused simplification of signals at δ 5.25 (H-4") and 5.15 (H-1").

The 2", 3"-, 4"-triacetylation of xylose was also consistent with the ^{13}C NMR data (Table 1). The upfield shifts of C-1" (-1.6 ppm) and C-5" (-2.3 ppm) signals in comparison with those of myricetin 3-*O*-xylopyranoside (**7**) were due to acetoxy groups at C-2" and C-4" positions. Thus, based on the above chemical and spectral considerations, compound **1** was characterised as myricetin 3-*O*-β-2", 3", 4"-triacetylxylopyranoside.

Compound **2** UV spectrum in MeOH and changes in addition of shift reagents (Mabry *et al.*, 1970; Markham, 1982) suggested that it is a flavonol with 3-hydroxy group. Acid hydrolysis afforded quercetin, glucose and rhamnose which were identified by TLC. The presence of quercetin was further supported by ^1H NMR spectrum which exhibited 2H AX and 3H ABX system (Manguro *et al.*, 1997). Furthermore, two anomeric protons at δ 5.40 (J = 1.1 Hz) and 4.75 (J = 7.7 Hz) signified an inner rhamnose and a terminal glucose, a fact corroborated by the correlation spectroscopy via the long range coupling (HMBC) between rhamnose anomeric proton (δ 5.40) and the carbon (δ 135.60) assigned to C-3, and between the other anomeric proton (δ 4.75) and a C-4" at δ 82.30. In the positive ion FAB mass spectrum, the compound showed a molecular ion peak at *m/z* 779 $[M + H]^+$ corresponding to molecular formula $C_{35}H_{38}O_{20}$. This together with other prominent peaks at *m/z* 533 $[M\text{-}246 + H]^+$ (loss of glucose and two acetoxy groups) and 303 $[M\text{-}246\text{-}230\text{-}H]^+$ (loss of rhamnose, glucose and four acetoxy groups) indicated the presence of diacetylglucopyranosyldiacetylrhamnopyranoside biose. The interglycosidic linkage of the biose was determined by HMBC experiment which showed long range coupling between the anomeric proton of the terminal glucose (δ 4.75) and the residual rhamnose C-4" at δ 82.30. This was further supported by NOESY correlations between a proton signal at δ 3.50 in a multiplet assigned to H-4" of the residual sugar unit and the anomeric proton of terminal glucose at δ 4.75. The positions of the acetoxy groups in the biose were determined by a combination of ^1H, ^{13}C and HMBC techniques. In the ^1H NMR, the downfield shifts at δ 5.20, 4.95 and 4.70 were ascribed to glucose H-$6'''_B$, H-$6'''_A$ and H-3''' while those at δ 4.90 and 5.02 represented rhamnose H-2" and H-3", respectively. The 2", 3", 3''', 6''' tetra-acetylation of the biose was also consistent with the ^{13}C NMR data (Table 1). The upfield shift of C-1" (-1.7 ppm) and C-4" (-2. 1 ppm) in rhamnose together with those of C-2''' (-2.2 ppm) , C-4''' (-2.7 ppm) and C-5''' (-2. 3 ppm) in glucose in comparison with kaempferol 3-*O*-β-glucopyranosyl-(1→4)-α-rhamnopyranoside (Markham *et al.*, 1992) indicated acetoxylation at C-2" and C-3" in rhamnose, and C-3''' and C-6''' in glucose.

On this basis, compound **2** was concluded as quercetin 3-*O*-β-3''', 6'''-diacetylglucopyranosyl-(1→4)-α-2", 3"-diacetylrhamnopyranoside.

Compound **3** exhibited UV data which suggested the presence of substituted 3-hydroxy flavonol (Markham *et al*, 1970). The chemical shift, both in ^1H and ^{13}C NMR data identified the agycone as myricetin. Rhamnose and xylose were identified by TLC after acid hydrolysis. In the FAB mass spectrum, the compound showed a molecular ion peak at *m/z* 597 $[M + H]^+$, corresponding to $C_{26}H_{28}O_{16}$. This

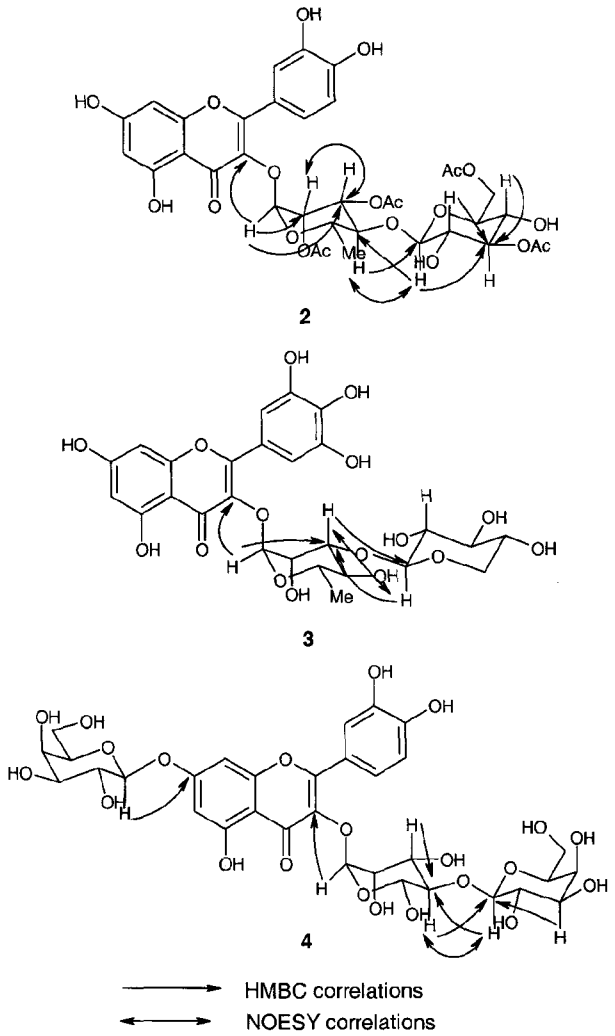

Fig. 1. Pertinent correlations observed in the HMBC and NOESY spectra of compounds **2**, **3** and **4**.

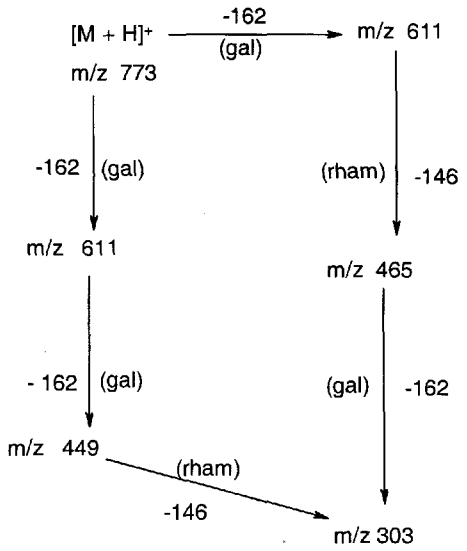

Fig. 2. Fragmentation of compound **4** in FAB MS.

together with other peaks at *m/z* 465 [M- 132 + H]$^+$ (loss of xylose), 319 [M - 146 -132 + H]$^+$ (loss of rhamnose and xylose) suggested a biose such as xylopyranosylrhamnopyranoside or rhamnopyranosylxylopyranoside substituted at C-3 of the aglycone. The interglycosidic linkage and the sequence of the biose was determined using ^{1}H - ^{1}H NOESY experiment (Fig. 1) whereby a peak at δ 3.52 in a multiplet assigned to H-3" of rhamnose showed a cross peak with the anomeric proton of xylose (δ 4.50), and the fact confirmed by HMBC correlations (Fig. 1). The results led to the conclusion that the biose is xylopyranosyl-(1→3)-rhamnopyranoside. Based on the above evidences, compound (**3**) was identified as myricetin 3-*O*-β-xylopyranosyl-(1→3)-α-rhamnopyranoside.

Compound **4**, a yellow powder gave ^{13}C NMR spectrum which exhibited the upfield and downfield shifts of quercetin consistent with 3- and 7-*O*-diglycosylation (Agrawal, 1989). Acid hydrolysis gave galactose and rhamnose as the sugar residues (confirmed by co-spoting with authentic samples). The UV spectrum of the compound in methanol and with addition of shift reagents confirmed lack of free 3- and 7-hydroxyls. This was further supported by HMBC correlations between the anomeric proton at δ 5.60 and the peak at δ 164.70 (C-7), and the other anomeric proton at δ 5.20 and C-3 (135.60). The results of FAB mass spectrum (Fig. 2) coupled with anomeric peaks at δ 5.60 (J = 7. 7 Hz), 5.20 (J = 0. 9 Hz) and 4.40 (J = 7.8 Hz) indicated that a galactose and a rhamnose are present as residual sugars while the other galactose as a terminal one (as galactopyranosylrhamnopyranoside). In the NOESY spectrum, H-4" (δ 3.40) of residual rhamnose showed a cross peak with anomeric proton (δ 4.40) of the terminal galactose, thus indicating that the biose is galactopyranosyl-(1→4)-rhamnopyranoside. Therefore, on the basis of spectroscopic evidences, compound **4** was concluded as quercetin 3-*O*-β-galactopyranosyl (1→4)-α -rhamnopyranoside-7-*O*-β -galactopyranoside.

Other flavonols isolated from the same extract included quercetin (**5**), myricetin (**6**), quercetin 3-*O*-xylopyranoside (**7**), quercetin 3-*O*-α-rhamnopyranoside (**8**), myricetin 3-*O*-rhamnoside (**9**), myricetin 3-*O*-galactopyranoside (**10**) and myricetn 3-*O*-rutinoside (**11**).

Acknowledgements

L. O. A. Manguro is grateful to Alexander von Humboldt Foundation for a Research visit to the University of Munich, Germany. Mr S. G. Mathenge of Botany Department, University of Nairobi is thanked for identification and

collection of plant materials.

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(Accepted July 15, 2002)