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Further Flavonol Glycosides from *Myrsine africana* Leaves

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Abstract – A new flavonol glycoside, quercetin 3-rhamnosyl (1 → 3) galactoside [5] was isolated from the leaves of *M. africana*. The known compounds kaempferol 3-rutinoside [1], 3'-O-methylquercetin 3-rutinoside [2], quercetin 3-rutinoside [3], and quercetin 3-rhamnosyl (1 → 6) galactoside [4] were also isolated for the first time from this plant. Their structures were determined by chemical and spectroscopic methods.

Key words – *Myrsine africana*, Myrsinaceae, Flavonol glycoside, Quercetin 3-rhamnosyl (1 → 3) galactoside.

Myrsine africana L (Myrsinaceae) is an undershrub to a small tree, 1 to 5 m high, wide spread in upland dry forest and rocky hill sides (Beentje, 1994). The plant is reported to have anthelmintic and antimicrobial properties (Kokwaro, 1976). Previous studies have led to the isolation of long chain alkyl-1, 4-benzoquinones (Midiwo, 1988), anthraquinones (Xia-Hua and McLaughlin, 1989; Midiwo and Manguro, 1993), and a triterpenoid saponin (Kupchan *et al*, 1969) form this source. In a recent paper (Manguro and Midiwo, 1996), we reported the isolation of a new flavonol glycoside along with nine other flavonoids and gallic acid. As part of our continuing investigations for flavonoid compounds from the leaves of the plant, we have now isolated and characterized five more flavonoids including a component described here for the first time.

Experimental

General – UV spectra were recorded on a 8452A Hewlett Packard Array spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Brucker WM instrument operating at 250 and 62.5 MHz, respectively. Preparative high performance liquid chromatography (HPLC) was performed on a Bischoff instrument using a model pump connected to 785 A programmable absorbance detector and a programme monitor 8252 dual pen recorder. EIMS were measured using 70 eV MAT 311 A Varian Bremen instrument. Silica gel 70-230 mesh (Merck) was used for gravity column chromatography while for low pressure column (flash) chromatography, silica gel 60 G (0.02-0.7 mm) was applied. Silica gel for column and TLC plates were impregnated with 2% oxalic acid solution.

Plant material – *M. africana* leaves were obtained from Kithembe hills in the Machakos District, Kenya in February, 1990, and voucher specimens (Manguro and Mwangangi 90/2) are deposited at the University of Nairobi, Botany Department Herbarium.

Extraction and Isolation – The defatted dry leaves (5 kg) were extracted with MeOH (7.5 l × 3) for one week. The extracts were combined and evaporated under vacuum to dryness to yield a dark green residue 350 g.

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A portion of the extract (120 g) was chromatographed on an open column eluting with CH_2Cl_2-MeOH (9:1) (increasing MeOH) and lastly with MeOH, collecting 250 ml each (110 fractions). The corresponding eluates were combined depending on TLC profiles. Fractions 1-35 were evaporated to give 7 g which was subsequently subjected to repeated low pressure column chromatography using CH_2Cl_2-MeOH (85:15) gradient and collecting 10 ml each to afford **1** (28 mg) and **2** (19 mg). Fractions 36-85 (11 g) were found to contain mainly a single component and further purification as described above gave 80 mg more of **2**.

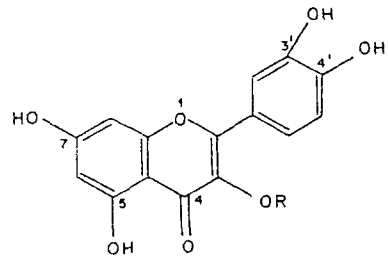
Fractions 86-120 afforded 11 g of gummy material after evaporation *in vacuo* and was rechromatographed on an open column with CH_2Cl_2-MeOH mixture of increasing polarity and lastly with MeOH giving 30 fractions of 100 ml each. The eluates 1-40 afforded **3** in 60 mg. The remaining fractions were finally purified by semi-preparative HPLC on reverse phase (RP-18) using MeOH-H_2O (7:3), affording **4** (25 mg) and **5** (13 mg).

Quercetin 3-rhamnosyl (1 → 3) galactoside (5). Amorphous powder. UV (CH_3OH) λ_{max} 358, 300, 258, (+$AlCl_3$) 432, 304, 272, (+$AlCl_3$/HCl) 400, 300, 270, (+NaOMe) 410, 328, 272, (+NaOAc) 380, 320, 274, (+NaOAc/H_3BO_3) 380, 262 nm. ^{1}H NMR ($CDCl_3$+one drop DMSO-d_6) δ ppm. 12.6(1H, br s, 5-OH), 10.7(1H, br s, 7-OH), 9.6(1H, br s, 3'-OH), 8.9(1H, br s 4'-OH), 7.8(1H, d, J=2.4 Hz, H-2'), 7.5(1H, dd, J=8.6, 2.4Hz, H-6'), 6.8 (1H, d, J=8.7 Hz, H-5'), 6.4 (1H, d, J=1.9 Hz, H-8), 6.2(1H, d, J=1.9 Hz, H-6), 5.5(1H, d, J=7.6 Hz, H-1"), 4.8(1H, d, J =1.4 Hz, H-1'''), 3.9 (1H, dd, J=11.2, 6 Hz, H-6"$_A$), 3.7(1H, dd, J=9.1, 7.4 Hz, H-2'''), 3.5-2.9 (8H, m, H-2", H-3", H-4", H-5", H-6"$_B$, H-3''', H-4''', H-5''', over lapping sugar signals), 1.0 (3H, d, J=6.4 Hz, CH_3 -6'''). ^{13}C NMR (CD_3OD) δ ppm. 158.7(C-2), 135.4(C-3), 179.4(C-4), 163.0 (C-5), 99.8(C-6), 165.7(C-7), 94.7(C-8), 158(C-9), 104.8(C-10), 122.1(C-1'), 116.0(C-2'), 144.5(C-3'), 148.8(C-4'), 115.7(C-5'), 121.6(C-6'), 105.3(C-1"), 75.4 (C-2"), 82.8(C-3"), 71.3(C-4"), 76.5(C-5"), 62.2(C-6"), 99.5(C-1'''), 70.2(C-2'''), 70.8(C-3'''), 72.4(C-4'''), 70.1(C-5'''), 17.9(C-6'''), MS(70 eV) m/z(%) 302(100), 284(4), 273(15), 227(10), 153 (20), 137(25), 69 (25). FABMS m/z 611 [M+H]$^+$, 303 [aglycone+H]$^+$, 154, 138.

Acid hydrolysis – Compound **5** (10 mg) in a mixture of 8% HCl (2 ml) and MeOH (20 ml) was refluxed at 100 ℃ for 2 h. The reaction mixture was reduced *in vacuo* to dryness, dissolved in H_2O (3 ml) and neuralized with NaOH. The neutralized product was 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 100 ℃ for 5 min. The sugar were identified after comparison with authentic samples.

Results and discussion

Compound **5** was obtained as a yellow powder. In the positive ion FABMS, it displayed a molecular ion peak at m/z 611 [M+H]$^+$, analyzing for $C_{27}H_{30}O_{16}$ formula and an aglycone peak at m/z 303 [quercetin+H]$^+$. Acid hydrolysis of the compound released galactose and rhamnose identified by TLC after comparison with authentic sugar samples. The UV spectrum and its changes after addition of common shift reagents (Mabry *et al*, 1970 and Markham, 1982) indicated that the compound is a quercetin derivative with free hydroxyl groups at C-5, C-7, C-3' and C-4'. This result was confirmed by ^{1}H NMR spectrum downfield peaks at δ 21.6, 10.7, 9.6 and 8.9 which upon addition of D_2O disappeared corresponding to 5-OH, 7-OH, 3'-OH



5 R=Rha(1 → 3)Gal

and 4'-OH, respectively and the characteristic 2H AX and 3H ABX system found in the aglycone pattern of quercetin. Furthermore in the ^{1}H NMR spectrum two anomeric protons at δ 5.5 and 4.8 were observed in each doublet signifying an inner galactose and a terminal rhamnose, respectively. The β-configuration of galactose (diaxial coupling) and the α-configuration of rhamnose (diequtiorial coupling) were evidenced by their respective coupling constants. The position of attachment of rhamnose to galactose was deduced from ^{13}C NMR data. The C-3" of galactose was shifited downfield at δ 82.8 while the anomeric carbon of rhamnose underwent an upfield shift at δ 99.5. The chemical shif values were in good agreement with the reported data for isorhamnetin 3-glucosyl (1 → 3) galactoside (Tomas-Lorente *et al*, 1992 and Mizuno *et al*, 1992), a fact which was corroborated by ^{1}H-^{13}C long range correlation spectral data whereby the key signal to prove interglycosidic linkage was that from galactose C-3". Thus, on the basis of spectroscopic data, 5 was concluded to be quercetin 3-rhamnosyl (1 → 3) galactosicde.

The known compounds **1-4** were identified by physical, chemical and spectroscopic methods as well as comparison of their spectral data with those in the literature (Bilia *et al*, 1993; Bader *et al*, 1993 and D'Agostino *et al*, 1992).

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