A New Acetylated Flavonoid Glycoside from *Myrsine africana* L

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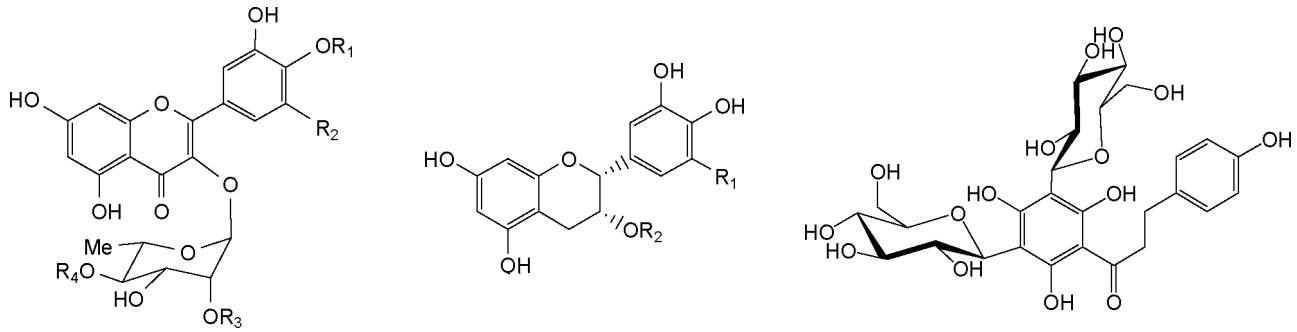
Myrsine africana L. is a small shrug plant of the Myrsinaceae family, and distributed in Africa, Europe and the Shanxi, Guangxi, Gansu, Xizang provinces of China. *Myrsine african* L. has been used traditionally for the treatment of diarrhea, rheumatism, toothache, pulmonary tuberculosis, and relieving hemorrhage.[1] Previous phytochemical investigations on *M. africana* L. have resulted in the isolation of flavonoids,[2,3,4] benzoquinones,[5,6] and triterpenoids.[7] Our chemical constituents study on the stems of *M. africana* L. led to the isolation of ten flavonoid compounds, including a new ones, mearnsetin 3-(2", 4"-diacetylrhamnoside) (**1**), along with nine known flavonoids **2**-**10** (Figure 1).

The dried and powdered stems of *M. africana* L. were extracted with 95% EtOH. After concentration under reduced pressure, the extract was suspended in H_2O and partitioned successively with petroleum ether, $CHCl_3$, EtOAc, and BuOH. The EtOAc-soluble fraction was separated by repeated chromatographic procedures to give a new acetylated flavonoid glycoside **1**, along with seven known flavonoids **2**, **3**, and **5**-**9**. Repeated column chromatography of the BuOH-soluble fraction resulted in the isolation of two known flavonoids **4** and **10**. Depending on chromatographic and spectroscopic analysis, **2**-**10** were identified as quercitrin (**2**),[8] myricitrin (**3**),[9] mearnsitrin (**4**),[9] myricetin-3-*O*-(4"-*O*-acetyl)-a-L-rhamnopyranoside (**5**),[10] mearnsetin-3-*O*-(4"-*O*-acetyl)-a-L-rhamnopyranoside (**6**),[9] (−)-epicatechin (**7**),[11] (−)-epigallocatechin (**8**),[12] (−)-epigallocatechin-3-*O*-gallate (**9**),[13] and 3',5'-di-*C*-*β*-glucopyranosyl phloretin (**10**).[14]

Compound **1**, obtained as yellow amorphous powder, had the molecular formula $C_{26}H_{26}O_{14}$ as deduced by the HR-ESI-MS (*m/z*: 585.1191, $[M + Na]^+$, calcd. 585.1220 for $C_{26}H_{26}O_{14}Na^-$). The IR bands exhibited the present of hydroxyl groups (3411 cm^{-1}), carbonyl group (1731 cm^{-1}), and aromatic rings (1612 and 1442 cm^{-1}). Acidic hydrolysis of **1** gave L-rhamnose as the sugar moiety. The ^{1}H-NMR spectrum (Table 1) of **1** showed signals for a mearnsetin unit,[15] an *a*-rhamnose fragment, and two acetyl groups (δ_H 2.09 and 2.04, each 3H, s). The former aglycone unit was characterized by a pair of *meta*-coupled protons at δ_H 6.45 and 6.39 (each 1 H, d, J = 2.1 Hz), two aromatic protons at δ_H 6.87 (2H, s), and a methoxyl (δ_H 3.88, 3H, s). The sugar moiety comprised an anomeric proton at δ_H 5.55 (1H, d, J = 1.8 Hz), one methyl (δ_H 0.79, 3H, d, J = 6.0 Hz), and four methines at δ_H 5.47 (1H, dd, J = 3.3, 1.8 Hz), 4.76 (1H, t, J = 9.9 Hz), 4.10 (1H, dd, J = 9.4, 3.6 Hz), and 3.28 (1H, dd, J = 9.9, 6.0 Hz). The ^{13}C-NMR and DEPT spectral (Table 1) displayed 26 carbon signals, including a flavone skeleton (14 olefinic and one conjugated carbonyl carbons), two acetyls (δ_C 172.9, 172.2, 21.4, and 21.3), a methoxyl (δ_C 56.9), and one rhamnose signals (99.4, 73.4, 68.9, 75.5, 69.9, 18.1). The above evidences suggested **1** to be diacetylated mearnsetin rhamnoside. The linkage position of the rhamnosyloxyl and the two acetyls were determined on



1 R_1=CH_3, R_2=OH, R_3=R_4=$COCH_3$
2 R_1=R_2=R_3=R_4=H
3 R_1=R_3=R_4=H, R_2=OH
4 R_1=CH_3, R_2=OH, R_3=R_4=H
5 R_1=R_3=H, R_2=OH, R_4=$COCH_3$
6 R_1=CH_3, R_2=OH, R_3=H, R_4=$COCH_3$

7 R_1=R_2=H
8 R_1=OH, R_2=H
9 R_1=OH, R_2=galloyl

10

Figure 1. Structures of flavonoids isolated from *M. africana* L.

Table 1. ^{1}H- and ^{13}C-NMR spectral data of **1** (400 and 100 MHz, *J* in Hz, CD_3OD)

position	δ_H	δ_C	position	δ_H	δ_C
2		160.8	1"	5.55 (d, 1.8)	99.4
3		137.1	2"	5.47 (dd, 3.3, 1.8)	73.4
4		175.6	3"	4.10 (dd, 9.4, 3.6)	68.9
5		163.1	4"	4.76 (t, 9.9)	75.5
6	6.39 (d, 2.1)	97.7	5"	3.28 (dd, 9.9, 6.0)	69.9
7		165.5	6"	0.79 (d, 6.0)	18.1
8	6.45 (d, 2.1)	96.6	2"- O<u>C</u>OCH$_3$		172.2
9		157.9	2"- OCO<u>CH$_3$</u>	2.09 (s)	21.3
10		109.1	4"- O<u>C</u>OCH$_3$		172.9
1'		122.6	4"- OCO<u>CH$_3$</u>	2.04 (s)	21.4
2'	6.87 (s)	109.7			
3'		147.5			
4'		138.0			
5'		147.5			
6'	6.87 (s)	109.7			
4'-OMe	3.88 (s)	56.9			

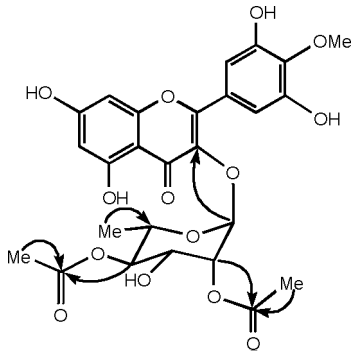


Figure 2. Key HMBC correlations of **1** (H→C).

C-3, C-2" and C-4" on the basis of HMBC cross-peaks of δ_H 5.55 (H-1")/δ_C 137.1 (C-3), δ_H 5.47 (H-2")/δ_C 172.2 (C-2"-O<u>C</u>OCH$_3$), and δ_H 4.76 (H-4")/δ_C 172.9 (C-4"-O<u>C</u>OCH$_3$) (Figure 2). Therefore, the structure of **1** was established to be mearnsetin 3-(2",4"-diacetylrhamnoside).

The antioxidant activity of **1** was evaluated by free radical scavenging activity against DPPH radical. The IC_{50} of **1**, defined as the necessary concentration at which the DPPH radical generated by the reaction systems was scavenged by 50%, was 14.5 μM.

The known compounds were identified by comparing their spectral data with reported in the literatures. Furthermore, compounds **4**-**10** were isolated from the genus of *Myrsine* for the first time.

Experimental

General Procedures. Optical rotation was measured with Perkin-Elmer-341 polarimeter. IR was obtained on a Nicolet-Magna-750-FTIR spectrometer, KBr pellets, in cm^{-1}. UV was determined on a Varian CARY 300 Bio spectrometer, λmax in nm (log ε). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) was run on a Bruker-DRX-400 spectrometer. ESI- and HR-ESI-MS were recorded on LCQ-Deca and Q-Tof Ultima mass spectrometers, respectively.

Plant Material. The stems of *M. africana* L. were collected in March 2005 from Dali of Yunnan Province, P. R. China, and was authenticated by Dr. Ji Huang of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (No. 20050308) was deposited at the Herbarium of Shanghai Institute of Materia Medica.

Extraction and Isolation. The dried and powdered stems of *M. africana* L. (6 kg) were extracted with 95% ethanol (50 L, 2 d × 3) by maceration. The solvent was evaporated under reduced pressure, and the residue (650 g) was suspended in H_2O and then partitioned successively with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. The EtOAC-soluble part (55 g) was subjected to column chromatography over silica gel (2 kg), eluting with the mixture of chloroform and methanol in an order of increasing polarity to give 7 fractions (Fr.1-Fr.7). Fr.4 was further chromatographed over silica gel column and ODS columns to afford compounds **1** (42 mg), **5** (190 mg), and **7** (10 mg). Fr.5 was separated over silica gel column eluting with chloroform-methanol (8:1 v/v) to give 5 subfractions (Frs.5.A -5.E). Fr.5.A was further purified over ODS-A gel and Sephadex LH-20 columns to afford compound **6** (58 mg). By using the same procedure, **2** (127 mg) and **8** (21 mg) were isolated from Fr.5.B and Fr.5.C, respectively. Re-chromatography of Fr.6 over silica gel coulmn with eluant of chloroform-methanol (8:1 v/v) yielded 5 subfractions (Frs.6.A-6.E). Fr.6.B and Fr.6.E, were further chromatographed over ODS-A gel and Sephadex LH-20 columns to give **9** (363 mg), and **3** (390 mg), respectively. The *n*-BuOH-soluble part (155 g) was subjected to column chromatography (macroporous resin (i.d. 10 × 80 cm), EtOH/H_2O (v/v) 0:100, 10:90, 30:70, 50:50, 70:30, 95:5); Frs.A-F. Fr.C (30% EtOH, 30 g) was separated by column chromatography (SiO_2, $CHCl_3$/MeOH 100:0 → 0:100) to give 8 fractions (Frs. C.1-C.8). Fr.C.5 was repeatedly chromatographed over silica gel, Sephadex LH-20, and ODS gel columns to give **4** (43 mg), **10** (38 mg).

Compound **1**: Yellow amorphous powders. $[\alpha]_D^{22}$ −189.7 (c = 0.310, MeOH); UV λ_{max} (MeOH): 221 (log ε 4.79), 268 (log ε 4.12), 332 (log ε 4.35). IR (KBr) ν_{max} 3411, 2941, 1731, 1612, 1442, 1371 cm^{-1}; ESI-MS *m/z*: 333 $[M+H-188]^-$, 563 $[M+H]^-$, 1147 $[2M+Na]^+$, 561 $[M-H]^-$, 1123 $[2M-H]^-$. HR-ESI-MS: *m/z* 585.1191 $[M + Na]^-$ (calcd. 585.1220 for $C_{26}H_{26}O_{14}Na^-$). ^1H- and ^{13}C-NMR data see Table 1.

Acid Hydrolysis of 1. Acid hydrolysis of **1** and sugar identification was conducted according to a standard procedure. In brief, **1** (*ca.* 2.0 mg) in 2 N HCl/dioxane (1:1 v/v; 2 mL) was refluxed for 2 h. On cooling, the mixture was neutralized with $NaHCO_3$. After extraction with AcOEt, the aq. layer was concentrated by blowing with N_2. The residue was purified by CC (Sephadex LH-20; MeOH/H_2O 1:1 v/v) to give the sugar mixture. The purified sugar and standard L-rhamnose (Sigma, USA) were treated with l-cysteine methyl ester hydrochloride (2 mg) in pyridine (1 mL) at 60 °C for 1 h. Then, the soln. was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.02 mL) at 60 °C for 1 h. The supernatant was applied to GLC analysis (Supelco; 230 °C, N_2). L-rhamnose (t_R 16.7 min) was detected from **1** by comparing its retention time with that of the authentic sample.

DPPH Radical Scavenging Assay. The DPPH radical scavenging assay was performed as reported previously with slight modification.[16] In brief, 20 μL of the new compound at final concentrations of 6.25 μM to 200 μM was added to 180 μL of a 150 μM MeOH solution of DPPH in a well of 96-well plate. The absorbance of reaction mixture was measured after 30 min of incubation at room temperature in dark using a microplate reader (TECAN Austria Gmbh, Austria). Controls containing methanol instead of the antioxidant solution and blanks containing methanol instead of DPPH solution were also made. The scavenging activity of the DPPH radical by the samples was calculated according to the following equation: DPPH scavenging activity (%) = (1 - (Abs. of sample-Abs. of blank)/Abs. of control) × 100. The percentage of scavenging activity was plotted against the sample concentration to obtain the IC_{50}.

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