Flavonoid Glycosides from *Melandrium firmum*

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Abstract□Linarin and schaftoside were isolated from *Melandrium firmum* (Caryophyllaceae) and characterized by spectral data.

Keywords□*Melandrium firmum,* Caryophyllaceae, flavone glycosides, linarin, schaftoside.

Melandrium firmum is a medicinal plant which has been used as a remedy for anurea, breast cancer, gonorrhea and disease of lactation in the various parts of East Asia.[1] It was previously reported that the butanol soluble fraction from methanol extract caused hepatotoxic activities including significant prolongation of hexobarbital-induced sleeping time, elevation of serum transaminase activities and severe histopathological changes in hepatic cells when administered intraperitoneally.[2]

In course of chemical study of the hepatotoxic constituents of this plant, two flavone glycosides were isolated.

Compound **1**, mp 248-50°, gave positive $FeCl_3$, Zn-HCl, Mg-HCl and Molisch tests, indicating to be flavonoid glycoside. The UV spectrum of compound **1** exhibited typical absorption maxima of flavone at 270 nm and 330 nm. The bathochromic shift of band I in the presence of $AlCl_3$ or $AlCl_3$/HCl indicated the presence of free 5-hydroxyl group. The ^{1}H-NMR spectrum of compound **1** showed a singlet at δ6.85(1H) assigned to the proton at C-3, two meta-coupled doublets (J = 2Hz) at δ6.44 (1H) and 6.78(1H), and two ortho-coupled doublets (J = 9Hz) at δ7.12(2H) and 8.02(2H), which indicated that the flavone moiety was oxygenated at C-5, C-7 and C-4′. In addition, a singlet at δ3.83(3H) indicated the presence of one methoxyl group.

The ^{1}H-NMR spectrum of its acetate showed six aliphatic and one aromatic acetyl signals in the region of δ1.09-2.04 and at δ2.42 indicating the presence of six alcoholic and one phenolic hydroxyl groups. Two anomeric proton signals at δ4.21 and 5.24 indicated that two moles of sugars were linked, one of which was assumed to be rhamnose by the diagnostic methyl signal at δ1.14(3H).

Acid hydrolysis of compound **1** yielded an aglycone, mp 246-8°, D-glucose and L-rhamnose as the sugars. The NaOAc spectrum of the aglycone showed bathochromic shift of band II unlike that of compound **1**, indicating the presence of free 7-OH group, therefore, attachment site of sugar moiety was 7-hydroxyl group.

Thus one methoxyl group must be located at C-4′, which was further supported by the presence of peaks at *m/z* 132 (RDA fragment with B ring) and *m/z* 117 (132-CH_3) in the mass spectrum of the aglycone. Therefore, the aglycone of compound **1** was identified as acacetin. The ^{13}C-NMR data of compound **1** supported that rutinosyl moiety was attached to 7-position of acacetin. From these evidences, compound **1** was identified as linarin and confirmed by direct comparison with an authentic sample (mmp, TLC, UV and NMR)[3].

Compound **2**, mp 224-6° gave positive $FeCl_3$, Mg-HCl tests and negative Molisch test. The UV spectrum of compound **2** showed typical absorption maxima of flavone at 274 nm and 335 nm. The bathochromic shift of band I or II in $AlCl_3$ spectrum and the shift still remaining with the addition of HCl indicated those of 5-hydroxy flavone. The ^{1}H-NMR spectrum of compound **2** showed two anomeric proton signals at δ4.81 and 4.70 indicating the presence of 2 moles of sugars in the compound. Compound **2** remained unchanged on acid hydrolysis, indicating to be C-glycoside. Two singlets at δ6.78 (1H) and 13.79 (1H), and two ortho-coupled doublets (J = 8.5Hz) at δ6.91 (2H) and 8.10 (2H) in the ^{1}H-NMR spectrum showed the presence of a proton at C-3, and hydroxyls at C-5 and C-4′, respectively. Proton signals for A ring were not found, suggested the full substitution of A-ring of flavone moiety. It was, thus, suggested that com-

pound **2** be 6,8-di-C-glycosyl 5,7,4′-trihydroxyflavone. The C^{13}-NMR spectrum of compound **2** confirmed this suggestion and gave information on the kind of C-linked sugars to be D-glucose and L-arabinose by the comparison with those of the reference compounds[4]. The attachment sites of sugars were deduced by mass data of permethyl ether of compound **2**. The peak for M^+-175 (loss of glucose) at m/z 529 was higher than that of M^+-131 (loss of arabinose) at m/z 573, indicating that D-glucose linked to C-6 and L-arabinose linked to C-8 of apigenin on the basis of the preferential fragmentation of C-6 sugar residue.[4] The configuration of D-glucose and L-arabinose was determined to be β- and α-linkages, respectively.

Therefore, compound **2** was characterized as schaftoside and confirmed by direct comparison with authentic sample (mmp, TLC, UV and MS).[5]

EXPERIMENTAL METHODS

Melting points were determined on a Mitamura-Riken apparatus and are uncorrected. The UV spectra were run on Gilford 2600 UV-Vis spectrophotometer and IR spectra were determined in KBr pellets on a Perkin-Elmer 283 B spectrophotometer. The NMR spectra were recorded on a Varian FT-80A spectrometer with TMS as internal standard and chemical shifts are given as ppm. Mass spectra were taken on a Hewlett-Packard Model 5985B GC/MS system. Optical rotations were measured on Rudolph Autopol® III automatic polarimeter.

Extraction and Isolation

The powdered whole plants of *Melandrium firmum* (4.75kg) were refluxed with MeOH. The MeOH extract (190g) was partitioned with Hexane: 10% aqu. MeOH (1:1) and the aqu. layer was extracted with $CHCl_3$, EtOAc and BuOH, successively. The BuOH soluble fraction was subjected to SiO_2 column chromatography ($CHCl_3$-MeOH, gradient) to yield linarin (**1**). One of subfractions was repeatedly chromatographed using SiO_2($CHCl_3$: MeOH: H_2O = 7:5:1) and Sephadex LH-20 (70% MeOH) columns to give schaftoside (**2**).

Lanarin (1)

White powder from 50% MeOH, mp 248-50°. $FeCl_3$, Mg-HCl, Zn-HCl and Molisch tests: positive; TLC (Silicagel; $CHCl_3$:MeOH:H_2O = 25:6:0.6): Rf 0.35; IR ν_{max}^{KBr} (cm^{-1}): 3400 (OH), 1660 (α,β-unsaturated ketone), 1605, 1490 (aromatic C=C), 1000-1100 (C-O-C); UV λ_{max}^{MeOH} nm (logε): 270(4.30), 330(4.40); with NaOMe: 294(4.43), 370(3.93); with NaOAc: 270(4.30), 330(4.40); with NaOAc + H_3BO_3: 270(4.30), 330(4.40); with $AlCl_3$: 276(4.26), 301(4.21), 347(4.42), 383(4.32); with $AlCl_3$ + HCl: 279(4.28), 301(4.27), 340(4.42), 383(4.22).

^{1}H-NMR (80 MHz, DMSO-d_6) δ: 1.09(3H, d, J = 6 Hz, CH_3 of rhamnose), 3.83(3H, s, -OMe), 4.53(1H, brs, anomeric H of rhamnose), 5.10(1H, d, J = 5.5 Hz, anomeric H of glucose), 6.44(1H, d, J = 2 Hz, H-6), 6.78(1H, d, J = 2 Hz, H-8), 6.85 (1H, s, H-3), 7.12(2H, d, J = 9 Hz, H-3′ & H-5′), 8.02(2H, d, J = 9 Hz, H-2′ & H-6′); ^{13}C-NMR (20 MHz, DMSO-d_6) δ: 181.8(C-4), 163.8(C-2), 162.9 (C-4′), 162.3(C-9), 161.0(C-5), 156.8(C-7), 128.3 (C-2 and 6′), 122.6(C-1′), 114.6(C-3′ and 5′), 105.4(C-10), 103.7(C-3), 100.4(C-1″), 100.0(C-1‴), 99.8(C-6), 94.8(C-8), 76.3(C-3″), 75.7(C-5″), 73.0(C-2″), 72.0(C-4‴), 70.7(C-3‴), 70.3(C-2‴), 69.6(C-4″), 68.2(C-5‴), 66.0(C-6″), 55.4(-OMe), 17.6(C-6‴).

Acetylation of 1

A sample (30 mg) was treated with Ac_2O and pyridine (1 m*l* each) for overnight and worked up as usual. The reaction product was crystallized from 50% MeOH to give colorless needles.

mp 115-7°; IR ν_{max}^{KBr} (cm^{-1}): 1760 (C=O), 1256 (C-O); ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.14 (3H, d, J = 6 Hz, CH_3 of rhamnose), 1.90-2.04 (18H, each all s, 6 × OAc), 2.42 (3, s, C_5-OAc), 3.88 (3H, s, -OMe), 4.71 (1H, brs, anomeric H of rhamnose), 5.24 (1H, d, J = 7 Hz, anomeric H of glucose), 6.52 (1H, s, H-3), 6.64 (1H, d, J = 2 Hz, H-6), 6.97 (1H, d, J = 2 Hz, H-8), 7.01 (2H, d, J = 9 Hz, H-3′ & H-5′), 7.81(2H, d, J = 9 Hz, H-2′ & H-6′).

Acid hydrolysis of 1

Twenty miligram of **1** was refluxed with 5% H_2SO_4 (20 m*l*) for 4 hrs. After cooling, the reaction mixture was filtered. The ppt was crystallized from MeOH to give pure aglycone as needles, mp 246-8°. IR ν_{max}^{KBr} (cm^{-1}): 3400 (OH), 1660 (α,β-unsaturated ketone), 1600-1650 (C=C), 1605, 1500 (aromatic C=C); UV λ_{max}^{MeOH} nm (logε): 271 (4.23), 330 (4.26); with NaOMe: 278 (4.44), 297 (sh. 4.27), 369 (4.14); with NaOAc: 278 (4.38), 298 (sh. 4.24), 352 (4.12); with NaOAc + H_3BO_3: 271 (4.23), 330 (4.25); with $AlCl_3$: 278 (4.21), 297 (sh. 4.18), 304 (4.22), 346 (4.27), 384 (4.16); with $AlCl_3$ + HCl: 280 (4.20), 297 (sh. 4.19), 304 (4.22), 340 (4.24), 382 (4.03); MS, m/z (re. int.): 284 (M^+, 100), 283 (M^+-H, 15.7), 269 (M^+-CH_3, 2.9), 256 (M^+-CO, 4.9), 241 (256-CH_3, 13.7), 152 (RDA fragment with A ring,

10.8), 134 (152-OH, 2.9), 132 (RDA fragment with B ring, 31.4), 124 (152-CO, 11.8), 117 (132-CH_3, 10.8); ^{1}H-NMR (80 MHz, DMSO-d_6) δ: 3.86 (3H, s, -OMe), 6.21 (1H, d, J = 2 Hz, H-6), 6.51 (1H, d, J = 2 Hz, H-8), 6.85 (1H, s, H-3), 7.11 (2H, d, J = 9 Hz, H-3′ & H-5′), 8.04 (2H, d, J = 9 Hz, H-2′ & H-6′), 12.9 (1H, s, C_5-OH). The filtrate was neutralized with $BaCO_3$, filtered and concentrated in vacuo. D-glucose and L-rhamnose were identified by TLC (precoated cellulose, pyridine:EtOAc:HOAc:H_2O = 36:36:7:21, Rf 0.24 for glucose and Rf 0.46 for rhamnose).

Schaftoside (2)

Yellowish powder from 80% MeOH, mp 224-26°. $FeCl_3$, Mg-HCl, Zn-HCl tests: postive, Molisch test: negative; TLC (Si gel; $CHCl_3$:MeOH:H_2O = 13:10:2): Rf 0.42; $[\alpha]_D^{24}$ = +65 (C = 0.02, MeOH); IR ν_{max}^{KBr} (cm^{-1}): 3350 (OH), 1650 (α,β-unsaturated ketone), 1580, 1510, 1470 (aromatic C = C), 830 (para-disubstituted benzene ring); UV λ_{max}^{MeOH} nm (log ε): 274 (4.28), 335 (4.28); with NaOH: 284 (4.30), 335 (4.12); with NaOAc: 283 (4.37), 358 (4.15); with NaOAc + H_3BO_3: 274 (4.26), 335(4.31); with $AlCl_3$: 280 (4.23), 306(4.15), 355 (4.29), 386 (4.21); with $AlCl_3$ + HCl: 279 (4.19), 304 (4.18), 346 (4.28), 381 (4.13); ^{1}H-NMR (80 MHz, DMSO-d_6) δ: 4.70 (1H, d, J = 5.65 Hz, anomeric H of arabinose), 4.81 (1H, d, J = 5.07 Hz, anomeric H of glucose), 6.78 (1H, s, H-3), 6.91 (2H, d, J = 8.5 Hz, H-3′ & H-5′), 8.10 (2H, d, J = 8.5 Hz, H-2′ & H-6′), 13.79 (1H, s, C_5-OH); ^{13}C-NMR (20 MHz, DMSO-d_6) δ: 182.4 (C-4), 164.2 (C-2), 161.4 (C-7), 161.3 (C-4′), 159.6 (C-5), 154.6 (C-9), 129.3 (C-2′, 6′), 121.5 (C-1′), 116.3 (C-3′, 5′), 108.7 (C-6), 104.5 (C-8), 104.1 (C-10), 102.6 (C-3), 81.5 (C-5″), 79.0 (C-3″), 75.3 (C-3‴), 74.8 (C-1‴), 73.8 (C-1″), 71.2 (C-2″), 71.0 (C-5‴), 70.4 (C-4″), 69.3 (C-4‴), 69.0 (C-2‴), 61.0 (C-6″).

Permethylation of 2

3 mg of sample was permethylated using Brimacombe's method[6] and followed by the usual work up.

MS, *m/z* (rel. Int.): 704 (M^+, 3.8), 689 (M^+-CH_3, 6.5), 673 (M^+-31, 100), 659 M-45, 2.2), 657 (M^+-47, 2.3), 641 (673-CH_3OH, 2.0), 601 (M-103, 5.9), 585 (M-119, 2.3), 573 (M^+-131, 3.5), 559 (M^+-145, 3.1), 541 (M^+-163, 23.6), 529 (M^+-175, 23.8), 515 (M^+-189, 8.7), 499 (M^+-205, 7.9).

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