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# MYRICETIN-3′-METHYL ETHER-7-GLUCOSIDE FROM *LIMONIUM SINUATUM*

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*Limonium sinuatum* (L.) Mill. (*Statice sinuata* L.) is a rough, hairy plant of the family Plumbaginaceae (1). In our previous communications, we reported the presence of a series of flavonoids in its flowers and leaves (2-4). The present paper documents the isolation and structure elucidation of two flavonol glycosides from the flowers of the title plant; a new glycoside, myricetin-3′-methyl ether-7-*O*-B-D-glucopyranoside, and isorhamnetin-7-*O*-B-D-glucopyranoside. Their uv data (band I, 378 and 384 nm, respectively) suggest that they are flavonol derivatives rather than flavones (5). Both compounds give dark green color reactions with alcoholic $FeCl_3$ and bright yellow colors with boric acid in $Ac_2O$ (6), characteristic of the strongly hydrogen-bonded orthohydroxyl carbonyl system. The latter is also supported by intense absorptions at 3390-3500 (OH) and 1655-1670 $cm^{-1}$ (γ-pyrone) in their ir spectra as well as bathochromic shifts in both bands I and II with $Zrocl_2$. The two compounds gave deep yellow fluorescence with $ZrOCl_2$ and citric acid (7), proving the presence of free OH at position C-3, which is further confirmed by a bathochromic shift in band I with $AlCl_3$ (+55 and 60 nm, respectively). Each also gave a blue color test on the addition of bicarbonate to their respective Shinod's reduction products (8), indicating the presence of free OH at position C-4′. This is supported by a bathochromic shift in band I with NaOMe.

Both compounds were completely hydrolyzed with emulsin, indicating a β-linkage between the aglycone and the sugar moiety. This is supported by intense absorption bands at 880 $cm^{-1}$ in the ir spectra. The sugar moiety in both was identified by cochromatography with an authentic sample of glucose. The presence of three absorptions at 1080, 1060, and 1035 $cm^{-1}$ in their ir spectra suggests the pyranose structure of glucose. Both liberated aglycones gave a positive color test with vanillin hydrochloric reagent, indicating the presence of free OH groups at positions C-5 and C-7. The two glycosides neither responded to this test nor gave any shift in band II with NaOAc, whereas the two aglycones gave a bathochromic shift (+10 and 8 nm, respectively) in band II, suggesting the possibility of the sugar moiety at positions C-7 in the two glycosides.

Hence, it is considered that the two isolated compounds are flavonol glycosides containing free OH groups at positions C-3, C-5, and C-4′ with glucose in the pyranose form β-linked at position C-7.

The aglycone obtained by acid hydrolysis of the first compound was identified as isorhamnetin by its color reactions, mp, spectral analysis, acetate formation, degradative studies, and cochromatography with an authentic sample. Therefore, the structure of the first compound is isorhamnetin-7-O-β-D-glucopyranoside. The pmr data (5) of that isolated compound confirms the suggested structure. A literature survey showed that isorhamnetin-7-O-glucoside was recently isolated from *Clibadium sessile* (Compositae) (10); however, our report is the first record on the presence of isorhamnetin-7-O-glucoside in the family Plumbaginaceae.

The liberated aglycone from the second compound had mp 336-340° and $M^+$ at *m/z* 332. Color reactions and uv spectral data with different diagnostic

reagents (5) proved the presence of five free OH groups at positions: 3,5,7,4′, and 5′. The aglycone did not develop any green color with alkali, indicating the absence of a vicinal trihydroxy system in the B-ring (11). Pmr data showed: a three proton signal at δ 3.7 ($OCH_3$) and four doublets (1 H each) with coupling constant 2.5 hz and centered at δ 6.14 (H-6), 6.28 (H-8), 7.46 (H-2′), and 7.6 (H-6′). Thus, the structure of the aglycone is probably: 3,5,7,4′,5′-pentahydroxy-3′-methoxy flavone (myricetin-3′-methyl ether). Ms fragmentation of the aglycone (12) is in good agreement with this suggestion. Demethylation of the aglycone to yield myricetin further supports the suggested structure. Therefore, the structure of the second compound is myricetin-3′-methyl ether-7-O-β-D-glucopyranoside. Pmr and cmr data of the isolated glycoside (13,14) support the suggested structure.

Myricetin-3′-methyl ether has a very limited natural distribution. Prior to its present discovery in *Limonium sinuatum*, it had been reported in *Larix sibrica* and *Limnanthes douglasii* (15). The presence of methoxylated myricetin glycosides in the family Plumbaginaceae has been previously reported (12,15): myricetin-5-methyl ether-3-galactoside (leaf, *Ceratostigma plumbaginoides* Bunge) and myricetin-7-methyl ether-3-rhamnoside (leaf, *Plumbago europea* L.). Thus, the occurrence of myricetin-3′-methyl ether-7-*O*-β-D-glucopyranoside in the flowers of *Limonium sinuatum* is not surprising. This is the first definitive report of this compound as a natural product.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Unicam SP 1750, ir spectra as KBr pellets on a Perkin-Elmer 720, mass spectra at 70 eV on an AEI MS 902, and pmr spectra at 90 MHz in DMSO-$d_6$ on a Perkin-Elmer R 32. Silica gel Merck was used for column chromatography, cellulose Merck for tlc, and Whatman No. 1 for pc (ascending). System I, $CHCl_3$-MeOH (5:1 v/v) was used for tlc; System II, *n*-BuOH-HOAc-$H_2O$ (4:1:2 v/v) for pc. Melting points were determined with a Kofler hot stage apparatus and are uncorrected. The flowers were collected in April 1980, from plants grown in the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Assiut, Egypt. The plant was identified by the late Prof. Dr. F.Y. Amin, Professor of Floriculture, Faculty of Floriculture, Faculty of Agriculture, Assiut University

EXTRACTION AND ISOLATION.—The defatted flowers (0.5 kg) were extracted with MeOH and the extract was concentrated.

Using the methanolic extract, a silica gel column (700 g), eluted with $CHCl_3$-MeOH mixtures in increasing polarities, resolved the two compounds.

HYDROLYSIS.—Acid hydrolysis was done by refluxing each glycoside with 0.5 N $H_2SO_4$ for 2 h. Aglycones were extracted with $CHCl_3$. The sugar moiety in the hydrolysate was examined on pc in system II.

Each glycoside (5 mg) was separately hydrolyzed with emulsin (5) at 30-40° for 48 h. Liberation of glucose was revealed by cochromatography with an authentic sample.

ISORHAMNETIN-7-*O*-β-D-GLUCOSIDE.—Mp 215-217°; tlc, system I, Rf=0.5; uv (EtOH) 255 sh, 273, 295 sh, 378 nm; +NaOMe 288, 327, 434 nm; +NaOAc 274, 330, 390 nm; +NaOAc/$H_3BO_3$ 255, 272, 290, 378 nm; +$AlCl_3$ 262 sh, 272, 358, 433 nm; +$AlCl_3$/HCl 262 sh, 273, 358, 433 nm; +$ZrOCl_2$ 282, 355 sh, 435 nm; ir 3420-3500, 1700, 1655, 1615, 1560, 1385, 1250, 1160, 1120, 1080, 1060, 1035, 880, and 810 $cm^{-1}$. Pmr δ 6.17 (1H, d, $J$=2.5 Hz, H-6), 6.28 (1H, d, $J$=2.5 Hz, H-8), 7.00 (1H, d, $J$=8.5 Hz, H-5), 7.70 (1H, d, $J$=2.5 Hz, H-2′), 7.93 (1H, dd, $J$=2.5 and 8.5 Hz, H-6′), 5.00 (1H, d, $J$= Hz, glucosyl H-1″), 3.35-3.6 (6H, m, 6H of glucose), and 3.7 (3H, S, $OCH_3$). Acid hydrolysis yielded isorhamnetin and glucose).

Isorhamnetin obtained on acid hydrolysis had: mp 305-307°; pc, system II, Rf=0.88; uv (EtOH) 264, 320, 370 nm; demethylation with HI, gave quercetin; tetraacetate, mp 208-210°.

MYRICETIN-3′-METHYL ETHER-7-*O*-β-D-GLUCOSIDE.—Mp 232-235°; tlc, system I, Rf=0.3; uv (EtOH) 256, 269, 293 sh, 384 nm; +NaOMe 328, 440 sh, dec.; +NaOAc 256, 271, 295 sh, 387 nm; +NaOAc/$H_3BO_3$ 262, 290 sh, 395 nm; +$AlCl_3$ 272, 446 nm; +$AlCl_3$/HCl 264, 370, 438 nm; +$ZrOCl_2$ 274, 390 sh, 438 nm; ir 3390-3500, 3020, 1705, 1670, 1615, 1555, 1390, 1255, 1160, 1125, 1080, 1060, 1035, and 880 $cm^{-1}$; pmr 6.17 (1H, d, $J$=2.5 Hz, H-6), 6.26 (1H, d, $J$=2.5 Hz, H-8), 7.50 (1H, d, $J$=2.5 Hz, H-2′), 7.62 (1H, d, $J$=2.5 Hz, H-6′), 4.94 (1H, d, $J$=7 Hz, glucosyl H-1″), 3.3-3.5 (6H, m, 6H of glucose),

and 3.71 (3H, s, $OCH_3$), cmr (DMSO-$d_6$) δ 175.75, s (C4); 165.49, s (C5); 163.88, s (C7); 160.66, s (C9); 156.00, s (C4′); 147.34, s (C2); 145.79, s (C3′); 136.11, s (C3); 121.18, s (C1′); 119.32, d [C6′); 110.52, d (C2′); 107.00, s (C5′); 102.98, s (C10); 102.25, (C1″); 98.14, d (C6); 93.40, d (C8); 75.86 (C5″); 74.29, (C4″); 73.29 (C3″); 70.25 (C2″); 63.71 (C6″); 55.89, q (OMe).

Acid hydrolysis yielded the aglycone, myricetin-3′-methyl ether, and glucose.

Myricetin-3′-methyl ether obtained on acid hydrolysis had mp 336-340°; pc, system II, Rf=0.8; uv (EtOH) 255, 268, 292 sh, 382 nm; +NaOMe 320 dec.; +NaOAc 258, 276, 295 sh dec.; +NaOAc/$H_3BO_3$ 260, 290 sh, 395 nm; +$AlCl_3$ 275, 450 nm. Myricetin-3-methyl ether-pentaacetate, mp 198-200°; pmr δ 3.70 (1H, S), 6.14 (1H, d), 6.28 (1H, d), 7.46 (1H, d), and 7.60 (1H, d); ms *m/z* 332 ($M^+$, 9), 318 ($M^+$-$CH_3$ with H· transfer, 99), 317 ($M^+$-$CH_3$, 13), 316 ($M^+$-0,9), 301 ($M^+$-OMe, 5), 289 ($M^+$-$CH_3CO$, 7), 167 ($B_2^+$, 3), 153 ($A_1^+$, 26), 136 ($B_2^+$-OMe, 16), 137 ($A_1^+$-0.7), 107 ($A_1^+$-0, -C 0,7), demethylation with HI, gave myricetin.

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