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FLAVONOIDS OF *ERICA VERTICILLATA*

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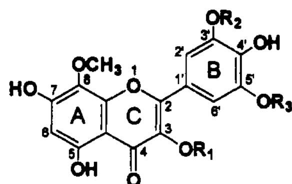
ABSTRACT.—Three new flavonol glycosides were isolated from the aerial parts of *Erica verticillata*, along with two known flavonoids. The structures of the new compounds were established as 3,5,7,3′,4′,5′-hexahydroxy-8-methoxyflavone-3-*O*-α-L-rhamnopyranoside [**1**], 3,5,7,3′,4′-pentahydroxy-8,5′-dimethoxyflavone-3-*O*-α-L-rhamnopyranoside [**2**], and 3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxyflavone-3-*O*-α-L-rhamnopyranoside [**3**], by means of spectroscopic and chemical techniques. The known compounds were identified as quercetin and myricetin.

The genus *Erica* (Ericaceae) consists of 500 species of shrubs and perennial herbs, all of which are found in dry places in South Africa, and some also in southern and western Europe. Among these, three *Erica* species are endemic and restricted to Greece (1,2). A survey of the literature has indicated that *E. verticillata* Forsk. (*E. manipuliflora* Salisb.), growing in Greece, has not been investigated phytochemically for its flavonoid content. This paper reports the isolation and characterization of three new flavonol glycosides, together with the flavonoid aglycones quercetin and myricetin, from the aerial parts of *E. verticillata*.

Repeated cc of the EtOAc-soluble portion of the MeOH extract resulted in the isolation of five compounds. Compounds **1–3** were obtained as pale yellow amorphous powders and their identification was based on uv, ms, and ^{1}H-nmr spectral data. The uv spectra of **1–3** in MeOH (Table 1) were characterized by



1 R_1=α-L-Rha, R_2=R_3=H
1a R_1=R_2=R_3=H
2 R_1=α-L-Rha, R_2=H, R_3=CH_3
2a R_1=R_2=H, R_3=CH_3
3 R_1=α-L-Rha, R_2=R_3=CH_3
3a R_1=H, R_2=R_3=CH_3

major bands that resembled those of 3-*O*-substituted flavonols (3–5). Compounds **1–3** all possess free hydroxyl groups at C-5 and C-7 of the A ring, indicated by the bathochromic uv shifts induced by NaOMe (new band in the region 320–330 nm), NaOAc, and $AlCl_3$/$AlCl_3$+HCl (Table 1). Moreover, the ^{1}H-nmr spectra of **1–3** (Table 2) exhibited signals suggesting that they are rhamnosides. The latter suggestion was confirmed by acid hydrolysis producing the respective aglycones (**1a**, **2a**, **3a**) and L-rhamnose.

In the uv spectrum of the less polar compound [**3**], the bathochromic shift induced by NaOMe (+65 nm without decrease in intensity relative to band I in MeOH) indicated the presence of a free OH-4′ group. The uv absorption spectrum, on addition of $AlCl_3$+HCl and NaOAc+H_3BO_3, suggested the absence of either an A- or B-ring ortho-dihydroxy system. The absence of a free OH-3 was concluded from the uv spectrum in NaOMe (no decomposition in relation to the presence of the free OH-4′ and no decreased intensity of band I). The ^{1}H-nmr spectrum of **3** showed a two-proton singlet at δ 7.17 ppm for equivalent protons at C-2′ and C-6′. A singlet at δ 6.14 ppm was assigned to an A-ring aromatic proton. The presence of three methoxyl groups was deduced by a six-proton singlet at δ 3.86 ppm and a three-proton singlet at δ 3.81 ppm. Two methoxyl groups must be located on the B ring, forming with the free OH-4′ a

TABLE 1. Uv Data for Flavonoid Glycosides **1–3** and Their Aglycones **1a–3a**.[a]

Solvent	Compound					
	1	**1a**	**2**	**2a**	**3**	**3a**
MeOH	256 sh, 270, 360	257, 306, 379	256 sh, 272, 358	256, 270 sh, 333, 379	256 sh, 273 355	254, 272, 337, 377
MeONa	274, 324, 410 dec	269 sh, 289 sh, 323 dec	279, 329, 420	257, 326 dec	280, 327, 420	254 sh, 333, 445 dec
AcONa	280, 328, 403	270, 339 dec	281, 329, 405	256, 341 dec	281, 329, 419	341, 440 dec
AcONa+H_3BO_3	266, 379	262, 399	265, 377	262, 399	273, 355	255, 273, 338, 379
$AlCl_3$	277, 312, 366, 437	273, 466	279, 311, 366, 445	274, 470	281, 312, 363, 417 sh	267, 315, 369, 439
$AlCl_3$+HCl	279, 310, 362, 418	269, 307 sh, 371, 441	281, 310, 359, 408	268, 306 sh, 369, 441	281, 311, 358, 408 sh	268, 315, 365, 440

[a]Expressed as λ max (nm).

symmetrically substituted B ring. The eims of **3** exhibited an aglycone ion $[A]^+$ at *m/z* 376, indicating a flavonoid skeleton with four hydroxyl and three methoxyl groups (6). Fragment ions at *m/z* 139 and *m/z* 181, attributable to the retro-Diels-Alder rearrangement fragments $[A_1-43]^+$ and $[B_2]^+$, respectively, showed the presence of one methoxyl and two hydroxyl groups in the A ring, and one hydroxyl and two methoxyl groups in the B ring as well.

All these findings permitted the functional group assignments of the B ring as 3′,5′-dimethoxy-4′-hydroxy and of the A ring as 5,7-dihydroxy-6- or 8-methoxy. The remaining question of the location of the methoxyl group in the A ring was answered by analysis of the ms, uv, and ^{1}H-nmr data: the fragmentation ion $[A-Me]^+$ at *m/z* 361 (100) and the absence of any $[A-H_2O]^+$ ion in the eims of **3** are in agreement with substitution at C-8 (4,7). The 5,7,8-trioxygenation pattern was unambiguously assigned to **3** according to the high-field ^{1}H-nmr signal of the isolated proton at δ 6.14 ppm, identified as H-6 (4,8–10); H-8 in a 5,7-dihydroxy-6-OMe A-ring system should resonate at lower field in DMSO-d_6 (ca. δ 6.60–6.70) (11–13). Furthermore, band I appeared in the $AlCl_3$ uv spectrum of **3** at 417 nm and in the $AlCl_3$+HCl spectrum of **3** at 408 nm (+62 and +53 nm, respectively, relative to band I in MeOH). These large shifts are in accord with an 8-methoxyl rather than a 6-methoxyl group (14).

The only position that remained for the linkage of the rhamnose moiety was C-3 of the aglycone. This was confirmed by the chemical shift of the single anomeric proton (H-1″) at δ 5.24 ppm of the ^{1}H-nmr spectrum of **3**. In the same spectrum the diequatorial coupling (J=2 Hz) observed between H-1″ and H-2″ indicated the α-configuration (3,4). Confirmation of this linkage was supported by acid hydrolysis of **3**, producing aglycone **3a** and L-rhamnose. The uv spectral data of **3a** (Table 1) clearly demonstrated the presence of a free OH-3; decomposition of the NaOMe and NaOAc uv spectra occurred due to the 3,4′-dihydroxy-3′-methoxy system (3–5). From the data described above, the structure of **3** was concluded to be 3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxyflavone-3-*O*-α-L-rhamnopyranoside.

The uv, ^{1}H-nmr, and ms spectral data of **2** were comparable with those of **3**, suggesting the same substitution pattern for the A ring and the absence of a methoxyl group in the B ring. Thus, the appearance of a second peak for band II at 272 nm in the uv spectrum of **2** in MeOH indicated a 3′,4′-ortho-dihydroxy system (3–5). The bathochromic uv shifts observed on addition of NaOAc+H_3BO_3 and $AlCl_3$/$AlCl_3$+HCl (+19 and +87/+50 nm, respectively, in relation to the band I in MeOH) offered further support

for a B-ring ortho-dihydroxy system. This was confirmed by means of the ^{1}H-nmr spectrum of **2** which showed two one-proton doublets ($J=2$ Hz) at δ 7.05 ppm and 7.08 ppm corresponding to H-2′ and H-6′, respectively, and indicating an unsymmetrically substituted B ring. In the same spectrum two methoxyl groups were identified as the two three-proton singlets at δ 3.81 ppm and 3.83 ppm. Thus, the B-ring substitution pattern must be assigned as 3′,4′-dihydroxy-5′-methoxy. The eims of **2** exhibited an aglycone ion peak $[A]^+$ at *m/z* 362, suggesting a flavonoid with five hydroxyl and two methoxyl groups (6). The fragmentation pattern in the eims of **2** (see Experimental) showed the location of one methoxyl and two hydroxyl groups in the B ring, with the A ring being identical to that of **3**. The location of the methoxyl group in the A ring at C-8 and the linkage of the rhamnose at C-3 followed arguments similar to those used for **3**. Therefore, **2** is 3,5,7,3′,4′-pentahydroxy-8,5′-dimethoxyflavone-3-*O*-α-L-rhamnopyranoside.

The uv spectrum of the most polar compound [**1**] in MeOH (double peak for band II at 270 and 256 nm) and after addition of NaOAc+H_3BO_3 (band I at +19 nm relative to band I in MeOH) indicated an ortho-dihydroxy system in the B ring. Moreover, **1** showed a hypsochromic shift of only 19 nm in band I in the presence of $AlCl_3$+HCl relative to band I in the $AlCl_3$ spectrum, favoring three adjacent hydroxyls, as found in myricetin-type B rings (3–5). As also described for **3**, this symmetrical substitution of the B ring was confirmed by the ^{1}H-nmr spectrum of **1**, which exhibited a two-proton singlet at δ 7.00 ppm, characteristic of 2′,6′- equivalent protons. A three-proton singlet at δ 3.87 ppm corresponded to a methoxyl group. The eims of **1** did not show any molecular or aglycone ion due to rapid decomposition of the molecule. However, certain fragment ions produced via the retro-Diels-Alder rearrangement (see Experimental) indicated the presence of three hydroxyl groups in the B ring and one methoxyl and two hydroxyl groups in the A ring. The positive fdms and dcims/ NH_3 of **1** suggested a rhamnoside of a flavonoid aglycone with one methoxyl and six hydroxyl groups: fragment ions $[M+Na]^+$ at *m/z* 517, $[M+H]^+$ at *m/z* 495, $[A+Na]^+$ at *m/z* 371, and $[A+H]^+$ at *m/z* 349 in the positive fdms and $[M+H]^+$ at *m/z* 495, $[A+NH_4]^+$ at *m/z* 366, and $[A+H]^+$ at *m/z* 349 in the dcims, respectively. The A-ring substitution pattern and the rhamnose linkage of **1** were identical to those of **2** and **3**, as concluded in a manner similar to that adopted for **2** and **3**. The structure of **1** was thus assigned as 3,5,7,3′,4′,5′-hexahydroxy-8-methoxyflavone-3-*O*-α-L-rhamnopyranoside.

The structures of the two known compounds quercetin and myricetin were determined by comparing chemical and spectroscopic (uv, ^{1}H-nmr) data with data found in the literature (3–5) and on the basis of comparison with authentic samples.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were obtained on a Hitachi U-2000 spectrophotometer. ^{1}H-Nmr spectra were recorded on a Bruker spectrometer operating at 200 MHz. Chemical shifts are given in ppm (δ) relative to TMS. Eims spectra were determined on a VJ TS-250 instrument at 70 eV. Positive-fdms and dcims spectra were recorded on a MAT-95 instrument. Polyamide (SC-6-MN) and Sephadex LH-20 (Pharmacia) were used for cc. Tlc analysis was performed on cellulose precoated aluminum sheets, Art. 5552, Merck (0.1-mm thickness) with $CHCl_3$-AcOH-H_2O (10:9:1) as solvent. The spots were visualized by uv light (254 and 366 nm) with exposure to NH_3 and by spraying with NA (Naturstoffreagenz-A) in MeOH.

PLANT MATERIAL.—*Erica verticillata* was collected in June 1987 from the area of Livadia, Greece. A voucher specimen is deposited at the Laboratory of Botany, Department of Biology, School of Sciences, Aristotle University of Thessaloniki.

EXTRACTION AND ISOLATION.—Dried leaves

and flowers (2.5 kg) of *E. verticillata* were successively extracted with petroleum ether, $CHCl_3$, and MeOH. The MeOH extract (573 g) was redissolved in boiling H_2O and the H_2O-soluble portion was then sequentially extracted with $CHCl_3$, EtOAc, and *n*-BuOH. A portion (4.3 g) of the EtOAc extract (5.4 g) was subjected to chromatographic fractionation using a polyamide column and elution with H_2O/MeOH (step gradient). Combined fractions (650 mg) eluted with H_2O-MeOH (60:40) were further chromatographed on a Sephadex LH-20 column using MeOH as eluent. Fractions of 10 ml were collected and combined on the basis of tlc. Fractions 20–24 (40 mg) were repeatedly chromatographed on a Sephadex LH-20 column eluted with MeOH-H_2O (80:20) to afford pure compounds **1** (15 mg), **2** (10 mg), and **3** (7 mg). A fraction (190 mg) obtained from the polyamide column by elution with H_2O-MeOH (50:50) yielded a mixture of two flavonoid aglycones. The two compounds, quercetin (22 mg) and myricetin (13 mg), were separated after repeated cc using a Sephadex LH-20 column eluted with MeOH-H_2O (80:20).

3,5,7,3',4',5'-Hexahydroxy-8-methoxyflavone-3-O-α-L-rhamnopyranoside [**1**].—Uv spectral data, see Table 1; ^{1}H-nmr data, see Table 2; eims *m/z* $[A_1+H]^+$ 183 (5), $[A_1-Me]^+$ 167 (6), $[A_1-CO]^+$ 154 (5), $[B_2]^+ + [A_1-HCO]^+$ 153 (13.5), $[B_1+H]^+$ 151 (15.5), $[A_1-COMe]^+$ 139 (16), $[B_2-Me]^+$ 138 (19), $[B_1-Me]^+$ 135 (23), $[B_2-CO]^+$ 125 (30), $[A_1-2CO-Me]^+$ 111 (48); positive fdms *m/z* $[M+Na]^+$ 517 (100), $[M+H]^+$ 495 (10), $[M]^+$ 494 (4), $[A+Na]^+$ 371 (48), $[A+H]^+$ 349 (35), $[A]^+$ 348 (55), $[A-Me]^+$ 333 (2), $[A_1]^+$ 182 (2); dcims *m/z* $[M+H]^+$ 495 (12), $[A+NH_4]^+$ 366 (14), $[A+H]^+$ 349 (100).

3,5,7,3',4'-Pentahydroxy-8,5'-dimethoxyflavone-3-O-α-L-rhamnopyranoside [**2**].—Uv spectral data, see Table 1; ^{1}H-nmr data, see Table 2; eims *m/z* $[A]^+$ 362 (62), $[A-Me]^+$ 347 (100), $[A-HCO]^+$ 333 (4), $[A-COMe]^+$ 319 (3), $[A_1-Me]^+ + [B_2]^+$ 167 (3), $[B_1+H]^+$ 165 (3), $[B_2-Me]^+$ 152 (2), $[A_1-COMe]^+ + [B_2-CO]^+$ 139 (7), $[B_1-HCO]^+$ 135 (3), $[B_1-COMe]^+$ 121 (3).

3,5,7,4'-Tetrahydroxy-8,3',5'-trimethoxyflavone-3-O-α-L-rhamnopyranoside [**3**].—Uv spectral data, see Table 1; ^{1}H-nmr data, see Table 2; eims *m/z* $[A]^+$ 376 (77), $[A-Me]^+$ 361 (100), $[B_2]^+$ 181 (8), $[B_1+H]^+$ 179 (5), $[A_1-Me]^+$ 167 (9), $[B_1-Me]^+$ 163 (4), $[A_1-HCO]^+ + [B_2-CO]^+$ 153 (8), $[B_1-HCO]^+$ 149 (8), $[A_1-COMe]^+$ 139 (17), $[B_1-COMe]^+$ 135 (6), $[A_1-2CO-Me]^+$ 111 (14).

HYDROLYSIS OF **1–3**.—Acid hydrolysis of flavonoid glycosides **1–3** was carried out with a 1% solution of HCl under reflux for 1 h, yielding, in turn, flavonoid aglycones **1a**, **2a**, **3a** (uv spectra, see Table 1), and rhamnose. The aglycone **1a** was unstable in solution and on storage; all operations were carried out as quickly as possible. The sugar was identified by chromatographic comparison with authentic markers, using the following tlc system: precoated Si gel 60 F_{254} plates (0.2 mm thickness, Art. 5554, Merck) with a 0.3 M solution of NaH_2PO_4 with Me_2CO-H_2O (9:1) as mobile phase (double development). The spots were visualized by spraying with 4-aminohippuric acid reagent after heating at 110° for 10 min and reheating under the same conditions.

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TABLE 2. ^{1}H-Nmr Data for Flavonoid Glycosides **1–3**.[a]

Proton	Compound		
	1	**2**	**3**
H-6	6.34, s	6.28, s	6.14, s
H-2'	7.00, s	7.05, d (2)	7.17, s
H-6'	7.00, s	7.08, d (2)	7.17, s
H-1"	5.22, d (2)	5.23, d (2)	5.24, d (2)
Me-6"	0.90, d (6), 3H	0.82, d (6), 3H	0.80, d (6), 3H
OMe	3.87, s, 3H	3.81, s, 3H 3.83, s, 3H	3.81, s, 3H 3.86, s, 6H

[a]Spectra recorded in DMSO-d_6 at 200 MHz; chemical shifts (δ) in ppm, coupling constants (*J* values) in parentheses in Hz.

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