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FLAVONOL GLYCOSIDES FROM *ERICA CINEREA*[1]

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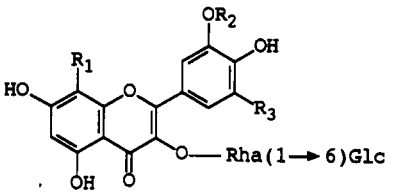
ABSTRACT.—In addition to quercetin 3-rutinoside [**1**], isorhamnetin 3-rutinoside [**2**], and myricetin 3-rutinoside [**3**], a new flavonol glycoside was isolated from fresh heather flowers of *Erica cinerea*. Its structure was established as gossypetin 3-[α-L-rhamnopyranosyl (1→6) β-D-glucopyranoside] [**4**] by spectroscopic analysis.

Our previous studies on the Me_2CO extract of *Erica cinerea* L. fresh heather flowers have led to the isolation of polyphenolics such as flavone, flavonol, and dihydroflavonol monoglycosides (2,3). The *n*-BuOH-soluble part of this extract yielded large amounts of rutin along with other minor flavonol glycosides. This paper reports the isolation and the structural elucidation of two rare isorhamnetin- and myricetin 3-rutinosides [**2, 3**], as well as the new gossypetin 3-rutinoside [**4**].

On the basis of spectroscopic data, compounds **1–3** were identified as quercetin 3-rutinoside [**1**], isorhamnetin 3-rutinoside [**2**] and myricetin 3-rutinoside [**3**] (4,5). These glycosides have not been reported previously from fresh *E. cinerea* heather flowers.

Compound **4** exhibited a similar chromatographic behavior to that of myricetin 3-rutinoside [**3**] on Si gel tlc, but a slightly more polar mobility on cellulose. The difference was more evident by hplc. The ^{1}H-nmr spectrum displayed only four aromatic protons in addition to the glycosidic protons belonging to rutinose. Its uv spectra ran in the common shift reagents indicated a flavonol 3-conjugated structure as for **1–3** (5,6). In the ^{1}H-nmr spectrum, the presence of



1 $R_1=R_2=R_3=H$
2 $R_1=R_3=H$; $R_2=Me$
3 $R_1=R_2=H$; $R_3=OH$
4 $R_1=OH$; $R_2=R_3=H$

only one proton at δ 6.26, in the region 6.0–6.5 ppm, indicated that the A-ring was either 5,6,7- or 5,7,8-trihydroxylated, the 5-OH group being at δ 12.02. Furthermore, the B-ring was 3′,4′-dihydroxylated as suggested by the signals for H-2′, H-5′ and H-6′ occurring at δ 7.61 (br s), δ 6.84 (d, J=8.2 Hz) and δ 7.63 (dd, J=8.2 and 2.1 Hz) respectively. The ^{13}C-nmr data confirmed the ^{1}H-nmr analysis for the aromatic region and identified the aglycone part in this molecule as gossypetin. This result was deduced from both the C-6 signal (δ 98.6) being similar to those of compounds **1–3**, and the C-8 quaternary peak (δ 128.4) (7). The B-ring was, as expected, 3′,4′-dihydroxylated according to the two upfield quaternary signals at δ 144.7 (C-3′) and δ 148.4 (C-4′) (7,8). As in the case of rutinosides **1–3**, only four protons (H-1″, H-3″, H-6″$_A$ and H-1‴) along with the methyl group of the rhamnose residue, could be identified in the ^{1}H-nmr spectrum (DMSO-d_6). The

[1]Part 6 in the series "Phytochemistry of the Ericaceae." For Part 5, see Simon *et al.* (1).

^{13}C-nmr spectrum showed that **4** had an inner β-D-glucopyranose and a terminal rhamnose. The diagnostic downfield shift of 5.9 ppm for the C-6 of glucose pointed to the interglycosidic linkage at this position (7,8). Finally, the significant differences of +9.8 ppm for C-2, +1.5 ppm for C-4, and −2.3 ppm for C-3 indicated that glycosylation took place at the 3-position (4, 7–9). Thus, compound **4** was deduced as gossypetin 3-[α-L-rhamnopyranosyl (1→6) β-D-glucopyranoside].

Among the flavonol glycosides isolated from *E. cinerea*, rutinose appears to be a common disaccharide unit. While quercetin 3-rutinoside is a widespread flavonoid in the plant kingdom, isorhamnetin 3-rutinoside has been reported in two families only: the Liliaceae, in *Narcissus tazetta* (10) and *Lilium auratum* (11), and the Balanitaceae, in *Balanites aegyptiana* (12). The characterization of both myricetin- and gossypetin-3-rutinoside is of special note, because the former can be regarded as a rare secondary metabolite that has only been reported from *Solanum soukupii* (Solanaceae) (13,14), and the latter is a new natural product.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Tlc was carried out on microcrystalline cellulose plastic sheets (Merck) and Si gel 60 F-254 plastic sheets (Merck), while cc was achieved on Sephadex LH 20 (Pharmacia). Purification was performed either by reversed-phase mplc (Lichroprep RP-18, 25–40 μm, 15×200 mm) or by semi-prep. hplc on a Waters model equipped with a 510 pump, a variable wavelength detector and a μ-Bondapak C-18 column (10 μm, 25×100 mm) (Waters). Acid hydrolysis and recording of uv spectra with the usual shift reagents were performed according to standard procedures (5,6). Sugars were analyzed by tlc on Si gel with EtOAc-H_2O-MeOH-HOAc (13:3:3:4) and visualized by spraying with *p*-anisidine phthalate. Chromatographic mobilities relative to compounds **1–4** were recorded in four systems: system 1 [Si gel F-254, EtOAc-HCOOH-HOAc-H_2O (100:5:5:10)], system 2 [cellulose F-254, *n*-BuOH-HOAc-H_2O (4:1:5, upper phase)], system 3 [cellulose F-254, HOAc-H_2O (15:85)], and system 4 [radial μBondapak 10 μ (8×100 mm), MeOH-H_2O (40:60), 1 ml·min^{-1}]. The nmr spectra were measured at 200 MHz for ^{1}H nmr and 50 MHz for ^{13}C nmr. The solvent signal was used as reference.

PLANT MATERIAL.—As reported previously (3).

ISOLATION AND PURIFICATION.—The general extraction procedure was previously reported (2). The *n*-BuOH fraction (13 g) of the Me_2CO extract was introduced on a Sephadex LH-20 column eluted by MeOH. The middle fraction (3 g) containing compounds **1–4** was submitted to a second Sephadex LH-20 column (MeOH). From the first fraction, 13 mg of isorhamnetin 3-rutinoside [**2**] was obtained following purification by reversed-phase mplc with 40% aq. MeOH. The middle fraction yielded 2 g of rutin [**1**], which was further purified by reversed-phase mplc with 30% aq. MeOH. Treatment of the last fraction (150 mg) by reversed-phase hplc with 40% aq. MeOH led to the isolation of 13 mg of myricetin 3-rutinoside [**3**], and 19 mg of gossypetin 3-rutinoside [**4**].

Quercetin 3-rutinoside [**1**].—Yellow powder. Identification was based on a combination of hydrolysis results and spectral properties (uv, ^{1}H- and ^{13}C nmr) in agreement with literature data (4,7). Chromatographic mobilities: R_f 0.15 (system 1), R_f 0.30 (system 2), R_f 0.47 (system 3), R_t 12.8 min (system 4).

Isorhamnetin 3-rutinoside [**2**].—Yellow powder. Identification was based on a combination of hydrolysis results and spectral properties (uv, ^{1}H- and ^{13}C nmr) in agreement with literature data (4,7). Chromatographic mobilities: R_f 0.19 (system 1), R_f 0.40 (system 2), R_f 0.49 (system 3), R_t 23.6 min (system 4).

Myricetin 3-rutinoside [**3**].—Yellow powder. Acid hydrolysis: myricetin, glucose, rhamnose. Chromatographic mobilities R_f 0.10 (system 1), R_f 0.16 (system 2), R_f 0.37 (system 3), R_t 8.2 min (system 4). ^{1}H nmr (DMSO-d_6, δ 2.49) δ 12.62 (1H, s, HO-5), 7.15 (2H, s, H-2′, 6′), 6.34 (1H, d, J=1.7 Hz, H-8), 6.17 (1H, d, J=1.7 Hz, H-6), 5.38 (1H, d, J=7.3 Hz, H-1″), 4.38 (1H, br s, H-1‴), 3.72 (1H, br d, J=9.8 Hz, H-6″$_A$), 3.50–3.15 (m, saccharide H), 3.04 (1H, t, J=9.2 Hz, H-3″), 0.99 (3H, d, J=6.1 Hz, H-6‴). ^{13}C nmr (DMSO-d_6, δ 39.5) δ177.3 (C-4), 164.2 (C-7), 161.2 (C-5), 156.4 (2C, C-2, 9), 145.4 (2C, C-3′, 5′), 136.7 (C-4′), 133.4 (C-3), 120.1 (C-1′), 108.6 (2C, C-2′, 6′), 103.8 (C-10), 101.0 (C-1″), 100.7 (C-1‴), 98.7 (C-6), 93.5 (C-8), 76.5 (C-5″), 76.1 (C-3″), 73.9 (C-2″), 71.8 (C-4‴), 70.5 (C-2‴ or C-3‴), 70.4 (C-2‴ or C-3‴), 70.0 (C-4″), 68.2 (C-5‴), 67.1 (C-6″), 17.7 (C-6‴).

Gossypetin 3-rutinoside [**4**].—Yellow amorphous powder; acid hydrolysis: glucose and

rhamnose. R_f 0.10 (system 1), R_f 0.10 (system 2), R_f 0.42 (system 3), R_t 2.7 min (system 4). Uv λ max (MeOH) nm 257 sh, 275, 342; ($AlCl_3$) 287, 400; ($AlCl_3$+HCl) 285, 360; (NaOH) 291, 364; (NaOAc) 285, 328, 385 sh; (NaOAc+ H_3BO_3) 250 sh, 285, 358. ^{1}H nmr (DMSO-d_6) δ 12.02 (1H, s, HO-5), 7.63 (1H, dd, J=8.2 and 2.1 Hz, H-6′), 7.61 (1H, br s, H-2′), 6.84 (1H, d, J=8.2 Hz, H-5′), 6.26 (1H, s, H-6), 5.34 (1H, d, J=7.4 Hz, H-1″), 4.37 (1H, br s, H-1‴), 3.70 (1H, br d, J=9.9 Hz, H-6″$_A$), 3.45–3.10 (m, saccharide H), 3.06 (1H, t, J=8.8 Hz, H-3″), 0.99 (3H, d, J=5.9 Hz, H-6‴). ^{13}C nmr (DMSO-d_6) δ 177.6 (C-4), 156.4 (C-2), 152.7 (2C, C-5, 7), 148.4 (2C, C-9, 4′), 144.7 (C-3′), 133.1 (C-3), 128.3 (C-8), 121.8 (C-6′), 121.4 (C-1′), 116.5 (C-5′), 115.1 (C-2′), 103.7 (C-10), 101.3 (C-1″), 100.7 (C-1‴), 98.5 (C-6), 76.4 (C-5″), 75.9 (C-3″), 74.1 (C-2″), 71.8 (C-4‴), 70.5 (C-2‴ or C-3‴), 70.3 (C-2‴ or C-3‴), 70.0 (C-4″), 68.2 (C-5‴), 67.0 (C-6″), 17.7 (C-6‴).

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