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TWO FLAVONOL CONJUGATES FROM *ERICA CINEREA*[1]

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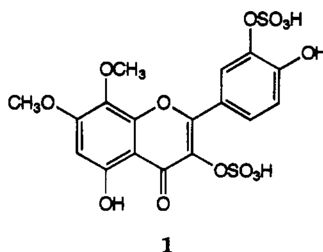
ABSTRACT.—The novel compound gossypetin 7,8-dimethyl ether 3,3′-disulfate [**1**] was isolated from the flowers of *Erica cinerea* along with the rare quercetin 3-β-D-(6-*p*-hydroxybenzoyl)-galactoside. The structure of **1** was established by spectroscopic methods.

Previous investigations on two selected species in the family Ericaceae, *Calluna vulgaris* L. and *Erica cinerea* L., afforded several rare phenolic compounds for the family. Investigation of the fresh aerial parts in the flowering stage gave flavonol glycosides and dihydroflavonol glycosides based on kaempferol, quercetin, and 8-hydroxydihydrokaempferol for *C. vulgaris* (1–4) and kaempferol, quercetin, limocitrin, dihydrokaempferol, dihydroquercetin, and dihydromyricetin for *E. cinerea* (5–7). In *C. vulgaris*, the diglycosides occurred as a pair of homologues corresponding to triacetylarabinofuranosyl- and triacetylarabinopyranosyl-glucosides, as well as the triacetylarabinopyranosyl galactoside. In contrast to *C. vulgaris*, the disaccharide in *E. cinerea* corresponded only to rutinose. We report here the isolation of two flavonoids not previously described from *E. cinerea*, namely, the novel gossypetin 7,8-dimethyl ether 3,3′-disulfate [**1**] and the rare quercetin 3-β-D-(6-*p*-hydroxybenzoyl)-galactoside.



1

The isolation of quercetin 3-β-D-(6-*p*-hydroxybenzoyl)-galactoside was achieved by five successive chromatographic procedures on the EtOAc-soluble part of the Me_2CO extract of *E. cinerea* flowers, using Sephadex LH 20 cc, polyamide-, cellulose-, and C_{18} reversed-phase mplc, and finally C_{18} reversed-phase hplc. The isolate was identified on the basis of its uv and ^{1}H-nmr data (8). Its structure was confirmed by the ^{13}C-nmr spectrum, which was similar to that of quercetin 3-β-D-galactoside except for the downfield shift of the C-6″ resonance by 3.1 ppm due to the *p*-hydroxybenzoate substituent (9,10). This is the second report of this product from a plant, which was previously isolated from *Ledum palustre*, another Ericaceae species (8).

The most polar compound obtained from *E. cinerea* flowers was **1**, which was

[1]Part 8 in the series "Phytochemistry of the Ericaceae." For Part 7, see Simon *et al.* (1).

isolated from the aqueous phase of the Me_2CO extract, and was present in smaller amounts than the other flavonol glycosides that occurred in this plant. It could be differentiated from them by both its higher polarity as well as the absence of any glycosidic moiety in the molecule. Such a polarity could only be explained by a dipolar form. The uv spectrum in the presence of the usual shift reagents suggested a 3-conjugated flavonol structure [λ max (MeOH) 272, 303 sh, and 353 sh nm]. Furthermore, the ratio of band II/ band I=2 was close to that exhibited by 8-*O*-substituted flavonol derivatives (11–13). Acid hydrolysis of **1** yielded gossypetin 7,8-dimethyl ether, which was identified by both chromatography and nmr spectroscopy in comparison with an authentic sample obtained from the hydrolysis of gossypetin 7,8-dimethyl ether 3-β-D-glucoside extracted from the same species (13). This result agreed with both the ^{1}H- and ^{13}C-nmr spectra (DMSO-d_6) for **1**. The negative-ion fab- and electrospray ms of **1** revealed the same fragments at *m/z* 505 $[M-H]^-$, *m/z* 425 $[M-H-80]^-$, and *m/z* 345 $[M-H-160]^-$. The successive losses of 80 and 160 mass units from the quasi-molecular ion, as well as the high polarity of the related compound (see Experimental), strongly suggested the presence of two sulfate groups in this molecule. They were unequivocally located at the 3- and 3′-positions on the basis of nmr analysis. The 3-sulfate function was indicated in the ^{13}C-nmr spectrum by the significant upfield displacement for the ipso carbon (Δδ −3.4 ppm) when compared with quercetin (14). In the B ring, the C-2′- and C-6′ protons exhibited uncommon deshielding (δ 8.01 and 8.09) produced by 3′-*O*-substitution and particularly by 3′-sulfation (9,14). Additional evidence for this substitution pattern came from the ^{13}C-nmr data. The chemical shift values of the carbons assigned to the B ring were found to be similar to those of quercetin 3,3′-disulfate. The ortho carbons C-2′ (δ 122.9; Δδ +7.6 ppm) and C-4′ (δ 151.9; Δδ +4.3 ppm) as well as the para carbon C-6′ (δ 126.9; Δδ +6.9 ppm) were affected by the expected downfield shift corresponding to the 3′-sulfation (15–17). On the basis of the nmr data obtained for gossypetin 7,8-dimethyl ether 3,3′-disulfate [**1**], it is suggested that the quercetin 3,3′-disulfate assignments (15–17) be revised as follows: H-2′ (δ 8.03 instead of 7.87), C-2′ (δ 123.1 instead of 121.6), and C-1′ (δ 121.6 instead of 123.1).

Flavonoid sulfates are known to occur in ca. 250 species belonging to 17 dicotyledonous and 15 monocotyledonous families. However, their presence has been discussed more in relation to saline habitats than to taxonomic considerations (18,19). As members of the Ericaceae grow on acid soils, it is therefore surprising that representative genera such as *Rhododendron* and *Vaccinium*, which have received considerable phytochemical attention, have not been found to accumulate such metabolites. This is probably due to the instability of the sulfate ester linkage.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Tlc, hplc, and uv analyses were carried out as previously reported (5). Chromatographic mobilities were recorded in six 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)], system 4 [radial Novapak 4 μm (8×100 mm), MeOH-H_2O (10:90), 1 ml/min], system 5 [radial μBondapak 10 μm (8×100 mm), MeOH-H_2O (50:50) 1 ml/min], system 6 [radial Novapak 4 μm (8×100 mm), linear gradient from MeOH-H_2O (3:7) to MeOH in 30 min (1 ml/min)]. The nmr spectra were measured at either 300 or 200 MHz for ^{1}H and 75 or 50 MHz for ^{13}C. The solvent (DMSO-d_6) signal was used as reference (^{1}H δ 2.49; ^{13}C δ 39.5). The fabms spectrum was taken on a VG-ZAB-SEQ spectrometer with a thioglycerol matrix, and the electrospray ms spectrum was made on a MS-engine.

PLANT MATERIAL.—The plant material used was previously described (5).

EXTRACTION AND ISOLATION.—The general

extraction procedure was previously reported (5). The residual aqueous phase (10 g) of the Me_2CO extract was submitted to a Sephadex LH-20 column, which was eluted by EtOH-H_2O (1:1) to give seven fractions. The third fraction (3 g), which contained compound **1**, was treated by reversed-phase mplc (10% aqueous MeOH) to afford seven fractions. The new fraction 3 yielded 5 mg of gossypetin 7,8-dimethyl ether 3,3'-disulfate [**1**] following purification by reversed-phase hplc with 20% aqueous MeOH. The EtOAc-soluble portion (5 g) of the Me_2CO extract gave an impure compound (15 mg) following successive chromatographic separations on a Sephadex LH-20 column (MeOH), polyamide mplc (EtOH gradient in toluene), and cellulose mplc (*n*-BuOH-AcOH-H_2O, 4:1:5). The final purification of quercetin 3-β-D-(6-*p*-hydroxybenzoyl)-galactoside (12 mg) was carried out by reversed-phase hplc (system 5).

Gossypetin 7,8-dimethyl ether 3,3'-disulfate [**1**].—Yellow powder; uv λ max (MeOH) 272, 303 sh, 328, 353 sh nm; ($AlCl_3$) 282, 308, 340, 405 nm; ($AlCl_3$+HCl) 278, 307 sh, 341, 361, 403 nm; (NaOH) 243 sh, 277, 305 sh, 353, 388 nm; (NaOAc) 262, 271, 305 sh, 399 nm; (NaOAc+H_3BO_3) 277, 297 sh, 323, 353 sh, 401 sh nm; fabms (negative-ion) *m/z* 544 $[M-H+K]^-$, 543 $[M-2H+K]^-$, 528 $[M-H+Na]^-$, 527 $[M-2H+Na]^-$, 505 $[M-H]^-$, 489 $[M-2H-Me]^-$, 463 $[M-2H+K-SO_3]^-$, 447 $[M-2H+Na-SO_3]^-$, 425 $[M-H-SO_3]^-$, 411 $[M-SO_3-Me]$, 379, 345 $[M-H-2SO_3]^-$, 344 $[M-2H-2SO_3]^-$, 339, 325, 311; electrospray ms (negative mode) *m/z* 543 $[M-2H+K]^-$ (100), 527 (36), 505 $[M-H]^-$ (5), 463 $[M-2H+K-SO_3]^-$ (92), 447 $[M-2H+Na-SO_3]^-$ (68), 425 $[M-H-SO_3]^-$ (42), 346 (19), 345 $[M-H-2SO_3]^-$ (25), 329 (29), 314 (13); ^{1}H nmr (300 MHz) δ 12.54 (1H, s, OH-5), 8.09 (1H, dd, *J*=8.7 and 2.2 Hz, H-6'), 8.01 (1H, d, *J*=2.2 Hz, H-2'), 6.89 (1H, d, *J*=8.7 Hz, H-5'), 6.56 (1H, s, H-6), 3.90 (3H, s, OMe-7), 3.81 (3H, s, OMe-8); ^{13}C nmr (75 MHz) δ 178.0 (C-4), 158.0 (C-7), 156.0 (C-2 and C-5), 151.9 (C-4'), 147.6 (C-9), 140.6 (C-3'), 132.2 (C-3), 128.3 (C-8), 126.9 (C-6'), 122.9 (C-2'), 121.6 (C-1'), 116.7 (C-5'), 104.4 (C-10), 95.6 (C-6), 61.2 (OMe-8), 56.4 (OMe-7); chromatographic mobilities: R_f 0 (system 1), R_f 0.75 (system 2), R_f 0 (system 3), R_t 13 min (system 4).

Gossypetin 7,8-dimethyl ether.—Acid hydrolysis of compound **1** (3 mg), in 4 N HCl for 10 min at 60°, afforded the corresponding aglycone. Purification was achieved by liquid-liquid extraction with Et_2O followed by Sephadex LH-20 cc packed with MeOH. Uv λ max MeOH 256, 274, 327, 377 nm; ^{1}H nmr (200 MHz) δ 12.22 (1H, s OH-5), 7.63 (1H, br d, *J*=8.6 Hz, H-6'), 7.61 (1H, br s, *J*=2.2 Hz, H-2'), 6.85 (1H, d, *J*=8.6 Hz, H-5'), 6.56 (1H, s, H-6), 3.91 (3H, s, OMe-7), 3.80 (3H, s, OMe-8), ^{13}C nmr (50 MHz) δ 176.5 (C-4), 157.7 (C-7), 155.7 (C-5), 147.8 (C-4'), 147.2 (C-2 or C-9), 146.5 (C-9 or C-2), 144.9 (C-3'), 135.8 (C-3), 128.3 (C-8), 121.8 (C-6'), 121.0 (C-1'), 115.8 (C-5'), 115.3 (C-2'), 103.5 (C-10), 95.1 (C-6), 61.2 (OMe-8), 56.5 (OMe-7); chromatographic mobilities: R_f 1 (system 1), R_f 0 (system 2), R_f 1 (system 3), R_t 26 min (system 6).

Quercetin 3-β-D-(6-p-hydroxybenzoyl)-galactoside.—Yellow powder; ^{13}C nmr (50 MHz) δ aglycone: 177.4 (C-4), 164.6 (C-7), 161.2 (C-5), 156.3 (C-9 or C-2), 155.9 (C-2 or C-9), 148.5 (C-4'), 144.9 (C-3'), 133.2 (C-3), 121.9 (C-6'), 121.1 (C-1'), 115.8 (C-5'), 115.1 (C-2'), 103.6 (C-10), 98.9 (C-6), 93.5 (C-8), β-galactosyl: 101.3 (C-1), 73.1 (C-3 or C-5), 72.9 (C-5 or C-3), 71.0 (C-2), 68.3 (C-4), 63.1 (C-6), *p*-hydroxybenzoyl: 165.2 (C-7), 161.9 (C-4), 131.0 (C-2, -6), 115.1 (C-3, -5), 120.1 (C-1); chromatographic mobilities: R_f 0.85 (system 1), R_f 0.26 (system 2), R_f 0.86 (system 3), R_t 14.4 min (system 5).

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LITERATURE CITED

1. A. Simon, A.J. Chulia, M. Kaouadji, and C. Delage, *Phytochemistry*, **36**, 1043 (1994).
2. D.P. Allais, A. Simon, B. Bennini, A.J Chulia, M. Kaouadji, and C. Delage, *Phytochemistry*, **30**, 3099 (1991).
3. A. Simon, A.J. Chulia, M. Kaouadji, D.P. Allais, and C. Delage, *Phytochemistry*, **32**, 1045 (1993).
4. A. Simon, A.J. Chulia, M. Kaouadji, D.P. Allais, and C. Delage, *Phytochemistry*, **33**, 1237 (1993).
5. B. Bennini, A.J. Chulia, M. Kaouadji, and F. Thomasson, *Phytochemistry*, **31**, 2483 (1992).
6. B. Bennini, A.J. Chulia, M. Kaouadji, and C. Delage, *Phytochemistry*, **33**, 1233 (1993).
7. B. Bennini, A.J. Chulia, and M. Kaouadji, *J. Nat. Prod.*, **57**, 181 (1994).
8. G.G. Zapesochnaya and T.T. Pangarova, *Dolk. Bolg. Akad. Nank.*, **33**, 933 (1980).
9. E. Breitmaier and W. Voelter, "Carbon-13 NMR Spectroscopy," VCH, Weinheim, Germany, 1990.
10. P.K. Agrawal, Ed. "Studies in Organic Chemistry. 39. Carbon-13 Nmr of Flavonoids," Elsevier, Amsterdam, 1989.
11. B. Voirin, *Phytochemistry*, **22**, 2107 (1983).
12. E. Wollenweber, D. Marx, J. Favre-Bonvin,

B. Voirin, and M. Kaouadji, *Phytochemistry,* **27**, 2673 (1988).
13. B. Bennini, A.J. Chulia, and M. Kaouadji, *Phytochemistry,* **38**, 259 (1995).
14. K.R. Markham and V.M. Chari, in: "The Flavonoids: Advances in Research." Ed. by J.B. Harborne and T.J. Mabry, Chapman and Hall, London, 1982, Chapter 2.
15. D. Barron and R.K. Ibrahim, *Phytochemistry,* **26**, 1181 (1987).
16. D. Barron, L.D. Colebrook, and R.K. Ibrahim, *Phytochemistry,* **25**, 1719 (1986).
17. J.L. Cabrera, H.R. Juliani, and E.G. Gross, *Phytochemistry,* **24**, 1394 (1985).
18. D. Barron, L. Varin, R.K. Ibrahim, J.B. Harborne, and C.A. Williams, *Phytochemistry,* **27**, 2375 (1988).
19. C.A. Williams and J.B. Harborne, in: "The Flavonoids: Advances in Research Since 1986." Ed. by J.B. Harborne, Chapman & Hall, London, 1994, Chapter 8.

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