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THE REVISED STRUCTURE OF TWO FLAVONOL 3- AND 4′-MONOGLUCOSIDES FROM *ERICA CINEREA**

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Key Word Index—*Erica cinerea*; Ericaceae; flavonol monoglucosides; gossypetin 7,8-dimethyl ether 3-glucoside; gossypetin 7,8-dimethyl ether 4′-glucoside.

Abstract—The structures of two flavonoid glucosides provisionally described previously from the fresh heather flowers of *Erica cinerea* need revision. They are now established as gossypetin 7,8-dimethyl ether 3-glucoside and gossypetin 7,8-dimethyl ether 4′-glucoside.

INTRODUCTION

As part of our investigation on flavonoids of the Ericaceae, we recently reported the occurrence of acylated flavonol diglycosides in *Calluna vulgaris* (L.) Hull [2–5] as well as flavonol diglycosides in *Erica cinerea* [6]. From the latter species, we have also isolated two new flavonol monoglucosides whose structures had been assigned only tentatively [7]. As a result, the previously proposed structures need to be revised to gossypetin 7,8-dimethyl ether 3- and 4′-glucoside instead of limocitrin 3- and 4′-glucoside. The structure correction of these is reported herein.

RESULTS AND DISCUSSION

Products **1** and **2** for which tentative structures have been published previously [7] were re-isolated from the fresh heather flowers of *Erica cinerea*. Treatment of the aqueous acetone extract first by liquid–liquid partitioning (petrol to *n*-BuOH) and then by combination of chromatographic separations on successive Sephadex LH 20 and polyamide columns afforded flavonol glucosides **1** and **2** for which final purification was achieved by reverse phase HPLC (see Experimental). The data for the new compounds are compiled in Tables 1 (^{1}H NMR) and 2 (^{13}C NMR).

Compound **1** from *E. cinerea* was suggested in a preliminary communication to have 5,7,4′-trihydroxy-8,3′-dimethoxy-3-glucosyl substitution [7]. Analysis of the new spectroscopic data clearly shows a 5,3′,4′-trihydroxy-7,8-dimethoxy-3-glucosyl flavonol structure, i.e. gossypetin 7,8-dimethyl ether 3-glucoside. This result is supported by the absence of a significant shift on addition of sodium acetate (see Experimental) which thus indicates 7-*O*-substitution [8, 9].

Consequently, the 3′-OH group, must be free as proved by the important UV shift on addition of sodium acetate and boric acid ($\Delta\lambda_1$ 26 nm). A similar result is also obtained first in aluminium trichloride medium ($\Delta\lambda_1$ 78 nm) and then on addition of hydrochloric acid ($\Delta\lambda_1$ 44 nm). These UV data demonstrate the presence of the 3′,4′-dihydroxy system in this molecule [8, 9]. Discrimination between the 3- and the 7-substitution by either a

Table 1. ^{1}H NMR spectra of flavonoid glucosides **1** and **2***

H	1	2
6	6.57 *s*	6.56 *s*
2′	7.63 *br s*	7.73 *d* (2)
5′	6.86 *d* (8.8)	7.27 *d* (8.5)
6′	7.67 *dd* (8.8–2)	7.63 *dd* (8.5–2)
HO-5	12.48 *s*	12.21 *s*
MeO-7	3.90 *s*	3.90 *s*
MeO-8	3.81 *s*	3.82 *s*
1″	5.46 *d* (7.2)	4.85 *d* (6.3)
2″	3.40–3.10 *m*	3.40–3.15 *m*
3″	3.40–3.10 *m*	3.40–3.15 *m*
4″	3.40–3.10 *m*	3.40–3.15 *m*
5″	3.08 *m*	3.40–3.15 *m*
6″$_A$	3.40 *m*	3.49 *dd* (11.5–5.4)
6″$_B$	3.57 *br d* (11.3)	3.73 *br d* (11.5)

*At 400 MHz in DMSO-d_6 (δ2.49).

*Part 9 in the series 'Phytochemistry of the Ericaceae'. For Part 8, see ref. [1].

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Table 2. ^{13}C NMR spectra of flavonoid glucosides **1** and **2***

C	1	2
2	156.3	147.6
3	133.2	136.4
4	177.7	176.4
5	156.3	155.8
6	95.7	95.2
7	158.0	157.8
8	128.3	128.3
9	148.7	146.4[a]
10	104.2	103.4
MeO-7	56.5	56.5
MeO-8	61.1	60.9
1′	121.2	125.2
2′	115.2	115.0
3′	144.9	146.8[a]
4′	147.7	146.2
5′	116.0	116.0
6′	121.7	119.3
1″	100.9	101.4
2″	74.0	73.2
3″	76.4	75.9
4″	69.9	69.8
5″	77.5	77.2
6″	60.9	60.7

*At 75.46 MHz in DMSO-d_6 (δ39.5).
[a]Assignments may need interchanging.

methoxy group or a glucosyl residue is based on the comparative analysis of the ^{13}C NMR of reference compounds with either ether linkages (OMe, OGlc) at C-3 and C-7 or with free hydroxyl groups at these positions (Table 3). According to the chemical shifts for C-3 (δ133.2) and C-6 (δ95.7), it is clearly established that the 3-position is glucosylated and the 7-position methylated. Compound **1** is therefore gossypetin 7, 8-dimethyl ether 3-glucoside, a new natural product.

As previously reported, **2** is assumed to be the 4′-glucosylated isomer of **1** lacking *ortho*-dihydroxyl substitution in the B-ring. This is shown in the UV spectrum by the changes after addition of shift reagents pointing to the presence of free hydroxyl groups at C-3, C-5 and C-3′. The UV shifts for band I and band II in alkaline media (NaOAc: 48 nm and − 5 nm; NaOH: 57 nm and 0 nm) are in agreement with a 4′-OH and a 7-OH both included in an ether linkage [8, 9]. As stated for **1**, the C-7 is bearing a methoxyl group according to both the ^{1}H and ^{13}C NMR chemical shifts at δ6.56 and 95.2 for CH-6 (Tables 1–3). In contrast to **1**, the 3-hydroxyl is free as supported by the downfield shift of C-3 (δ136.4) and the upfield shift of C-2 (δ147.6) [10, 11]. The remaining glucosyl residue is located at C-4′ as proved by both the downfield shift of the *para*-carbon C-1′ (δ125.2) and the *ortho*-proton H-5′ (δ7.27) (Tables 1–3). Consequently, **2** is gossypetin 7,8-dimethyl ether 4′-glucoside, another new natural product.

Gossypetin derivatives seem to be regular compounds in the Ericaceae [12–15]. We have previously reported in *E. cinerea* gossypetin 3-rutinoside [6], gossypetin 8-methyl ether 3-glucoside and 3-galactoside [7] as well as gossypetin 7, 8-dimethyl ether 3,3′-disulphate [1]. The two newly identified products described herein increase the list of compounds based on the gossypetin pattern in *E. cinerea*.

EXPERIMENTAL

The plant material was previously reported [7].

General procedure. TLC was carried out on microcrystalline cellulose plastic sheets (Merck) and silica gel 60 F-254 plastic sheets (Merck). CC was achieved on Sephadex LH 20 (Pharmacia). Purification was performed 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 sprectra with the usual shift reagents were made according to standard procedures [8, 9]. Sugars were analysed by TLC on silica gel with EtOAc–H_2O–MeOH–HOAc (13:3:3:4) and vizualized by spraying with *p*-anisidine phthalate. Chromatographic mobilities relative to glycosides **1** and **2** were recorded in 4 systems: system 1 (silica gel F-254, EtOAc–HCOOH–HOAc–H_2O = 20:1:1:2), system 2 [cellulose F-254, *n*-BuOH–HOAc–H_2O = 4:1:1:5 (upper phase)], system 3 (cellulose F-254, HOAc–H_2O = 3:17), system 4 [radial μBondapak 10μ (8 × 100 mm),

Table 3. ^{13}C NMR differentiation between 3-*O*-methyl, 3-*O*-glucosyl, 7-*O*-methyl and 7-*O*-glucosyl flavonols in reference to quercetin (**3**) taken from ref. [10]*

C	1	2	3	4	5	6	7
3	133.2	136.4	135.6	133.3	135.9	133.2	138.9
6	95.7	95.2	98.2	98.8	98.9	99.1	95.7
7	158.0	157.8	163.9	164.2	162.8	157.7	158.0
10	104.2	103.4	103.0	104.2	104.7	103.4	104.9
MeO-7	56.5	56.4					56.5
MeO-8	61.1	60.9				60.9	60.9

*In DMSO-d_6 (δ39.5); **4**: quercetin 3-glucoside [10]; **5**: quercetin 7-glucoside [10]; **6**: gossypetin 8-methyl ether 3-glucoside [7]; **7**: 5, 2′, 5′-trihydroxy 3, 7, 8-trimethoxyflavone [16].

MeOH–H_2O = 2:3, 1 ml min^{-1}]. NMR spectra were measured at 400 MHz for ^1H and 50 MHz for ^{13}C.

Extraction and isolation. The general extraction procedure was previously reported [7]. The ethyl acetate soluble fr. (11 g) of the acetone extract was first treated by CC on Sephadex LH 20 with methanol. The middle fr. (8 g) containing **1** and **2** was then submitted to a polyamide column eluted with a toluene–MeOH gradient. Flavonol glucosides **1** and **2** were present together in the ahead fr. [toluene–MeOH (17:3 to 3:1)] corresponding to 118 mg. A part of this fr. was submitted to a further treatment by Sephadex LH 20 CC in MeOH. The reported compounds were concentrated in two different frs. Each flavonol glucoside was then purified by semi-prep. reverse phase HPLC with 32% aq. MeOH for glycoside **1** and 36% aq. MeOH for glycoside **2**. This procedure led to the isolation of pure **1** (14.5 mg) and **2** (5 mg).

Gossypetin 7,8-dimethyl ether 3-glucoside (**1**). Yellow powder; acid hydrolysis: glucose. Chromatographic mobilities: R_f 0.55 (system 1), R_f 0.66 (system 2), R_f 0.54 (system 3), R_t 11.6 (system 4). UV λ_{max} MeOH nm: 261, 270sh, 299sh, 361; ($AlCl_3$) 280sh, 308sh, 360sh, 407, 439; ($AlCl_3$ + HCl) 272, 308, 360, 405sh, 432sh; (NaOH) 270sh, 406; (NaOAc) 268, 302sh, 388sh, 285sh, 405sh; (NaOAc + H_3BO_3) 265, 302sh, 387. ^1H NMR: see Table 1. ^{13}C NMR: see Table 2.

Gossypetin 7,8-dimethyl ether 4′-glucoside (**2**). Yellow powder; acid hydrolysis: glucose. Chromatographic mobilities: R_f 0.56 (system 1), R_f 0.64 (system 2), R_f 0.15 (system 3), R_t 15.9 (system 4). UV λ_{max} MeOH nm: 259, 271, 315sh, 335sh, 370; ($AlCl_3$) 268 285sh, 307sh, 360, 440; ($AlCl_3$ + HCl) 268, 285sh, 307sh, 360, 440; (NaOH) 271, 426; (NaOAc) 265, 418; (NaOAc + H_3BO_3) 257, 272, 335, 378. ^1H NMR: see Table 1. ^{13}C NMR: see Table 1.

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