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HERBACETIN AND GOSSYPETIN 3-GLUCURONIDE-8-GLUCOSIDES FROM *ROEMERIA HYBRIDA*

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Abstract—Two new flavonols, herbacetin and gossypetin 3-*O*-β-D-glucuronide-8-*O*-β-D-glucosides, have been isolated from *Roemeria hybrida*.

Amongst the small number of herbacetin and gossypetin diglycosides reported 3,8-disubstitution is of rare occurrence [1]. However, herbacetin 3-glucoside-8-xyloside has been identified in *Rhodiola ksylorii* Crassulaceae [2] and herbacetin and gossypetin 3-sophoroside-8-glucosides *Equisetum hyemale* [3].

In a study of the flavonoids of Papaveraceae, two glycosides were encountered in *Roemeria hybrida* (L). DC. which appeared to be new. They were identified as herbacetin 3-β-D-glucuronide-8-β-D-glucopyranoside **1** and gossypetin 3-β-D-glucuronide-8-β-D-glucopyranoside **2**. Both compounds gave glucose and glucuronic acid, together with their respective aglycones, on acid hydrolysis. Negative ion FAB-MS of **1** revealed a molecular ion at *m/z* 640 and a stronger ion at *m/z* 639 [M − 1], and the spectrum of **2** revealed a molecular ion at *m/z* 656 together with a stronger ion at *m/z* 655 [M − 1]. These data confirm the diglycosidic nature of both **1** and **2**. 3,8-Diglycosylation patterns for **1** and **2** are evident from the absorption spectra which show the 5,7,3′ and 4′-hydroxyls to be unsubstituted and which are identical with those previously published for 3,8-di-glycosylated herbacetin and gossypetin [3].

That the glucuronic acid residue is attached to the 3-hydroxyl in each case was indicated by paper chromatographic analysis of the products of mild acid hydrolyses. The monoglycosides formed appeared yellow fluorescent in UV (360 nm) light indicating that the 3-hydroxyls were unsubstituted [4], and possessed R_fs in H_2O of less than 0.1 compared with R_fs of 0.55 and 0.45 for **1** and **2**, respectively. Such low R_f values are not consistent with these products being monoglucuronides (which possess R_f values of about 0.5 [4, 5]), and further, the product from **1** was chromatographically indistinguishable from authentic herbacetin 8-glucoside in BAW, 15% HOAc and H_2O. Additional support for a 3-linked glucuronic acid moiety is found in the negative ion mass spectrum, in which the expected [4] ready loss of the 3-linked sugar resulted in the appearance of major ions at *m/z* 463 (in **1**) and *m/z* 479 (in **2**). Both of these fragments represent the loss of one glucuronic acid residue. No fragment ions were visible representing the loss of a glucose moiety from the molecular ion.

On the basis of the above data, the structures for **1** and **2** are proposed as the 3-*O*-glucuronide-8-*O*-glucosides of herbacetin and gossypetin respectively. The ^{13}C NMR spectrum of **1** provided confirmation of key structural features and in addition proved that both sugars are β-linked to the aglycone and are in the pyranose form. The expected [4] glucose and glucuronic acid signals are visible in the 61–78 ppm range with the glucuronic acid carbonyl resonance at 169.4 ppm. The C-1 signals of these sugars also appear in the expected positions for an 8-hydroxyl linked glucose (106.6 [3]) and a 3-hydroxyl linked glucuronic acid (102.4 [5]). The presence of 8-*O*-glycosylation is confirmed by the chemical shifts of C-9 at 148.7 ppm and C-5 at 157.3 ppm since in 8-hydroxyflavonols these resonances appear at *ca* 144 and 152 ppm respectively [3].

EXPERIMENTAL

Plant material. Roemeria hybrida (L.) DC. was collected from Wadi Habis, West of Mersa Matrouh (17 March 1985). It was identified by Prof. Dr M N El-Hadidi, The Herbarium, Cairo University. Voucher specimens are deposited at the Herbarium, Cairo University.

Isolation and identification methods. Fresh aerial parts of *R. hybrida* were extracted with 70% EtOH and the conc. extract applied to a polyamide column (H_2O and increasing concentrations of EtOH as eluent) followed by further purification on Sephadex LH 20 (MeOH as eluent). Standard methods were used for the identification of both glycosides [4, 6, 7]. FAB-MS was recorded on a Kratos MS 50-TC-TA instrument (6–7 kV gun, XE beam, 2 mA, 8 kV source). Samples were dissolved in pure formic acid, 1 μl of this solution was mixed with 1 μl of glycerol and deposited on the probe top, as described in [8]. ^{13}C NMR spectroscopy was carried out at 30° on a Varian FT80A spectrophotometer using DMSO-d_6 as solvent.

Herbacetin 3-glucuronide-8-glucoside (**1**). R_f values: BAW = 0.17, H_2O = 0.55, 15% HOAc = 0.52, PhOH = 0.08. UV data (λ_{max} nm): MeOH = 274, 305sh, 317sh, 360; NaOMe = 280, 329, 410 (stable); $AlCl_3$ = 279, 310, 355, 407; $AlCl_3$-HCl = 279, 307, 349, 404; NaOAc = 269, 310, 308 sh, 380; NaOAc–H_3BO_3 = 274, 305 sh, 319, 362. ^{13}C NMR data (ppm): Aglycone; 154.5 (C-2), 132.7 (C-3), 176.0 (C-4), 157.3 (C-5), 99.0 (C-6), 157.3

(C-7), 127.6(C-8), 148.7 (C-9), 102.8 (C-10), 121.3 (C-1'), 131.6 (C-2', 6'), 115.2 (C-3', 5'), 159.7 (C-4'); Glucose; 106.6 (C-1), 74.4 (C-2), 76.7, 77.6 (C-3, 5), 70.0 (C-4), 61.0 (C-6); Glucuronic acid; 102.4 (C-1), 71.6 (C-2), 73.1 (C-3), 75.4 (C-4), 76.4 (C-5), 169.4 (C-6).

Gossypetin 3-glucuronide-8-glucoside (**2**). R_f values: BAW = 0.15, H_2O = 0.45, 15% HOAc = 0.45, PhOH = 0.04. UV data (λ_{max} nm): MeOH = 260 sh, 271, 366; NaOMe = 278, 329 sh, 421 (stable); $AlCl_3$ = 276, 305 sh, 365 sh, 426; $AlCl_3$-HCl = 273, 305 sh, 360, 414; NaOAc = 279, 325, 386; NaOAc–H_3BO_3 = 267, 387.

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