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A GOSSYPETIN GLUCURONIDE SULPHATE FROM THE LEAVES OF *MALVA SYLVESTRIS*

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Key Word Index—*Malva sylvestris*; Malvaceae; gossypetin 8-*O*-β-D-glucuronide-3-sulphate; ^{1}H NMR and ^{13}C NMR.

Abstract—The new natural product gossypetin 8-*O*-β-D-glucuronide-3-sulphate was isolated as a minor flavonoid constituent from the leaves of *Malva sylvestris*. The structure was established by chromatographic behaviour and acid hydrolysis yielding gossypetin, glucuronic acid and sulphate and confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy.

INTRODUCTION

Leaf extracts of *Malva sylvestris* L. are used in traditional Egyptian medicine for treating haemorrhoids. In a previous investigation [1], gossypetin 3-sulphate-8-*O*-β-D-glucoside and hypolaetin 3'-sulphate were identified as the major flavonoid constituents in the leaf tissue of *Malva sylvestris*. We now report the isolation and characterization of the new natural product, gossypetin 8-*O*-β-D-glucuronide 3-sulphate (**1**) from the same tissue. Structural elucidation was mainly by ^{13}C NMR spectroscopy. For this purpose, a synthetic sample [2] of gossypetin (3,5,7,8,3',4'-hexahydroxyflavone) was prepared and its ^{13}C NMR spectrum assigned using additive rules. All assignments were then confirmed through off-resonance experiment. It should be noted that, although ^{13}C NMR spectra of a number of flavonoids have been examined [3–6], only a few spectra have been recorded for 8-substituted flavonoids [6], and none for gossypetin or any of its glycosides. Also, no ^{13}C NMR data has been recorded for sulphated flavonoids.

RESULTS AND DISCUSSION

1 was isolated and purified from an aqueous ethanolic leaf extract of *Malva sylvestris* by a combination of polyamide and PC techniques. Chromatographic behaviour (dark brown on PC in UV), the results of normal and controlled acid hydrolysis, enzymic hydrolysis [7] and electrophoretic behaviour suggest that **1** is a gossypetin glucuronide monosulphate.

UV spectral analysis of **1** and of its acid hydrolysis product (**2**) in methanol and with diagnostic shift reagents [8, 9] (Table 1) indicates that **2** is gossypetin 8-*O*-β-D-glucuronide (positive shifts with all reagents and unstable NaOMe spectrum) and that **1** is the 3-sulphate derivative of **2** (hypsochromic shift of band I relative to that of **2** in MeOH and the relatively small $AlCl_3$ shift and stable NaOMe spectrum).

The ^{1}H NMR spectra of **1** and **2** show sharp signals overlapping with broad signals from hydroxylic protons. They are in accordance with the proposed structures. The signals of the protons 6-H, 6'H, 2'-H and 5'-H of **1** shifted upfield compared with **2**, an effect obviously caused by the sulphate group.

The ^{13}C NMR spectra (Table 2) confirm the proposed structures for **1** and **2**. Most of the chemical shift values for **2** are the same as for gossypetin or for β-D-glucopyranuronic acid. The attachment of the sugar moiety to C-8 follows from the upfield shift of the C-8 signal and the downfield shift of the signals of its *ortho*- and *para*-related carbons: C-7, C-9 and C-5. Similar shifts are well known from the work of Markham *et al.* [5]. The β-configuration is derived from the position of the anomeric carbon signal at 106 ppm [10]. In **1** the position of the sulphate group at C-3 is recognized from the upfield shift of the C-3 signal and the large downfield shift of the C-2 signal (all in comparison with **2**). Interestingly, the influence of a sulphate group on both the C-3 and C-2 signals is very similar to that of a sugar group, but produces a downfield shift of *ca* 3 ppm for the C-6 signal and an upfield shift of *ca* 4 ppm for the C-10 signal (compare with the chemical shift data of quercetin and quercetin 3-*O*-β-glucoside).

EXPERIMENTAL

NMR: Jeol FX 100, δ values; solvent DMSO-d_6; reference: signal of DMSO-d_6 set at 39.5 ppm which is the chemical shift in relation to $\delta_{TMS} = 0$. Atomic absorption: Varian 1000 spectrometer.

PC was carried out on Whatman No. 1 paper using solvent systems 1–5: 1—HOAc (HOAc–H_2O, 3:17); 2—BAW (*n*-BuOH–HOAc–H_2O, 4:1:5; top layer); 3—iPW (*iso*-PrOH–H_2O, 11:39); 4—Forestal (conc HCl–H_2O–HOAc, 3:10:30); 5—BPOH (C_6H_6–*n*-BuOH–pyridine–H_2O, 1:5:3:3; top layer). Solvent systems 1 and 2 were also used for PPC on Whatman No. 3 MM paper.

The ionic behaviour of the isolated compounds was examined by electrophoresis on 3 MM paper at pH 2 using HOAc–HCO_2H buffer (H_2O–HOAc–HCO_2H, 89.5:8:2.5) at 50 V/cm for 90 min.

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Table 1. Some properties of gossypetin 8-glucuronide-3-sulphate (1) and its hydrolysis products

	Electrophoretic* mobility (cm)	Chromatographic data R_f (×100)					UV spectral data λ_{max}^{MeOH} (nm)	UV spectral data $\Delta\lambda$ (nm)				
		H_2O	HOAc	BAW	iPW	Forestal		NaOAc†	NaOAc‡ + H_3BO_4	NaOMe‡	$AlCl_3$‡	$AlCl_3$ + HCl‡
1	2.9	48	66	28	58	—	262sh, 276, 370	18	16	57	22	32
2	0	06	18	33	40	—	260, 275, 382	18	10	60 (dec.)	68	60
Gossypetin	0	—	—	48	20	25	260, 278, 338, 386	20	10	39 (dec.)	—	—

*90 min at pH 2, 50 V/cm (see Experimental).
†Band II.
‡Band I.
sh, shoulder.

Table 2. ^{13}C NMR spectra of **1**, **2**, related flavonols and glucuronic acid*

Carbon No.	Quercetin†	Quercetin-† 3-*O*-*β*-D-glucoside	Gossypetin	Gossypetin-8-*O*-*β*-D-glucuronide (**2**)	Gossypetin-8-*O*-*β*-D-glucuronide-3-SO_3K (**1**)	*β*-Glucuronic‡ acid
2	146.9	156.5	146.6 (*s*)	148.0 (*s*)	157.6 (*s*)	
3	135.5	133.7	135.4 (*s*)	135.6 (*s*)	132.8 (*s*)	
4	175.8	177.6	176.1 (*s*)	175.8 (*s*)	175.9 (*s*)	
5	160.7	161.3	152.6 (*s*)§	156.4 (*s*)§	157.2 (*s*)	
6	98.2	98.8	98.1 (*d*)	98.3 (*d*)	101.8 (*d*)	
7	163.9	164.2	152.1 (*s*)§	156.4 (*s*)§	157.2 (*s*)	
8	93.3	93.6	122.3 (*s*)	121.1 (*s*)	122.0 (*s*)§	
9	156.2	156.5	144.8 (*s*)	148.3 (*s*)	148.5 (*s*)	
10	103.1	104.2	102.7 (*s*)	102.8 (*s*)	99.1 (*s*)	
1′	122.1	121.4	124.7 (*s*)	124.9 (*s*)	127.6 (*s*)	
2′	115.5	115.3	115.5 (*d*)‖	115.8 (*d*)‖	114.7 (*d*)‖	
3′	145.0	144.8	145.0 (*s*)	144.7 (*s*)	145.0 (*s*)	
4′	147.6	148.5	147.7 (*s*)	147.7 (*s*)	147.8 (*s*)	
5′	115.6	116.5	115.3 (*d*)‖	115.9 (*d*)‖	116.4 (*d*)‖	
6′	120.0	121.6	120.2 (*d*)	120.2 (*d*)	121.3 (*d*)§	
1″				106.2	107.1	97.2
2″				71.5	71.4	71.5
3″				73.8	73.6¶	74.4
4″				75.4	74.1¶	75.5
5″				76.0	77.3	75.9
6″				170.2	172.8	170.4

* Letters in parentheses refer to the splitting of the signal in off-resonance experiment.
† Data from ref. [5].
‡ Data from ref. [11].
§ Data may be reversed.
‖ Data may be reversed.
¶ Data may be reversed.

Gossypetin was prepared according to [2]. Leaves were extracted with aq. EtOH. The extract was concd, applied to a polyamide column and eluted with H_2O followed by H_2O–MeOH (1:9) to yield a fraction containing mainly **1**.

Gossypetin 8-*O*-*β*-D-glucuronide 3-sulphate (**1**) was purified by PPC, mp (uncorr.) 239° (dec.). For R_f values see Table 1. Acid hydrolysis (1 N HCl) at 100° yielded gossypetin (mp, mps, co-PC and UV spectral data, Table 1) and glucuronic acid (co-PC). The hydrolysate gave a white ppt. with $BaCl_2$ assumed to be $BaSO_4$. Enzymic hydrolysis with *β*-glucuronidase gave a negatively charged intermediate (dark brown on electrophoretograms in UV, migrating 2.3 cm) which, on acidification with 0.05 N HCl gave gossypetin. Controlled acid hydrolysis of **1** (0.05 N HCl) at 100° gave **2** (yellow on PC in UV), which gave gossypetin on hydrolysis with *β*-glucuronidase. An aq. soln of **1** was found to contain K as shown by atomic absorption spectroscopy and by the formation of a yellow ppt. with Na cobaltinitrite. Found *S*, 5.4%. Calc. for $C_{21}H_{18}KO_{17}S$: *δ*, 5.2%. UV spectral data of **1** and **2** are recorded in Table 1.

^1H *NMR* (**1**). 12.42 ppm (*s*, 5-OH); 9–11 (*br*, OH); 7.62–7.84 (*m*, 2″-H, 6″-H); 6.7 (*d*, J = 8 Hz, 5″-H); 5.67 (*s*, 6″-H); 5.24–5.36 (*m*, $\Delta\nu_{\frac{1}{2}}$ = 10 Hz, 1″-H); 4.42 (*d*, J = 6 Hz, 5″-H); 3.1–3.8 (*m*, 2″-H, 3″-H, 4″-H); 3.5–5 (*m*, overlapping with other protons, OH).

^1H *NMR* (**2**). 12.35 ppm (*s*, 5-OH), 9.4–10.5 (*m*, OH); 7.86 (*d*, J = 2.5 Hz, 2″-H); 7.62 (*d*, *d*, J_m = 2.5 Hz, J_o = 8 Hz, 6″-H); 6.8 (*d*, J = 8 Hz, 5″-H); 6.2 (*s*, 6″-H); 5.4 (*br*, 1″-H); 4–9 (*br*, 5″-H, OH); 3.1–3.8 (*m*, 2″-H, 3″-H, 4″-H). The ^{13}C NMR data are given in Table 2.

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