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THE FLAVONOIDS OF *PHRAGMITES AUSTRALIS* FLOWERS

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Key Word Index—*Phragmites australis*; Gramineae; flavone *C*-glycosides; swertiajaponin 3′-*O*-gentiobioside; swertiajaponin 3′-*O*-glucoside; rhamnetin glycosides.

Abstract—The major flavonoid constituents of *Phragmites australis* flowers are the *C*-glycosylflavones swertiajaponin, isoswertiajaponin and two new *O*-glycosides, the 3′-*O*-gentiobioside and the 3′-*O*-glucoside of swertiajaponin. Two unusual flavonol glycosides, rhamnetin 3-*O*-rutinoside and rhamnetin 3-*O*-glucoside, were also characterized from the same tissue.

INTRODUCTION

The flavonoids of a large number of Gramineae species have been documented [1–5]. Harborne and Williams [1] have reported tricin and *C*-glycosylflavones as the major leaf flavonoids of *Phragmites australis* (Cav.) Trin. ex Steudel (syn. *P. communis* Trin.) However, no other consituents of this plant have been identified and it was therefore of interest to make a more detailed study of *P. australis*, a species which grows wild in Egypt. The isolation and characterization of two new swertiajaponin X′-*O*-glycosides and two rhamnetin *O*-glycosides from the flowers of this plant are now reported.

RESULTS AND DISCUSSION

Two-dimensional PC of the direct aqueous ethanolic extract of the fresh flower tissue of *P. australis* revealed a complicated flavonoid profile dominated by flavone *C*-glycosides, which appear as dark purple spots in UV light. Fractionation of the extract on a polyamide column by successive elution with 10, 20, 30 and 40% aq. ethanol. respectively, gave four fractions (F_1–F_4). PPC then afforded pure samples of swertiajaponin 3′-*O*-gentiobioside (**1**) from F_1, swertiajaponin (**2**) from F_2, isoswertiajaponin (**3**) together with rhamnetin 3-*O*-rutinoside (**5**) from F_3 and swertiajaponin 3′-*O*-glucoside (**4**) and rhamnetin 3-*O*-glucoside (**6**) from F_4.

It is well known that flavone *C*-glycosides are the characteristic flavonoids of the Gramineae. The present study supports the findings of Harborne and Williams [1] that *C*-glycosylflavones are the major flavonoid constituents and it is of note that none of the flavone *C*-glycosides identified in this plant have been reported previously in the Gramineae [1–7]. Swertiajaponin has been found in *Swertia japonica* (Gentianaceae), *Iris* spp. (Iridaceae), *Tragopogon* spp. (Compositae) and *Cephalaria leucantha* (Dipsacaceae). More recently swertiajaponin has been reported in *Gnetum gnemon* (Gnetaceae) together with its isomer. This is the only previous report of isoswertiajaponin [8] in nature.

Flavonol glycosides are of rare occurrence in the Gramineae and are usually represented by simple quercetin and kaempferol glycosides [1, 6, 7]. The present paper reports the identification of two glycosides, the 3-*O*-rutinoside and the 3-*O*-glucoside of the rare flavonol rhamnetin from the flower tissue of *P. australis*. No rhamnetin glycosides have been reported before in the Gramineae. Rhamnetin 3-*O*-rutinoside was first isolated from *Suriana* (Simaroubaceae) [9], while the 3-*O*-glucoside was reported from *Thalictrum foetidum* (Ranunculaceae) [10]. Other rhamnetin glycosides of natural occurrence include the 3-*O*-xanthorhamnoside, the 3-*O*-(α-L-rhamnopyranosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactoside), which occur in *Rhamnus* spp. [11] and the 3-*O*-arabinoside, 3-*O*-diarabinoside and 3-*O*-galactoside from *Euphorbia amygdaloides* (Euphorbiaceae) [3]. Recently rhamnetin 3′-*O*-glucuronide-3,5,4′-trisulphate was characterized in leaves of *Tamarix aphylla* (Tamaricaceae) [12].

EXPERIMENTAL

Samples of the flowers of *Phragmites australis*, collected from Wadi-Hoof, south of Cairo, were authenticated by Dr. Vivi Tackholm, Prof. of Botany, Faculty of Science, Cairo University. The fresh plant material was then extracted with 75% aq. EtOH. PC on Whatman No. 1 paper was carried out using the following solvent systems: 1—HOAc (15% aq. HOAc); 2—BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, top layer); 3—PhOH (phenol–H_2O, 3:1); 4—CAcOH ($CHCl_3$–HOAc–H_2O; 13:6:1, lower layer); 5—Forestal (conc. HCl–HOAc–H_2O, 3:30:10); 6—BN(*n*-BuOH–2N NH_3, 1:1, top layer) and 7—BpOH (C_6H_6–*n*-BuOH–Py–H_2O, 1:5:3:3, top layer). Solvents 1 and 2 were used in 2D-PC, solvents 1,4 and 6 in PPC on Whatman 3MM paper and solvents 2 and 7 in sugar analysis. The purity of the isolated compounds was checked on Si gel TLC using solvent system EtOAc–Py–H_2O–MeOH, 16:4:2:1. Mps are uncorr. MS was carried out on a Varian MAT 112-EI spectrometer, 70 eV, direct inlet

Table 1. UV and chromatographic properties of the flavonoids of *Phragmites australis*

Flavonoid	Colour under UV	Chromatographic properties R_f values (×100)						λ_{max} in MeOH	UV spectral data Δλ (nm)				
		H_2O	HOAc	BAW	PhOH	CAcOH	Forestal		NaOAc*	NaOAc† + H_3BO_3	NaOMe†	$AlCl_3$†	$AlCl_3$† + HCl
1	d. purple	14	51	51	71	47	nd	245,‡ 270, 345.	0	0	65	45	40
2	d. purple	07	41	51	61	07	nd	245,‡ 255, 270, 347.	0	20	58	76	45
3	d. purple	04	26	42	44	02	nd	255, 270, 347.	0	18	52	72	49
4	d. purple	14	46	59	86	52	nd	245,‡ 272, 345.	0	0	59	45	40
5	d. brown	35	57	50	66	08	nd	256, 270,‡ 300,‡ 357.	0	11	58	55	45
6	d. brown	07	26	65		30	nd	255, 270,‡ 305,‡ 355.	0	11	55	55	46
Rhamnetin	yellow	nd	nd	81	68	78	58	257, 270,‡ 300,‡ 370	0	10	52	70	56

d. = Dark; nd = not determined.
* = Band II.
† = Band I.
‡ = Inflection.

and ^{1}H NMR determined on a Jeol-FX 100, 100 MHz FT NMR spectrometer using DMSO-d_6 as solvent and TMS as int. ref.

Swertiajaponin 3′-*O*-*gentiobioside* (**1**). R_f values (Table 1), on acid hydrolysis yielded glucose together with swertiajaponin as major aglycone and isoswertiajaponin as a minor one (coPC). Enzyme hydrolysis with β-glucosidase gave swertiajaponin and H_2O_2 oxidation yielded gentiobiose (coPC), thus confirming that **1** is swertiajaponin X′-*O*-gentiobioside. The UV spectral analysis of **1** (Table 1) proved substitution at position 3′ (no shift with NaOAc + H_3BO_3, large intense shift with NaOMe and relatively small shift with $AlCl_3$, slightly affected with HCl). This conclusion was confirmed by methylation with dimethylsulphate followed by alkali-fusion (at 200–205° for 3 min) to give isovanillic acid (coPC). It is thus concluded that **1** is swertiajaponin 3′-*O*-gentiobioside.

7-*O*-*Methyl*-6-β-D-*glucopyranosylluteolin, swertiajaponin* (**2**). Mp 263° (dec.), showed chromatographic properties (R_f-values, Table 1) similar to those of *C*-glycosylflavones with a luteolin nucleus. It yielded an equilibrium mixture of **2** with isoswertiajaponin on acid hydrolysis (coPC). Luteolin was obtained from **2** by hydrolytic cleavage with HI–phenol. **2** is therefore identified as a *C*-glycosylluteolin derivative. Its UV spectral data (Table 1) agreed with this conclusion but showed a substitution at position 7 (no shift with NaOAc). The sharp singlet shown on the ^{1}H NMR spectrum at δ 3.93 ppm proved that the substituent is a methoxyl group. Other proton signals in the same spectrum appeared at δ 6.42 (*s*, 3-H); 6.72 (*s*, 8-H); 7.6–7.84 (*m*, 2′-H and 6′-H); 6.95 (*d*, J = 8.5 Hz, 5′-H); 4.85 (*d*, J = 10 Hz, CH-1-glucosyl) and 3.35–3.8 (*m*, 6H, glucosyl). From the above data **2** is identified as 7-*O*-methyl 6-β-D-glucopyranosylluteolin (swertiajaponin, mp 265° (dec.) [3]). CoPC with an authentic sample of swertiajaponin further confirmed this identity.

7-*O*-*Methyl* 8-β-D-*glucopyranosylluteolin, isoswertiajaponin* (**3**). Mp 248° (dec.), $[\alpha]_D^{20}$ +92.6° in MeOH, showed R_f-values (Table 1) lower than those of swertiajaponin in all solvents. It yielded an equilibrium mixture of **2** + **3** on acid hydrolysis and gave luteolin on HI–phenol treatment. The UV spectral data of **3** (Table 1) are similar to those of swertiajaponin. From this and the above data **3** is identified as isoswertiajaponin [8].

Swertiajaponin 3′-*O*-β-D-*glucoside* (**4**). R_f-values (Table 1) on normal and mild acid hydrolysis yielded swertiajaponin and glucose. Enzymic hydrolysis of **4** with β-glucosidase gave swertiajaponin and H_2O_2 oxidation gave glucose. **4** showed UV spectral data (Table 1) similar to those of swertiajaponin 3′-*O*-gentiobioside and gave isovanillic acid on methylation and alkali-fusion. Thus **4** is identified as swertiajaponin 3′-*O*-β-D-glucoside. The related compound isoorientin 3′-*O*-β-D-glucoside has been identified in *Gentiana nivalis* (Gentianaceae [13]).

Rhamnetin 3-*O*-*rutinoside* (**5**). Mp 204° (dec.), R_f values (Table 1) on acid hydrolysis gave glucose, rhamnose and rhamnetin (coPC). Rutinose was released by H_2O_2 oxidation. The identity of the aglycone was confirmed through mp (294°, lit. 294–296° [14]) and MS analysis giving *m/e* 316 $(M)^+$ (major fragments: $(M-1)^+$; $(M)^+$; $(M-15)^+$; $(M-Me—CO)^+$; $(A_1)^+ = 166$; $(A_1-OH)^+ = 149$ and $(B_2)^+ = 137$ with relative abundance: 100, 17, 21, 11, 23, 61 and 30%, respectively). The identity was further confirmed by ^{1}H NMR of the aglycone, whereby proton signals appeared at: δ 3.94 (*s*, 3H, 7-OMe); 6.75 (*d*, J = 2.5 Hz, 8-H); 6.45 (*d*, J = 2.5 Hz, 6-H); 7.6–7.9 (*m*, 2′-H and 6′-H); 6.95 (*d*, J = 8.5 Hz, 5′-H). The UV spectral data of **5** (Table 1) together with the above data led to the conclusion that **5** is rhamnetin 3-*O*-rutinoside [9].

Rhamnetin 3-*O*-β-D-*glucoside* (**6**). R_f values (Table 1), on normal and mild acid hydrolysis gave glucose and rhamnetin. It gave rhamnetin on enzymic hydrolysis (β-glucosidase) and glucose on H_2O_2 oxidation. UV spectral analysis (Table 1) together with the above mentioned data led to the conclusion that **6** is rhamnetin 3-*O*-β-D-glucoside [10].

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FLOWER FLAVONOIDS OF *OPUNTIA* SERIES *OPUNTIAE*

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Key Word Index—*Opuntia*; Cactaceae; prickly-pear; quercetin; isorhamnetin; kaempferol; 3-glycosides.

As part of an investigation into the possible hybrid origin of the tetraploid prickly-pear, *Opuntia curvospina* Griffiths, we undertook the identification of the flavonoids from this species and its potential parent species. *Opuntia chlorotica* Engelmann & Bigelow and *O. phaeacantha* Englemann var. *major* Englemann have been suggested as the putative diploid and hexaploid parents, respectively, of *O. curvospina* [1]. Additional closely related taxa in series *Opuntiae* were also examined. These included the tetraploid *O. littoralis* (Englemann) Cockerell var. *martiniana* (L. Benson) L. Benson, and the hexaploids *O. phaeacantha* var. *discata* (Griffiths) L. Benson & Walkington and *O. littoralis* var. *littoralis.* All taxa were investigated for their flower flavonoids. Prior to this report, studies of *Opuntia* flavonoids have been few and mostly incomplete [2–8].

RESULTS AND DISCUSSION

The flowers of all 6 taxa of *Opuntia* series *Opuntiae* examined produce the same flavonoids. These are quercetin and isorhamnetin 3-glucosides and 3-rutinosides, isorhamnetin 3-rhamnosylgalactoside, and kaempferol 3-galactoside. These flavonoid profiles suggest close relationships among all 6 taxa. The chemical data indicate a slight divergence between *O. lindheimeri* Englemann and these taxa. *Opuntia lindheimeri* produces two flavonoids found in the group (isorhamnetin 3-rutinoside and 3-rhamnosylgalactoside) and two that are not (quercetin and isorhamnetin 3-galactosides) [6]. This species, which is also included in series *Opuntiae* (Pinkava, D. J., personal communication), is the only other member of the genus whose flavonoid profile has been completely determined. At this time the available data can only indicate the potential usefulness of chemotaxonomic studies of *Optunia* species. The lack of qualitative differences in flavonoids among the taxa reported here lends no support nor does it contradict the hypothesis that *O. curvospina* arose from the hybridization of *O. phaeacantha* var. *major* and *O. chlorotica.*

EXPERIMENTAL

Vouchers of all plant material are deposited in ASU. Flavonoids were isolated from 85% aq. MeOH extracts by Polyclar AT and Sephadex LH-20 column chromatography using the methods of Mabry *et al.* [9]. Individual compounds were characterized by standard UV–visible spectral analyses [9]. Monosaccharides were obtained using standard acid hydrolytic procedures [9] and disaccharides were recovered after H_2O_2 oxidation of the flavonoid moieties [10]. Sugars were identified by co-retention with standards using HPLC [11].