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FLAVONOIDS FROM *HAPLOPHYLLUM PEDICELLATUM*, *H. ROBUSTUM* AND *H. GLABRINUM*

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Key Word Index—*Haplophyllum pedicellatum*; *H. robustum*; *H. glabrinum*; Rutaceae; acylated flavonoids; gossypetin glycosides; isorhamnetin glycosides; quercetin glycosides.

Abstract—*Haplophyllum pedicellatum*, *H. robustum* and *H. glabrinum* all yielded the known compound gossypetin 8,3′-dimethyl ether 3-rutinoside. In addition the first two species afforded isorhamnetin and its 3-rutinoside. A new glycoside, gossypetin 8,3′-dimethyl ether 3-glucoside was obtained from *H. pedicellatum* together with the 3-malonylrutinoside, 3-malonylglucoside and 3-galactoside of isorhamnetin plus kaempferol 3-malonylglucoside. *H. robustum* yielded isorhamnetin 7-glucoside and 3-glucoside and quercetin 3-galactoside, while *H. glabrinum* was found to contain gossypetin 8-methyl ether 3-malonylrutinoside in addition to kaempferol and isorhamnetin 3-glucoside.

INTRODUCTION

Three Iranian species of *Haplophyllum*, *H. glabrinum* Bunge, *H. pedicellatum* Bunge ex Boiss. and *H. robustum* Bunge, were investigated for their flavonoids. The first two species were found to contain flavonol 3-malonylglycosides. The structure of a new 3-glucoside of gossypetin 8,3′-dimethyl ether from *H. pedicellatum* was established. This is the first flavonoid study of these three species of *Haplophyllum*.

RESULTS

The concentrates from the methanol extracts of the three *Haplophyllum* species were each fractioned over Polyclar columns. *H. pedicellatum* yielded gossypetin 8,3′-dimethyl ether 3-rutinoside (**1**) [1], isorhamnetin and its 3-rutinoside (**2**) [2] and 3-galactoside (**3**) [3], and the new compounds, gossypetin 8,3′-dimethyl ether 3-glucoside (**4**), the 3-malonylrutinoside (**5**) and 3-malonylglucoside of isorhamnetin (**6**) and kaempferol 3-malonylglucoside (**7**). *H. robustum* yielded six known compounds: **1** and **2**, isorhamnetin, its 7-glucoside (**8**) and 3-glucoside (**9**) and quercetin 3-galactoside (**10**) and the new gossypetin 8-methyl ether 3-malonylrutinoside (**11**).

The identification of the known glycosides was established by standard hydrolytic data as well as by UV spectral studies, ^{1}H NMR, MS and, except for **1**, direct comparison with authentic samples.

Acid hydrolysis (0.1 N TFA) of **4**, a new glycoside from *H. pedicellatum*, gave glucose (TLC comparison) and gossypetin 8,3′-dimethyl ether (UV, ^{1}H NMR and MS, as well as color reactions). The ^{1}H NMR spectrum of the TMSi ether of **4** (200 MHz, $CDCl_3$) confirmed the monoglucosyl moiety: six sugar protons between δ3.5–3.8 and the signal for H-1 of glucose at δ5.92, typical for flavonol 3-glucosides. All other NMR, UV and MS data (see Experimental) supported the proposed structure as the 3-glucoside of gossypetin 8,3′-dimethyl ether.

The acylated compounds **5**, **6**, **7** and **11** showed an extra singlet in their ^{1}H NMR spectra at δ3.5 for **5**, **7** and **11**, and at δ3.65 for **6**. An initial assumption was that the compounds contained glycosyl *O*-methyl ethers; however, MS analysis of the glycosides by the alditol-acetate method [4] did not support the presence of glycosyl *O*-methyl ethers in any of the natural products. Since the alditol-acetate method would have removed acyl functions, basic hydrolysis was carried out on each compound on the possibility that the extra signal might be associated with a group such as glycosyl malonic acid. Basic hydrolysis with 0.5 N potassium hydroxide yielded isorhamnetin 3-rutinoside from **5**, isorhamnetin 3-glucoside from **6**, kaempferol 3-glucoside from **7** and gossypetin 8-methyl ether 3-rutinoside from **11** (TLC comparisons). The UV spectra were unchanged for all compounds after basic hydrolysis. The basic hydrolysis product of **11**, gossypetin 8-methyl ether 3-rutinoside, was obtained in sufficient quantity for ^{1}H NMR; the spectra of the TMSi ether of this compound and **11** were essentially identical except that the spectrum of the hydrolysis product did not contain the δ3.5 signal present in the spectrum of **11**. These data were in accord with **11** being gossypetin 8-methyl ether 3-rutinoside acylated on the sugar with a group such as malonic acid. The ^{1}H NMR of the TMSi ether **11** gave the following signals: the rhamnosyl methyl signal appeared at δ0.88 (*d*, J = 7 Hz), 10 sugar proton signals were between δ3.55–3.9; malonyl signal at δ3.5; the signal for the H-1 of the rhamnosyl group was at δ4.63 (multiplet) and the signal for the H-1 of the glucosyl moiety was at δ5.92 (J = 7 Hz).

These basic hydrolytic studies of **5**, **6**, **7** and **11** suggest the presence of an acyl function, such as malonyl, in all of them. Unfortunately, insufficient quantities of the compounds were available for ^{13}C NMR studies to confirm the nature of the acylating group. FDMS of compound **11** gave a peak at m/z 748 (M + Na − H) consistent with the addition of $C_3H_3O_3$ to gossypetin 8-methyl ether 3-

Table 1. Chromatographic data for flavonoids from *Haplophyllum pedicellatum*, *H. robustum* and *H. glabrinum*

Compounds	R_f values in acetic acid		Colors*		
	15%	30%	UV	UV/NH_3	UV/NA
Gossypetin 8,3′-diOMe ether 3-rutinoside (**1**)	0.48	0.85	p	y	y–br
Isorhamnetin 3-rutinoside (**2**)	0.50	0.72	p	y	y
Isorhamnetin 3-galactoside (**3**)	0.35	0.66	p	y	y
Gossypetin 8,3′-diOMe ether 3-glucoside (**4**)	0.32	0.62	p	y	y–br
Isorhamnetin 3′-malonylrutinoside (**5**)	0.45	0.70	p	y	y–br
Isorhamnetin 3-malonylglucoside (**6**)	0.36	0.68	p	y	y
Kaempferol 3-malonylglucoside (**7**)	0.30	0.58	p	y	y
Isorhamnetin 7-glucoside (**8**)	0.27	0.55	y	y	y
Isorhamnetin 3-glucoside (**9**)	0.33	0.62	p	y	y
Quercetin 3-galactoside (**10**)	0.40	0.64	p	y	o
Gossypetin 8-OMe ether 3-malonylrutinoside (**11**)	0.54	0.75	p	y	o
Isorhamnetin	0.02	0.12	y	y	y

*Key; p = purple, y = yellow, y–br = yellow brown, o = orange, NA = naturstoffreagenz A.

rutinoside, as well as a peak at m/z 640 corresponding to the fragment ion for the latter. The FDMS of compounds **5**, **6** and **7** were not recorded, but the similarities in the NMR spectra indicate that the same acylating group is present in all these compounds. Whilst a more positive identification of the acylating group as malonic acid is still required in these cases, it is interesting to note that the malonic ester of awobanin has recently been proved to be the real anthocyanin present in *Commelina communis* [5].

EXPERIMENTAL

Plant material. Haplophyllum pedicellatum collected from Baluchestan (Iran) in April, 1979 (Voucher No. 350 Y.A.); *H. robustum* from Herman (900 km South of Tehran) in May, 1978 (Voucher No. 352, HRK); *H. glabrinum* from Bandar Abbas (1200 km south of Tehran) in April, 1978 (Voucher No. 349 Y.A.). Vouchers of all plants are deposited in the Herbarium of the Faculty of Pharmacy, University of Tehran.

Extraction, purification and identification of the flavonoids. The samples of *H. pedicellatum* (600 g), *H. robustum* (1600 g) and *H. glabrinum* (1500 g) were each defatted with petrol (40–60°) in a Soxhlet. The defatted samples were extracted with MeOH and the extracts concd *in vacuo*. The MeOH concentrates were each partitioned between $CHCl_3$ and H_2O and each of the aq. layers obtained were extracted with EtOAc. Upon evaporation of the EtOAc extracts 4.3 g, 7.1 g and 21.1 g of crude mixtures of flavonoids were obtained, respectively. The residues were fractioned over Polyclar columns using Egger's solvent (CH_2Cl_2–MeOH–MeCOEt–Me_2CO, 4:2:0.5:0.1); the polarity of eluent was increased by reducing the percentage of CH_2Cl_2. The following amounts of each compound were obtained from *H. pedicellatum*: gossypetin 8,3′-dimethyl ether 3-rutinoside (**1**, 6 mg), isorhamnetin 3-rutinoside (**2**, 15 mg), isorhamnetin 3-galactoside (**3**, 3 mg), isorhamnetin (5 mg), gossypetin 8,3′-dimethyl ether 3-glucoside (**4**, 15 mg), isorhamnetin 3-malonylglucoside (**6**, 8 mg), isorhamnetin 3-malonylrutinoside (**5**, 10 mg), kaempferol 3-malonylglucoside (**7**, 5 mg); from *H. robustum*: gossypetin 8,3′-dimethyl ether 3-rutinoside (**1**, 5 mg), isorhamnetin 3-rutinoside (**7**, 17 mg), isorhamnetin 7-glucoside (**8**, 6 mg), isorhamnetin 3-glucoside (**9**, 8 mg), quercetin 3-galactoside (**10**, 8 mg), isorhamnetin (5 mg); from *H. glabrinum*: gossypetin 8,3′-dimethyl ether 3-rutinoside (**1**, 30 mg), isorhamnetin 3-glucoside (**9**, 10 mg), kaempferol (15 mg) and gossypetin 8-methyl ether 3-malonylrutinoside (**11**, 35 mg). The alditol-acetate analyses were carried out on an SP2330 capillary column.

Gossypetin 8,3′-dimethyl ether 3-glucoside (**4**). UV λ_{max}^{MeOH} nm (relative intensities of bands in parentheses relative to the longest wavelength band being 1.0): 352 (1), 274 (sh), 257 (1.5); + NaOMe 420 (1 with an increase in intensity), 335 (0.1), 278 (1.2); + $AlCl_3$ and $AlCl_3$–HCl 415 (1), 363 (1.1), 305 (1), 280 (2); + NaOAc, 390 (1), 330 (0.2), 270 (1.5); + NaOAc–H_3BO_3, 352 (1), 272 (sh), 256 (1.4). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78 (1H, *d*, J = 2.5 Hz, H-2′), 7.58 (1H, *dd*, J = 2.5 Hz and 8 Hz, H-6′), 6.92 (1H, *d*, J = 9 Hz, H-5′), 6.25 (1H, *s*, H-6), 5.92 (1H, *d*, J = 7 Hz, H-1 glu), 3.9 (3H, *s*, OMe), 3.85 (3H, *s*, OMe). MS (70 eV) m/z: 346 $[M]^+$, 331 $[M-15]^+$ (greater intensity than the molecular ion peak, supporting an 8-OMe group); 183 $[A_1+1]^+$ and 151 $[B_2]^+$ indicated that one methoxyl group must be in ring A and one in ring B.

Gossypetin 8-methyl ether 3-malonylrutinoside **11**. UV λ_{max}^{MeOH} nm: 368 (1), 295 (sh), 263 (2); + NaOMe 420 (1, higher intensity) 332 (0.1), 290 (sh), 285 (2); + $AlCl_3$ 432 (1), 315 (sh), 280 (2); + $AlCl_3$–HCl, 412 (1), 363 (1.1), 300 (sh), 275 (2); + NaOAc, 380 (1), 330 (sh), 275 (1.5); + NaOAc–H_3BO_3 385 (1), 290 (sh), 264 (1.5). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.75 (2H, *m*, H-2′, H-6′), 6.98 (1H, *d*, J = 9 Hz, H-5′), 5.92 (1H, *d*, J = 7 Hz, H-1 glu), 4.63 (1H, *m*, H-1 rham), 3.96 (3H, *s*, OMe), 3.55–3.9 (sugar protons), 3.5 (2H, *s*, malonyl CH_2), 0.88 (3H, *d*, J = 7 Hz, Me-rham). FDMS m/z: 748 [M + Na − H], 640 [M − malonyl].

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