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FOUR FLAVONOID GLYCOSIDES FROM *PEGANUM HARMALA*

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Key Word Index—*Peganum harmala*; Zygophyllaceae; flavonoids; mono- and triglycoside derivatives of acacetin; cytisoside glycoside; ^{1}H and ^{13}C NMR; EI–MS and FAB–MS.

Abstract—The aerial parts of *Peganum harmala* yielded four new flavonoids: acacetin 7-*O*-rhamnoside, 7-*O*-[6″-*O*-glucosyl-2″-*O*-(3‴-acetylrhamnosyl)glucoside and 7-*O*-(2‴-*O*-rhamnosyl-2″-*O*-glucosylglucoside), and the glycoflavone 2‴-*O*-rhamnosyl-2″-*O*-glucosylcytisoside. Copyright © 1997 Elsevier Science Ltd

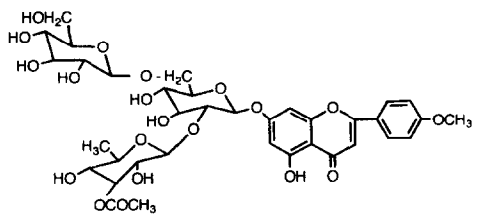
INTRODUCTION

An earlier chemical investigation of the leaves of *Peganum harmala* L. led to the isolation of peganetin [1]. The present communication describes the isolation and structural elucidation of four new glycosides. Pethes *et al.* [2] reported an acacetin rhamnoside in members of the Scrophulariaceae, but the position of the sugar was not determined.

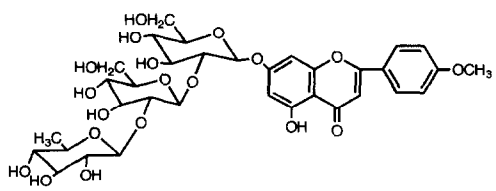
RESULTS AND DISCUSSION

The methanolic extract of the aerial parts of *P. harmala* was fractionated on a polyamide column. Purification of the compounds was achieved by a combination of silica gel TLC and sephadex LH-20. Four glycosides (**1**–**4**) (Fig. 1) were isolated and identified as follows.

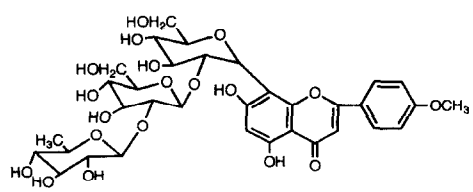
Acid hydrolysis of **1** afforded acacetin and rhamnose. This was confirmed by co-chromatography with authentic samples. The UV spectrum in methanol of the aglycone and changes observed after the addition of shift reagents [3], and the dark colour under UV light [3] suggested the presence of a free hydroxyl group at the C-5 position and that the 7-hydroxyl group was substituted. EI-mass spectrometry showed a molecular ion peak at *m/z* 430 in accordance with acacetin bearing one rhamnose moiety. The fragments at *m/z* 412, 394 and 284 were due to the loss of two successive molecules of water followed by the loss of the rhamnose unit. The ^{1}H NMR spectrum showed the expected signals of aromatic protons at δ 8.0 (*d*, J = 9 Hz) and δ 7.1 (*d*, J = 9 Hz) for H-2′,6′ and H-3′,5′. A singlet at δ 6.8 was assigned to H-3, whereas H-6 and H-8 appeared as two doublets at δ 6.3 and δ 6.5 (*d*, J = 2 Hz), respectively. The methoxy group appeared as a singlet at δ 3.86. A doublet at δ 1.1 (J = 6 Hz) was observed indicating the presence of one rhamnose methyl group, and the signal for the anomeric proton appeared at δ 5.2 (*d*, J = 2 Hz) confirming the α-configuration. Compound **1** is therefore identified as acacetin 7-*O*-α-rhamnoside.



acacetin 7-O-[6''-O-glucosyl-2''-O-(3'''-acetylrhamnosyl)]glucoside : 2



acacetin 7-O-[2'''-O-rhamnosy-2''-O-glucosyl)]glucoside : 3



2'''-O-rhamnosyl-2''-O-glucosyl-cytisoside : 4

Fig. 1. The flavonoid polyglycosides isolated from *Peganum harmala*.

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Acid hydrolysis of **2** released acacetin, glucose and rhamnose, all of which chromatographed with authentic samples. Mild acid hydrolysis gave acacetin 7-glucoside, as an intermediate, which was identified by ^{1}H NMR and acid hydrolysis yielding acacetin and glucose. The UV spectral shifts of **2** with standard reagents indicated an identical pattern to **1**, thus indicating that glycosylation was in position 7 only. Comparison of the ^{1}H NMR spectrum of **2** with that of **1** showed similarities, except that, in the ^{1}H NMR spectrum of **2**, there are two extra doublets ($J = 7.5$ Hz) at δ 5.35 and δ 4.6, and the anomeric proton of rhamnose appeared at δ 4.5. Thus **2** is a triglycoside of acacetin with one glucose moiety attached directly to the aglycone. Furthermore, a singlet representing three protons was exhibited at δ 1.85, suggesting that one of the sugar hydroxyl groups was acetylated. The signal for the methyl protons of the rhamnose unit appeared at δ 1.1 ($J = 6$ Hz). The characteristic double doublet signal ($J = 2$, $J = 10$ Hz) for one of the sugar protons at C-3 of a rhamnose moiety was shifted downfield from 3–4 to 5.03, indicating that it had an acetylated hydroxyl group [4, 5]. FAB-mass spectrometry of **2** showed a molecular weight of 796, confirming an acacetin nucleus with one rhamnose, one acetate and two glucose moieties. The fragmentation pattern [6] showed a peak at *m/z* 607 $[M - 189]^-$ due to the loss of acetylrhamnose. The further loss of glucose was indicated by peaks at *m/z* 445 $[M - 351]^-$ and *m/z* 283 $[M - 513]^-$. This confirms the presence of the acetyl group on the neohesperidoside fragment rather than on the gentiobioside or glucose fragment.

The ^{13}C NMR spectrum of **2** (Table 1) showed the presence of signals at δ 170.7 for one acetyl carbon (–OCOMe), δ 20.78 for one methyl carbon (–OCOMe) and signals for carbon atoms of one rhamnose moiety (see Table 1); these observations supported the ^{1}H NMR data. Furthermore, the signals at δ 98.32 and 77.74 were assigned to C-1″ and C-2″. This is in agreement with the observation that rhamnosylation of

Table 1. ^{13}C NMR of kaempferol 7-*O*-neohesperidoside and compounds **2**, **3** and **4**

Kaempferol 7-*O*-neohesperidoside*	δ			
C	δ	**2**	**3**	**4**
2	147.9	163	162.81	164.32
3	135.9	104.08	104.24	102.24
4	176.1	182.4	182.18	182.14
5	160.4	156.3	157.03	157.00
6	98.8	100.8	100.62	98.15
7	162.4	164.4	164.05	162.5
8	94.4	94.9	95.3	103.86
9	155.9	161.5	161.2	160.9
10	104.9	105.77	105.54	103.3
1′	121.6	122.9	122.83	122.5
2′	129.5	128.83	128.57	128.5
3′	115.5	115.1	114.86	114.8
4′	159.4	162.8	162.55	162.5
5′	115.5	115.1	114.86	114.8
6′	129.5	128.83	128.57	128.5
1″	98.4	98.32	98.35	70.6
2″	77.3	77.74	83.22	82.8
3″	77.1	76.74	76.56	77.14
4″	70.8	70.60	69.79	70.3
5″	76.09	76.03	77.44	82.6
6″	60.9	66.19	60.53	60.8
1‴	100.5	100.81	99.84	100.3
2‴	70.5	68.88	77.44	76.7
3‴	70.8	73.49	76.14	77.1
4‴	72.2	69.47	70.47	69.66
5‴	68.3	68.70	77.44	76.2
6‴	20.9	18.10	61.01	60.18
1⁗	–	102.69	102.5	99.77
2⁗	–	74.90	70.47	69.03
3⁗	–	76.32	70.85	70.6
4⁗	–	69.46	72.10	70.3
5⁗	–	76.03	68.46	68.3
6⁗	–	60.48	17.91	17.5
OCH_3	–	55.19	55.69	55.59
C=O (acetyl)	–	170.07	–	–
CH_3 (acetyl)	–	20.78	–	–

*^{13}C NMR of kaempferol 7-*O*-neohesperidoside were obtained from Markham *et al.* [8].

the neohesperidoside resulted in a 4 ppm downfield shift of the glucose C-2 signal and 2.2 ppm upfield shift of the glucose C-1 signal [7]. The ^{13}C NMR spectrum of **2** was also compared with that of kaempferol 7-*O*-neohesperidoside [8] (Table 1). Acetylation of a sugar hydroxyl shifts the signal of the carbon bearing the hydroxyl by about +2 ppm, and those of the two flanking carbon atoms by about −2 ppm [9, 10]. Thus the signals appearing at δ 68.88, 73.49 and 69.47 were assigned to C-2‴, C-3‴ and C-4‴, respectively. The signal at δ 66,19 was assigned to C-6″ due to the β-glucosidation of the glucose at C-6, while the signal which appeared at 60.48 was assigned to C-6‴. From the above data compound **2** is identified as acacetin 7-*O*-[6″-*O*-glucosyl-2″-*O*-(3‴-acetylrhamnosyl)]glucoside.

The UV and ^1H NMR spectra of compound **3** were identical to those of **2**, except for the absence of the acetyl group signals from the ^1H NMR spectrum of **3**. Thus **3** is a triglycoside of acacetin. The sugars were identified as glucose and rhamnose, as indicated by acid hydrolysis. The ^1H NMR spectrum of **3** indicated the presence of a glucose moiety directly attached to the aglycone nucleus (δ 5.35, *d*, $J = 7$ Hz). In the ^{13}C NMR spectrum of **3** (Table 1) the two signals of the glucose C-2 atoms were shifted downfield, suggesting a $1 \rightarrow 2$ linkage between the sugar moieties. The assignments of the sugar and the aglycone carbon atoms in Table 1 were based on those given in the literature [8, 11–13]. To clarify the position of the interglycosidic linkage a method described for the analysis of polysaccharides was used [14, 15], which has been used also for the structural determination of the sugar moieties in flavonoids [16, 17]. Permethylated **3** was hydrolysed and the methylated sugars were then reduced and acetylated. GLC analysis of the methylated alditiol acetates gave 1,2,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-glucitol and 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol, which corresponds to glucose substituted in position 2 with a terminal rhamnose (Table 2). Therefore, **3** is identified as acacetin 7-*O*-(2‴-*O*-rhamnosyl-2″-*O*-glucosyl)glucoside.

Acid hydrolysis of compound **4** released glucose, rhamnose and **4a**. The ^1H NMR spectrum of **4** showed three doublets at δ 5.2, 4.6 and 4.5 ($J = 7$, 7, 2 Hz), suggesting that **4** is a triglycoside. The ^1H NMR spectrum of **4a** showed a very close similarity to that of **4**, the only significant difference being the absence of the doublets at δ 4.6, 4.5 and 1.5 from the spectrum of **4a**. This suggested that **4a** is a *C*-glycoside and **4** is a triglycoside [3]. The ^1H NMR spectra of **4**, **4a**, **2** and **3** showed similarities, the differences being the absence of the doublet assigned to H-8 from the spectra of **4** and **4a**, suggesting that **4a** is an 8-*C*-glycoside. This was confirmed by the ^{13}C NMR spectrum of **4** and **4a**, wherein the signal assigned to C-8 was shifted downfield from 95.3 in **3** to 103.86 in **4** and **4a**, confirming that *C*-glycosylation was in position 8 of the aglycone moiety. Comparison of the sugar carbon signals of **4a** with that of vitexin [18b] showed similarities. The carbon values correspond well to each other and there are no significant differences between the glucose signals. Thus compound **4a** is identified as cytisoside (5,7-dihydroxy-4′-methoxy-8-*C*-glucosylflavone). The ^{13}C NMR spectrum of **4** was very similar to that of **3**. Thus, the signals which appeared at δ 70.6, 82.80, 100.3 and 76.7 were assigned to C-1″, C-2″, C-1‴ and C-2‴, respectively. This is in agreement with the observation that β-glucosylation (in the sophoroside) and rhamnosylation (in the neohesperidoside) exhibited upfield shifts of the glucose C-1 peak of about 2 and 2.2 ppm and a downfield shifts of about 8 and 4 ppm for the glucose C-2 signals [18a]. Thus compound **4** was identified as 2‴-*O*-rhamnosyl-2″-*O*-glucosyl-cytisoside.

EXPERIMENTAL

Plant material. Peganum harmala L. leaves were collected during May from Wadi Firan, Southern Sinai. A voucher specimen was deposited in the Herbarium of NRC, Cairo.

Extraction and isolation. The air-dried plant was extracted with 80% MeOH the concentrated extract was subjected to polyamide CC. The major components (**1–4**) were isolated by prep. TLC on silica gel GF_{254} (Merck) eluted with $CHCl_3$–MeOH–H_2O (65:45:12), and further purified using Sephadex LH-20 eluted with MeOH.

Acid hydrolysis. Glycosides were hydrolysed with 2 N HCl at 100° for 60 min.

Spectroscopic methods. UV spectra were obtained as described in literature [3]. EI–MS at 70 eV, ion source 200°. FAB-MS: the sample was suspended in triethanolamine and the target was bombarded with 7–8 kV Xe atoms. NMR spectra were measured in DMSO-d_6.

The permethylether was prepared with NaH, DMSO and CH_3I according to Hakomori's procedure [19]. The permethylated glycoside was hydrolysed with 8% H_2SO_4 at 100° for 1 hr. The acidic sugar soln was separated from the aglycone on a polyamide column

Table 2. GLC analysis of the methylated alditol acetates.

Alditol acetate	Retention times*	
	This work	Björndal *et al.* [20]
1,5-Di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol	0.46	0.47
1,2,5-Tri-*O*-acetyl-3,4,6-tri-*O*-methyl-D-glucitol	4.21	4.25

*Values are relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

and then reduced with $NaBH_4$ and acetylated with Ac_2O in pyridine, as described previously [13]. Retention times in GLC analysis are given in Table 2.

Compound **1**: *acacetin* 7-O-*rhamnoside*. UV (λ_{max} nm): MeOH, 267, 325; +NaOMe, 290, 377; +$AlCl_3$, 277, 298, 338, 383; $AlCl_3$ + HCl, 275, 298, 335, 383; +NaOAc, 267, 325; NaOAC + H_3BO_3, 267, 325. ^{1}H NMR: δ 8.0 (2H, *d*, J = 9 Hz, H-2′, 6′), 7.1 (2H, *d*, J = 9 Hz, H-3′, 5′), 6.8 (1H, *s*, H-3), 6.5 (1H, *d*, J = 2 Hz, H-8), 6.2 (1H, *d*, J = 2 Hz, H-6), 5.2 (1H, *d*, J = 2 Hz, H-1 rha), 3.85–3.50 (*m*, 4H rha), 3.90 (3H, *s*, –OMe), 1.1 (3H, *d*, J = 6 Hz, Me rha). EI-MS (rel. int. %): *m/z* 430 (13%), 412 (21%), 394 (18%), 284 (100%).

Compound **2**: *acacetin* 7-O-[6″-O-*glucosyl*-2″-O-(3‴-*acetylrhamnosyl*)]*glucoside*. UV (λ_{max} nm): MeOH, 268, 335; +NaOMe, 278, 370; +$AlCl_3$, 275, 295_{sh}, 343, 382, $AlCl_2$ + HCl, 275, 295_{sh}, 336, 382; +NaOAc, 268, 335; NaOAc + H_3BO_3, 268, 335. ^{1}H NMR: δ 8.05 (2H, *d*, J = 9 Hz, H-2′, 6′), 7.15 (2H, *d*, J = 9 Hz, H-3′, 5′), 6.95 (1H, *s*, H-3), 6.85 (1H, *d*, J = 2 Hz, H-8), 6.75 (1H, *d*, J = 2 Hz, H-6), 5.4 (1H, *d*, J = 7.5, H-1″), 5.2-4 (*m*, sugar protons, 15H *s*), 3.85 (3H, *s*, OMe), 1.85 (3H, *s*, –COMe), 1.1 (3H, *d*, J = 6 Hz, Me rham.). FAB-MS (negative mode) (rel. int. %): *m/z* 795 $[M-H]^-$ (8%), 607 $[M-189]^-$ (2%), 445 $[M^+-351$ (2%), 283 $[M^+-513]^-$ (6%). ^{13}C NMR: see Table 1.

Compound **3**: *acacetin* 7-O-(2‴-O-*rhamnosyl*-2″-O-*glucosyl*)*glucoside*. UV (λ_{max} nm): MeOH, 267, 325; +NaOMe, 285, 360; +$AlCl_3$, 277, 297, 337, 382; $AlCl_3$ + HCl, 276, 297, 335, 382; NaOAc, 267, 325; NaOAc + H_3BO_3, 267, 325. ^{1}H NMR: δ 8.05 (2H, *d*, J = 9 Hz, H-2′, 6′), 7.15 (2H, *d*, J = 9 Hz, H-3′, 5′), 6.95 (1H, *s*, H-3), 6.8 (1*H*, *d*, J = 2 Hz, H-8), 6.5 (1H, *d*, J = 2 Hz, H-6), 5.35 (1H, *d*, J = 7 Hz, H-1″), 4.9–4.0 (*m*, sugar protons, 15H *s*), 3.86 (3H, *s*, Ome), 1.1 (3H, *d*, J = 6 Hz, Me rham.). ^{13}C-NMR: see Table 1. The retention times of the methylated alditol acetates resulting from the acid hydrolysis of the permethylated **3** are given in Table 2.

Compound **4a**. *cytisoside*. ^{1}H-NMR: δ 7.9 (2H, *d*, J = 9 Hz, H-2′, 6′), 7.0 (2H, *d*, J = 9 Hz, H-3′, 5′), 6.75 (*s*, H-3), 6.44 (*s*, H-6), 5.2 (1H, *d*, J = 7 Hz, H-1″), 3.8 (3H, *s*, –OMe).

Compound **4**: 2‴-O-*rhamnosyl*-2″-O-*glucosylcytisoside*. UV (λ_{max} nm): MeOH, 267, 325; +NaOMe, 290, 377; +$AlCl_3$, 277, 298, 338, 383; $AlCl_3$ + HCl, 275, 298, 335, 383; NaOAc, 267, 325, NAOAc + H_3BO_3, 267, 325. ^{1}H NMR: δ 7.95 (2H, *d*, J = 9 Hz, H-2′, 6′), 7.1 (2H, *d*, J = 9 Hz, H-3′, 5′), 6.8 (1H, *s*, H-3), 6.45 (1H, *s*, H-6), 5.2 (1H, *d*, J = 7 Hz, H-1″), 4.6 (1H, *d*, J = 7 Hz, H-1‴), 4.5 (1H, *d*, J = 2 Hz, H-1⁗), 4.8–3.95 (*m*, sugar protons, 15H *s*), 3.85 (3H, *s*, OMe), 1.5 (3H, *d*, J = 6 Hz, Me rha.). ^{13}C-NMR: see Table 1.

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