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PEGANETIN, A NEW BRANCHED ACETYLATED TETRAGLYCOSIDE OF ACACETIN FROM *PEGANUM HARMALA*

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The flavonoids of the family Zygophyllaceae are presently under investigation with the aim of studying the chemosystematics of the family (1-3). *Peganum harmala* L. (Zygophyllaceae) belongs to the subfamily Peganoideae (4). The present study deals with the major flavonoid component of *P. harmala*, peganetin, a new natural product the structure of which is proposed to be acacetin-7-*O*-[rhamnosyl(1↦4″)-gluco-(1↦6″)-6‴-*O*-acetyl-sophoroside] [**1**]; **1** was isolated by preparative pc. Acid hydrolysis of **1** gave rise to acacetin, glucose, and rhamnose, all of which co-chromatographed with authentic samples. Moreover, the identity of the aglycone, acacetin, was confirmed by uv, ms, and ^{1}H nmr. The uv data indicated that glycosylation was at position 7 of acacetin (no shift of band 11 with NaOAc and absence of peak at 325 with NaOMe.

^{1}H-nmr of **1** showed a two proton doublet for H-2′ and H-6′ at δ 8.0 (J=9 Hz) coupled to another doublet at δ 7.2 (J=9 Hz) for H-3′ and H-5′. One peak is present as a singlet at δ 6.9 for H-3, whereas H-6 and H-8 appeared at δ 7.05 and 7.1 (J=2.5 Hz), respectively. A three-proton signal that appeared as a doublet (J=6.5 Hz) for the rhamnose methyl was present upfield at δ 1.6. The negative ion fabms showed a molecular weight of 958, which indicated an acacetin nucleus with three glucose, one rhamnose, and one acetate moieties. The fragmentation pattern (11,12) showed a peak at *m/z* 915 [M-43]$^-$ due to the loss of the acetate. The loss of acetylglucose and rhamnose was indicated by peaks at *m/z* 753 [M-205]$^-$ and 811 [M-147]$^-$, respectively. This confirms the existence of the acetyl group on the sophoroside fragment rather than the gentiobioside or rhamnose fragment (1↦2 linkage is weaker than a 1↦6 linkage).

The sugar sequence was determined through the ^{13}C-nmr data. The four anomeric carbon atoms appeared at 104, 102.4, 100.4, and 97.98 ppm. The signals at 97.98 and 83.0 ppm were assigned to C-1″ and C-2″. This is in agreement with a previous observation that β-glucosylation at C-2 (e.g., in sophoroside) in disaccharides and oligosaccharides exihibited an upfield shift of about 2.1 ppm for C-1 and a downfield shift of about 8 ppm for C-2 (5,6). In the case of quercetin 3-gentiobioside also isolated from the Zygophyllaceae (2), β-glucosylation of C-6 showed a downfield shift to 66-68 ppm (see Table 1). Thus, the signal which appeared at δ 68.2 ppm was assigned to C-6″. Furthermore, acetylation of a

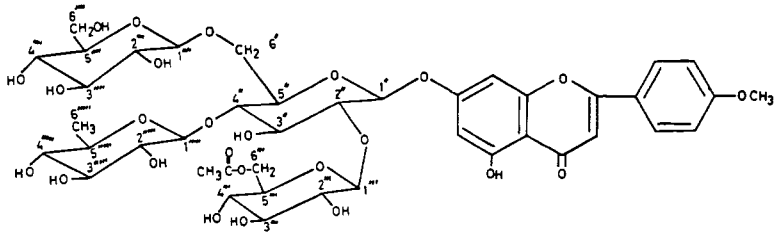

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TABLE 1. ^{13}C-nmr Data of Peganetin [1] and Quercetin 3-Gentiobioside

Carbon Atom	Compounds	
	[1]	Quercetin 3-gentiobioside
C-4	182.1, s	177.5 ,s
Me*C*O	170.3, s	
C-7	164.0, s	164.36, s
C-2	162.8, s	156.56, s
C-4′	162.5, s	148.53, s
C-5	161.3, s	156.56, s
C-9	157.0, s	161.37, s
C-2′	128.5, d	115.40, d
C-6′	128.5, d	121.83, d
C-1′	122.7, s	121.31, s
C-3′	114.7, d	144.92, s
C-5′	114.7, d	116.43, d
C-10	105.5, s	104.14, s
C-3	104.2, d	133.47
C-1‴	104.0, d	
C-1″	102.4, d	103.23, d
C-1⁗	100.4, d	
C-6	100.1, d	98.87, d
C-1″	97.9, d	101.09, d
C-8	95.2, d	93.80, d
C-2″	83.0, d	74.09, d
C-6″	68.2, t	68.24, t
C-6‴	62.7, t	60.90, t
C-6⁗	60.8, t	
O*Me*	55.5, q	
*Me*CO	20.3, q	
C-6⁗	17.7, q	

sugar hydroxyl causes a shift of the signal of the sugar carbon bearing the hydroxyl group downfield by ca. 2 ppm (7,8). Consequently, the signal at δ 62.7 ppm could be assigned to C-6‴, whereas the signal at δ 60.79 ppm could be assigned to C-6⁗. The rhamnose moiety is attached most probably at C-4″. The remaining signals for the other carbons atoms of the tetrasaccharide could not be assigned, as no ^{13}C-nmr models for tetrasaccharides were available (9,10). The results of the ^{13}C nmr are outlined in Table 1. This is the first report of a branched tetrasaccharide with an acetate group.

EXPERIMENTAL

PLANT MATERIAL.—*P. harmala* leaves were collected from Wadi Firan, South of Sinae; voucher specimens are deposited in the Herbarium of Cairo University.

EXTRACTION AND ISOLATION.—The plant material (150 mg) was extracted with EtOH-H_2O (7:3). The EtOH was removed under reduced pressure, and the aqueous concentrate was subjected to column chromatography on polyamide using H_2O and increasing concentrations of EtOH. The major component **1** (22 mg) was separated by preparative pc on Whatmann 3MM developed in *n*-BuOH-HOAc-H_2O (4:1:5) and further purified on Sephadex LH-20.

ELUCIDATION OF STRUCTURE [1].—Standard methods of uv, ms, ^{1}H nmr, ^{13}C nmr, and chemical analysis were employed. Acid hydrolysis in 2 N HCl yielded acacetin, glucose, and rhamnose. Rf, on Whatmann no. 1, *n*-BuOH-HOAc-H_2O (4:1:5), 0.2; H_2O, 0.9; uv λ max (MeOH) 266, 269, 326; NaOMe, 287, 379; $AlCl_3$, 274, 297, 338, 381; $AlCl_3$-HCl, 276, 299, 336, 382; NaOAc, 267, 279, 326; NaOAc-H_3BO_3, 267, 270, 328; fabms 958 [M], 957 [M-H]$^-$, 943 [M-15]$^-$, 915 [M-43]$^-$], 811 [M-147]$^-$, 735 [M-205]$^-$; ^{1}H nmr (in C_6D_6N) δ 1.6 (3H, d, rha-CH_3), 2.0 (3H, s, acetate), 6.9 (1H, s, H-3), 7.05 (1H, d, J=2.5 Hz, H-6), 7.1 (1H, d, J=2.5 Hz, H-8), 7.2 (2H, d, J=9.0 Hz, H-3′,5′), 8.0 (2H, d, J=9.0 Hz, H-2′,6′). For ^{13}C nmr see Table 1.

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