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Methoxyflavonoids from *Pinaropappus roseus*

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Three rare methoxyflavonoids, rhamnazin 3-O-rutinoside (**1**), rhamnocitrin 3-O-rutinoside (**2**) and isorhamnetin 3-O-rutinoside (**3**), were isolated from the methanolic extract of *Pinaropappus roseus* together with 2 well-known flavonoids, quercetin 3-O-rutinoside (=rutin) (**4**) and kaempferol 3-O-rutinoside (**5**). The structures of the isolated compounds were elucidated on the basis of UV, 1D and 2D NMR and FAB-Mass spectral analysis. Free radical scavenging activities of the isolated compounds were also demonstrated. This is the first report on the chemical composition of the genus *Pinaropappus*.

Key Words: *Pinaropappus roseus*, Asteraceae, rhamnazin 3-*O*-rutinoside, rhamnocitrin 3-O- rutinoside, isorhamnetin 3-*O*-rutinoside, flavonoids.

Introduction

Pinaropappus roseus is a Mexican plant. Decoctions of the aerial parts of the plant are used for the treatment of constipation in Mexico as a folk medicine[1]. There have been no reports on the chemical constituents of the genus *Pinaropappus* to date. Our preliminary studies showed the rich flavonoid contents of this plant. Flavonoids are phenolic substances isolated from a wide range of vascular plants, with thousands of individual compounds. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilatating actions[2]. However, most interest has been devoted to the antioxidant activities of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals[3]. With regard to the bioactivities of flavonoids and the wide usage of the plant in traditional medicine, free radical scavenger potentials of isolated compounds were determined by their protective effect against reactive oxygen species-induced impairment of the endothelium-dependent relaxation response in rat aortic rings, and were reported previously[4,5]. The present study deals with the isolation and characterization of 3 rare methoxyflavonoids together with 2 well-known flavonoid glycosides.

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Experimental

General Procedures: The UV spectra (λ_{max}) were recorded on a Shimadzu UV-240 spectrophotometer. NMR measurements were performed on a JEOL JNM-A 500 spectrometer with tetramethylsilane (TMS) as an internal standard. FAB-MS was recorded in an NBA matrix in the positive ion mode on a JEOL JMS-DX300 spectrometer. TLC plates using Silica gel 60 F_{254} and RP-18 F_{254} were obtained from Merck (Darmstadt, Germany). Medium pressure liquid chromatography (MPLC) was performed using a Lobar glass column packed with reversed-phase material (Sepralyte RP-18, 18.5 x 352 mm). High performance liquid chromatography (HPLC) was performed on an Asahipack ODP-50 column (10 x 250 mm).

Plant Material: *P. roseus* Less. (Asteraceae) was collected from the environs of Morelos in Mexico. It was identified by the head of the Morelos Ethnobotanical Garden and a voucher specimen is deposited in the Ethnobotanical Garden of the Mexico National Anthropology and History Museum.

Extraction and Isolation: Air dried aerial parts of the plant were extracted with MeOH at 50 °C (2X, 2 L). The combined extract was evaporated under vacuum nearly to dryness. H_2O (0.5 L) was added and H_2O insoluble material was removed by filtration. The filtrate was subjected to polyamide column chromatography and elution with H_2O, followed by increasing concentrations of MeOH to yield 2 main fractions, Frs. A and B. Fraction A was chromatographed over MPLC and eluted with increasing concentrations of MeOH (30→60%) to give compound **4** and a mixture of compounds **3** and **5**. **C**ompounds **3** and **5** were purified using HPLC by 15% acetonitrile. Fraction B was subjected to MPLC by stepwise elution with MeOH:H_2O (40:60→60:40) to give a mixture of compounds **1** and **2**. The mixture of **1** and **2** was rechromatographed over HPLC by 15% acetonitrile to give compounds **1** and **2** as pure forms.

Rhamnazin 3-*O*-rutinoside (1): Pale yellow, amorphous powder. FAB-MS m/z 638 $[M]^+$ (calcd. for $C_{29}H_{34}O_{16}$); UV λ_{max} (MeOH) nm: 259, 358; ^{1}H NMR (C_5D_5N, 500 MHz): Table 2; ^{13}C NMR (C_5D_5N, 125 MHz): Table 1[6].

Rhamnocitrin 3-*O*-rutinoside (2): Pale yellow, amorphous powder. FAB-MS m/z 608 $[M]^+$ (calcd. for $C_{28}H_{32}O_{15}$); UV λ_{max} (MeOH) nm: 257, 350; ^{1}H NMR (C_5D_5N, 500 MHz): Table 2; ^{13}C NMR (C_5D_5N, 125 MHz): Table 1[7].

Isorhamnetin 3-*O*-rutinoside (3): Pale yellow, amorphous powder. FAB-MS m/z 624 $[M]^+$ (calcd. for $C_{28}H_{32}O_{16}$); UV λ_{max} (MeOH) nm: 258, 349; ^{1}H NMR (CD_3OD, 500 MHz): Table 2; ^{13}C NMR (CD_3OD, 125 MHz): Table 1[8].

Quercetin 3-*O*-rutinoside (= Rutin) (4): Pale yellow, amorphous powder. UV, ^{1}H and ^{13}C NMR data were identical to those reported in the literature[8].

Kaempferol 3-*O*-rutinoside (5): Pale yellow, amorphous powder. UV, ^{1}H and ^{13}C NMR data were identical to those reported in the literature[9].

Results and Discussion

Compounds **1-5** (Figure 1) were obtained as pale yellow, amorphous powders, which gave a characteristic flavonoid color reaction on TLC using the vapor of ammonia. Their UV spectra showed absorption peaks (257-358 nm) arising from the flavonols and that suggested the glycosidation of the C-3 (OH) position.

Table 1. ^{13}C NMR spectral data for compounds **1-3*** (δ in ppm; 125 MHz).

C	DEPT	1	2	3
Aglycone				
2	C	158.1	158.4	156.4
3	C	135.1	135.0	133.3
4	C	178.8	178.8	179.6
5	C	162.3	162.3	162.0
6	CH	98.6	98.6	98.7
7	C	165.9	165.9	164.0
8	CH	92.6	92.6	93.9
9	C	157.4	157.4	156.2
10	C	106.3	106.3	105.0
1′	C	121.9	121.8	122.4
2′	CH	114.3	132.1	112.6
3′	C†	149.5	116.2	148.5
4′	C	150.3	161.9	135.1
5′	CH	116.4	116.2	114.5
6′	CH	123.7	132.1	124.0
7-OMe	CH_3	55.9	55.9	
3′-OMe	CH_3	56.1		56.8
Glucose				
1″	CH	103.8	104.3	105.0
2″	CH	76.0	76.2	76.0
3″	CH	77.5	77.7	77.4
4″	CH	71.5	71.7	71.7
5″	CH	78.5	78.6	78.3
6″	CH_2	68.3	68.5	68.6
Rhamnose				
1‴	CH	102.6	102.6	102.6
2‴	CH	72.5	72.1	72.2
3‴	CH	72.6	72.2	72.3
4‴	CH	73.9	73.8	73.9
5‴	CH	69.7	69.6	69.8
6‴	CH_3	18.5	18.4	17.9

*in C_5D_5N for **1-2**, CD_3OD for **3**
†CH for **2**

The FAB-MS of compound **1** exhibited the pseudomolecular ion $[M]^+$ at m/z 638, compatible with the molecular formula of $C_{29}H_{34}O_{16}$ and in good agreement with the presence of 3 methyl, 1 methylene, 15 methine and 10 quaternary carbon resonances in its ^{13}C NMR and DEPT spectra (Table 1). Its ^{1}H NMR spectrum (Table 2) showed expected signals for the 3, 5, 7, 3′ and 4′-pentasubstitute flavonoids in the aromatic region: 2 doublets at δ 6.57 and 6.64 (J= 1.8 Hz) for H-6 and H-8, and 3 signals as an ABX system at δ 7.43 (d, J= 8.5 Hz), 7.96 (dd, J= 8.5/1.8 Hz) and 8.42 (d, J = 1.8 Hz) for H-5′, H-6′ and H-2′, respectively. Two singlets at δ 3.73 and 3.93 (each 3H; δ_C 55.9 and 56.1) were assigned to the methoxy groups at the C-7 and C-3′ positions on the basis of HMBC correlations observed between the following protons and carbons: OCH_3 (δ 3.73, s) and C-7 of the A ring (δ 165.9, C), and OCH_3 (δ 3.93, s) and C-3′ of the benzoyl moiety (δ 149.5, C). The presence of 2 anomeric proton signals at δ 6.31 d (J= 7.3 Hz) and 5.35 br.s indicated its disaccharidic structure. ^{1}H and ^{13}C NMR signals assigned to the sugar moiety

Table 2. ^{1}H NMR spectral data for compounds **1-3*** (δ in ppm, 500 MHz).

H	**1**		**2**		**3**	
Aglycone						
6	6.57 d	(1.8)	6.57 d	(1.8)	6.11 d	(1.8)
8	6.64 d	(1.8)	6.62 d	(1.8)	6.28 br.s	
2′	8.42 d	(1.8)	8.49 d	(8.5)	7.94 d	(1.8)
3′			7.34 d	(8.5)		
5′	7.43 d	(8.5)	7.34 d	(8.5)	6.89 d	(8.5)
6′	7.96 dd	(8.5/1.8)	8.49 d	(8.5)	7.63 dd	(8.5/1.8)
7-OMe	3.73 s		3.70 s			
3′-OMe	3.93 s				3.94 s	
Glucose						
1″	6.31 d	(7.3)	6.11 d	(7.3)	5.14 d	(7.3)
2″	4.10-4.39†		4.10-4.39†		3.45 dd	(9.8/7.9)
3″					3.25†	
4″					3.24 t	(9.2)
5″					3.40 m	
6a″	4.53 dd	(11.6/1.2)	4.53 dd	(11.6/1.2)	3.81 dd	(11.6/1.8)
6b″	3.99 dd	(12.2/6.0)	4.06 dd	(11.6/6.0)	3.44 dd	(12.2/5.9)
Rhamnose						
1‴	5.35 br.s		5.31 br.s		4.52 d	(1.8)
2‴	4.10-4.39†		4.10-4.39†		3.63 dd	(3.1/1.8)
3‴					3.49 dd	(9.8/3.7)
4‴					3.25 t	(9.8)
5‴					3.35 dd	(3.1/1.8)
6‴	1.48 d	(6.1)	1.49 d	(6.1)	1.12 d	(6.1)

*in C_5D_5N for **1-2**, CD_3OD for **3**

†Signal patterns are unclear due to overlapping.

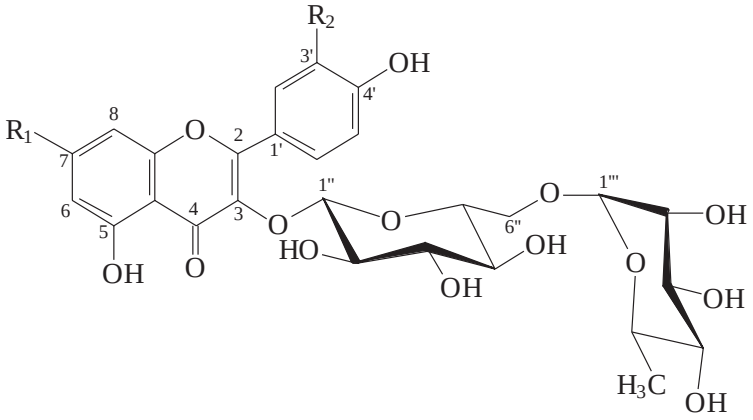

Figure 1. Isolated compounds from *Pinaropappus roseus* (**1-5**).

	R_1	R_2
1	OCH_3	OCH_3
2	OCH_3	H
3	OH	OCH_3
4	OH	OH
5	OH	H

showed that **1** should be composed of β-D-glucopyranose and α-L-rhamnopyranose units considering their chemical shifts and coupling constants. The secondary methyl signal at δ 1.48 (d, J = 6.1 Hz) supported the presence of a rhamnosyl unit. Chemical shifts of protons due to glucose and rhamnose moieties were assigned unambiguously from the COSY and HMQC experiments. Downfield shifts of C-6″ in the ^{13}C NMR spectrum and HMBC correlation between H-1‴ (δ 5.35 br.s) and C-6″ (δ 68.3) indicated that rhamnose was linked to C-6″ of the glucose, confirming the rutinose unit as a sugar moiety. The attachment of the rutinose moiety to the aglycone was determined from the correlations between H-1″ (δ 6.31 d) and C-3 (δ 135.1) of the aglycone (Figure 2). From these results, compound **1** was assumed to be rhamnazin 3-*O*-rutinoside and this was confirmed by the comparison of its spectral data with the literature values for rhamnazin 3-*O*-rutinoside[6].

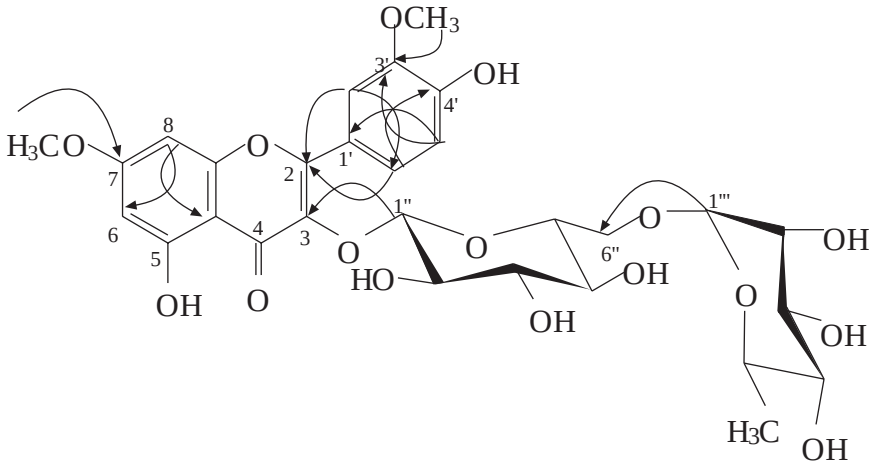

Figure 2. Selected HMBC correlations for compound **1.**

The molecular formula of compound **2** was established as $C_{28}H_{32}O_{15}$ by FAB-MS. Its ^{1}H and ^{13}C NMR spectra closely resembled those of **1** (Tables 1 and 2). The difference was the presence of 2 doublets centered at δ 7.34 (2H, d, J = 8.5 Hz) and 8.49 (2H, d, J= 8.5 Hz) assigned to the A_2B_2 system of B ring aromatic protons due to a *p*-hydroxybenzoyl group and the presence of only 1 methoxyl group at δ 3.70 (3H, s), which exhibited HMBC correlation with C-7 (δ 165.9) of the aglycone. Therefore, the structure of compound **2** was established as rhamnocitrin 3-*O*-rutinoside (Figure 1) and its spectral data were also superimpossable on those of the data published for rhamnocitrin 3-*O*-rutinoside[7].

The FAB-MS of **3** exhibited a pseudomolecular ion, $[M]^+$, at m/z 624, which was 14 mass units lower than that of **1,** compatible with the molecular formula of $C_{28}H_{32}O_{16}$. The ^{1}H and ^{13}C NMR spectra of **3** (Tables 1 and 2) were also similar to those of **1**, except for the absence of a methoxy group signal arising from the A ring. Thus, the structure of **3** was established as isorhamnetin 3-*O*-rutinoside (Figure 1) and was confirmed by the comparison of data published for isorhamnetin 3-*O*-rutinoside[8].

UV absorption together with the ^{1}H and ^{13}C NMR spectra of 2 major compounds (**4** and **5**) indicated that compounds **4** and **5** consisted of flavonol-3-*O*-glycosides. Their structures were determined as quercetin 3-*O*-rutinoside (**4**) and kaempferol 3-*O*-rutinoside (**5**) by comparison of their spectral data with those reported in the literature[8,9].

The common flavonols are of systematic interest in plant families because they occur in combined form as glycosides, and the nature of the sugars and the positions of their attachment to the flavonoid

nucleus are often specific to a particular plant group. Rhamnazin 3-*O*-rutinoside (**1**), rhamnocitrin 3-*O*-rutinoside (**2**) and isorhamnetin 3-*O*-rutinoside (**3**) are known compounds, but are not very common[10]. Free radical scavenger activity of compounds **1-5** was previously determined by their protective effect against reactive oxygen species-induced impairment of the endothelium-dependent relaxation response in rat aortic rings[4,5,11]. Pre-incubation of the aortic rings with rhamnazin 3-*O*-rutinoside (**1**), rhamnocitrin 3-*O*-rutinoside (**2**), isorhamnetin 3-*O*-rutinoside (**3**) and quercetin 3-*O*-rutinoside (**4**) did not prevent the electrolysis-induced inhibition of endothelium-dependent relaxation, and no significant activity was detected. However, kaempferol 3-*O*-rutinoside (**5**) was found to be moderately active in this system[4]. This study is the first report on the chemical composition of the genus *Pinaropappus*.

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References

1. J.R. Diaz, "Index y Sinonoma de las Plantes Medicinales de Mexico", Instituto Mexicana para el Estudio de las Plantes Medicinales. Mexico City (1976).
2. P. Pietta, **J. Nat. Prod., 63**, 1035-1042 (2002).
3. C.A. Rice-Evans, N.J. Miller, P.G. Bolwell, P.M. Bramley and J.B. Pridham, **Free Rad. Res., 22**, 375-383 (1995).
4. I. Saracoglu, U.S. Harput, P. Kelicen, I. Erdemli, K. Kojima and Y. Ogihara, XIth Symposium on Plant Originated Pharmaceutical Raw Materials, Ankara, Turkey 22-24 May (1996)
5. U.B. Ismailoglu, I. Saracoglu, U.S. Harput and I. Sahin-Erdemli, **J. Ethnopharmacol.**, **79**, 193-197 (2002).
6. H. Itokawa, Y. Oshida, A. Ikuta, H. Inatomi and S. Ikegami, **Phytochemistry, 20**, 2421-2422 (1981).
7. M. Krauze-Baranowska, **Acta Pol. Pharm., 53**, 53-54 (1996).
8. J.B. Harborne and T.J. Mabry, "The Flavonoids: Advances in Research". Chapman and Hall Ltd., London (1982).
9. T.J. Mabry, K.R. Markham and M.B. Thomas, "The Systematic Identification of Flavonoids", Springer-Verlag, Heidelberg (1970).
10. J.B. Harborne, "The Flavonoids: Advances in Research". Chapman and Hall Ltd., London (1986).
11. B. Gumusel, B.C. Tel., R. Demirdamar and I. Sahin-Erdemli, **Eur. J. Pharmacol., 306,** 107-112 (1996).