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New methoxyflavone glycosides from *Verbena bipinnatifida* Nutt.

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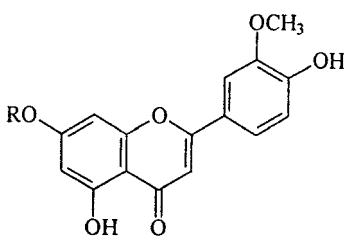
Two new methoxyflavone glycosides: chrysoeriol-7-*O*-(2,6-dirhamnosyl)-glucoside and chrysoeriol-7-*O*-neohesperidoside and seven other known flavonoids, were isolated and characterised from the aerial parts of *Verbena bipinnatifida* Nutt. (Verbenaceae). Their structures were elucidated by spectral and chemical methods of analysis.

1. Introduction

Plants of the family Verbenaceae are cultivated mainly from Egypt to Morocco. It is already reported that some *Verbena* species have medicinal uses [1]. The polyphenolic and flavonoid components of the flowers of some *Verbena* species have been thoroughly investigated [2–8]. One cafeoil glycoside and two irridoid glycosides were isolated from the aerial parts of *V. littoralis* [9]. The leaves of *V. officinalis* L. proved to contain important variety of flavonoids which were identified as 6-O-substituted flavones (sorbifolin, pedalitin and nepetin) [10] and another four O-glycosyl flavones: luteolin-7-glucoside, pedalitin-6-glucoside, apigenin-7-galactoside and 7-glucuronide [11]. *V. peruviana*, on the other hand, afforded 8 flavonoids: kaempferol, quercetin, luteolin, k-3-*O*-(3-acetylglucoside), k-3-glucoside, q-3-*O*-(3-acetylglucoside), 1–7-glucoside and 7-rutinoside [12]. From the aerial parts of *V. officinalis* L., Makboul [13] identified artemetin (5-hydroxy-3,6,7,3′,4′-pentamethoxy flavone), lupeol, β-sitosterol and ursolic acid, while Darnet [14] identified luteolin-7-diglucuronide. Pogani [15] found that *V. lonariensis* L. contains griselinoside, nodifloretin and small amounts of penduletin. Referring to *V. bipinnatifida* Nutt., nearly nothing was reported on flavonoid constituents in the literature, the only report was about the taxonomic difficulties which have been attributed to widespread hybridization [16].

2. Investigations, results and discussion

From the water extract of the aerial parts of *Verbena bipinnatifida* Nutt., nine flavonoid components were isolated including two new methoxyflavone glycosides, chrysoeriol-7-*O*-(2,6-dirhamnosyl)-glucoside (**1**) and chrysoeriol-7-*O*-neohesperidoside (**2**) together with seven known flavonoids namely: diosmetin-7-*O*-neohesperidoside, 7-*O*-glucuronide, 7-*O*-glucoside, chrysoeriol-7-*O*-rutinoside, 7-*O*-glucoside, diosmetin and chrysoeriol which were identified by R_f-values, colour reactions, co-chromatography matching with authentic samples, UV spectral analysis, chemical methods, ^{1}H- and ^{13}C NMR spectroscopy.



(**1**) R = 2,6-dirhamnosyl glucoside
(**2**) R = neohesperidose (rhamnosyl 1→ 6 glucoside)

The new natural glycoside **1** was identified through R_f-values, colour reactions, and UV spectral data which suggested that **1** is of a flavone type substituted at 7 and 3′ positions, since the addition of NaOAc and NaOAc/H_3BO_3 produced no shift in band II and I respectively, indicating the absence of a free 7-OH group in ring A or a free 3′,4′-dihydroxyl group in ring B. Addition of NaOMe led to a bathochromic shift in band I (54 nm) without decrease in intensity suggesting a substitution in position 3′. Complete acid hydrolysis of **1** yielded chrysoeriol as the aglycone (R_f-values, colour reactions and UV spectral data), glucose and rhamnose indicating that the sugar moiety was in position 7. β-Glucosidase enzymatic hydrolysis failed to give any intermediate i. e. glucose was directly attached to the aglycone and rhamnose was terminal, while using α-rhamnosidase afforded an intermediate which showed R_f-values and UV spectral data similar to those reported for chrysoeriol-7-*O*-glucoside.

Partial acid hydrolysis yielded chrysoeriol-7-rutinoside (identified by its R_f-values, UV spectral data and complete acid hydrolysis) i. e. the sugar moiety attached to position 7 of the aglycone consist of 1 mol of glucose and 2 moles of rhamnose attached to the glucose moiety. The ^{1}H NMR spectrum (Table 1) confirmed the above features and, in addition, revealed the glucosyl moiety to be β-linked to the aglycone chrysoeriol at 7-position (δ 5.5 ppm, d, J = 7 Hz) and rhamnosyl moieties to be α-linked to the glucose hydroxyl groups at 2″ and 6″ positions (δ 5.2 and 4.5 ppm, resp., d, J = 2 Hz) besides two singlet signals of the two methyl groups at δ 1.2 and 0.85 ppm. Finally the structure was confirmed as chrysoeriol-7-*O*-(2,6-dirhamnosyl) glucoside

Table 1: ^{1}H NMR spectral of compounds 1 and 2

	1	2
Aglycone		
H-6	6.43 d (2.5)	6.39 d (2.5)
H-3	6.44 s	6.4 s
H-8	6.8 d (2.5)	6.6 d (2.5)
H-2′	7.56 d (2.5)	7.58 d (2.5)
H-5′	6.88 d (8)	6.9 d (8)
H-6′	7.56 dd (2.5 & 8)	7.58 dd (2.5 & 8)
CH_3O-	3.87 s	3.87 s
Sugars		
H-1 Gluc.	5.5 d (7.5)	5.3 d (7.5)
H-1 Rha1-2Gluc.	5.2 d (2)	5.14 d (2)
CH_3 Rha1-2Gluc.	1.2 d (6)	1.28 d (6)
H-1 Rha1-6Gluc.	4.5 d (2)	
CH_3 Rha1-6Gluc.	0.85 d (6)	

Numbers in Parentheses denote coupling constants in Hz. Signals s = singlet, d = doublet; dd = double doublet

Table 2: ^{13}C NMR data of compounds 1 and 2

Carbons	Aglycone		Carbons	Sugars	
	1	2		1	2
2	164.2	164.2	Gluc. 1	98.5	100.7
3	103.5	103.5	2	77	79.2
4	181.9	181.9	3	77	76
5	161.5	161.5	4	70.9	70.4
6	99.2	99.6	5	76.4	77.1
7	162.3	162.9	6	66	60.7
8	95.0	95.6	Rha (1→6) 1	100.6	
9	156.9	156.9	2	70.6	
10	105.5	105.5	3	70.2	
1′	127.6	127.6	4	72.3	
2′	110.8	110.8	5	68.4	
3′	150.9	150.9	6	17.9	
4′	148.1	148.1	Rha (1→ 2)1	100.3	98.1
5′	116.0	116.0	2	71.5	70.0
6′	120.6	120.6	3	71.0	70.6
CH_3O-	56.2	56.2	4	73	73.1
			5	69.0	68.4
			6	17.8	17.7

through ^{13}C NMR data (Table 2) which gave signals similar to those of chrysoeriol with the anomeric carbons of the glucose moiety and the two rhamnose moieties at δ 98.5, 100.6 and 100.3 resp., together with the downfield shifts of C_2 and C_6 of the glucose at δ 77.0 and 66.0 ppm resp. (rather than the unsubstituted ones at δ 73.4 and 61.2 ppm) [19] confirming their substitution by the two rhamnose moieties.

UV spectral data, complete and enzymatic hydrolysis with R_f-values and colour reactions of the second new glycoside **2** revealed that it was also a 7-substituted chrysoeriol with the sugar neohesperidoside. It was further ensured by alkaline hydrogen peroxide oxidation [17]. The suggested structure was established by ^{1}H NMR spectroscopy where the positions of the signals of the H-1″ of glucose, H-1‴ of the rhamnose at δ 5.3 and 5.14 ppm, resp., and the signal of the methyl group of rhamnose at δ 1.28 ppm confirmed the structure [18]. And finally the sugar sequence was ensured through ^{13}C NMR data (Table 2).

3. Experimental

3.1. Instruments and materials

UV: UV/V Shimadzu spectrophotometer- UV 240; NMR: Jeol Fx 270 spectrometer; CC: Polyamide (6S Riedel De Häen AG-Seelze- Hannover), Sephadex LH-20 (Pharmacia); TLC: Silica gel G60 F254, 0.2 mm (Merck) and spray reagents: methanolic solution containing 1% $AlCl_3$, Neu reagent, Aniline hydrogen phthalate reagent; PC: Whatman No. 1 and 3 MM. Solvent system used (a) BuOH : AcOH : H_2O (4 : 1 : 5); (b) BuOH : AcOH : H_2O (6 : 1 : 2); (c) 15% AcOH and (d) H_2O.

Verbena bipinnatifida Nutt. herbs were collected from the Orman Garden-Giza. A voucher specimen is deposited in the National Research Center Herbarium (CAIRC). It was identified by Prof. Dr. Lotfy Boulos.

3.2. Extraction, isolation and purification

The dried *V. bipinnatifida* Nutt. herbs (500 g) were extracted several times with hot H_2O. The concentrated H_2O extract was chromatographed on a polyamide column, eluted first with H_2O and then with increasing amounts of EtOH; six fractions were obtained. All compounds isolated was further purified on Sephadex LH-20 column. Complete and controlled acid hydrolysis of glycosides (2 N HCl for 1 h and 0.1 N HCl, resp.) yield the aglycones and the sugar residues. All known isolated compounds were co-chromatographed with authentic samples. Enzymatic hydrolysis was achieved using β-glucosidase and α-rhamnosidase. Alkaline hydrogen peroxide oxidation was carried out according to Harborne [17]. All UV data were recorded in MeOH and in the presence of diagnostic reagents using the standard procedures [18].

3.3. Characteristics of glycosides

3.3.1. Chrysoeriol-7-O-(2,6-dirhamnosyl)glucoside (**1**)

Compound **1** was isolated from fraction III (20% ethanol) and was purified by preparative paper chromatography 3 MM paper using water for irrigation. R_f-values (X100) in (a) 32; (b) 23; (c) 27; (d) 70. Its colour is brown under UV light changing to bright yellow on exposure to ammonia vapours. UV data (MeOH, λ_{max}nm) 253, 265, 343; + NaOMe: 266, 397; + $AlCl_3$: 257, 272, 292 sh, 348, 379; + $AlCl_3$/HCl: 256, 270, 294 sh, 349, 380; + NaOAc: 260, 357 sh, 394; + NaOAc/H_3BO_3: 253, 265, 345. ^{1}H- and ^{13}C NMR spectral data see Tables 1 and 2.

3.3.1. Chrysoeriol-7-O-neohesperidoside (**2**)

Compound **2** was obtained from the fourth fraction (40% EtOH) and purified by elution technique on Whatman 3 MM paper using 6% AcOH for irrigation. R_f-values (X100) in (a) 32; (b) 24; (d) 30. Its colour is brown under UV light changing to faint yellow in NH_3/UV. UV data (MeOH, λ_{max}nm) 251, 265, 342; + NaOMe: 263, 400; + $AlCl_3$: 262, 296 sh, 378; + $AlCl_3$/HCl: 259, 296 sh, 342 sh, 378; + NaOAc: 260, 290 sh, 396; + NaOAc/H_3BO_3: 265, 344. ^{1}H- and ^{13}C NMR spectral data see Tables 1 and 2.

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