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Two flavonoid glycosides from *Chenopodium murale*

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Abstract

Two new triglycosides, kaempferol-3-*O*-{(4-β-D-apiofuranosyl)-α-L-rhamnopyranoside}-7-*O*-α-L-rhamnopyranoside and kaempferol-3-*O*-{(4-β-D-xylopyranosyl)-α-L-rhamnopyranoside}-7-*O*-α-L-rhamnopyranoside were isolated from the methanol extract of *Chenopodium murale*, together with a known diglycoside, kaempferol-3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside. The characterization of the three compounds was achieved by various spectroscopic methods.

Keywords: Chenopodium murale; Chenopodiaceae; Flavonoids; Flavonols; Kaempferol; Glycosides

1. Introduction

In the previous paper, the presence of flavonoids having dose-related hypotensive activity was reported in *Chenopodium murale* L. (Gohar & Elmazar, 1997). TLC and gravity column chromatography of the BuOH fraction afforded three flavonoids, one of them was identified as kaempferol-3,7-dirhamnoside. The identity of the other two compounds was not verified (Gohar & Elmazar, 1997). In this report, a full analysis of these flavonoids is presented.

2. Results and discussion

TLC and gravity column chromatography of the BuOH fraction of the methanolic extract of *Chenopodium murale* L. afforded three flavonoids. Kaempferol-3,7-dirhamnoside **1** in addition to compounds **2** and **3** were obtained (Gohar & Elmazar, 1997). Compound **2** was proved to be a mixture by NMR experiments. The TLC of **2** using chromatographic system A (Gohar & Elmazar, 1997), double development, resulted in resolution of **2** into two compounds (R_f 0.53 and 0.51). RpC18 — TLC of **2** using 40% aqueous methanol resolved it into two components which were isolated by preparative reversed phase HPLC using 45% aqueous methanol as mobile phase by isocratic elution (Carotenuto, Fattorusso, Lanzotti, Magno, de Feo, Cicala, 1997). The resolved compounds were noted as **2A** (R_t 2.82; TLC, system A, R_f 0.53, double run) and **2B** (R_t 7.55, TLC system A, R_f 0.51 double run). Compound **2A** was identified as kaempferol-3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnoside. Its identity was verified by comparison of its spectral data with those reported in the literature (Markham & Mahan Chari, 1982; Kowalewski & Wierzbicka, 1971; Mabry, Markham & Thomas, 1970; Gieger & Schwinger, 1980; Markham, Ternai, Stanley, Geiger & Mabry, 1978).

The IR spectrum of compound **2B** showed strong absorption bands at 3420 (OH), 1610 (C=O), 2950 (C–H), 1650 (C=C, aromatic), and broad band at 1130–1000 cm^{-1} indicating its glycosidic nature (Jain, Sarwar-Alam, Kamil, Ilyas, Niwa & Sakae, 1990). Its reaction (fluorescent yellow in UV with $AlCl_3$) and UV spectral data with diagnostic shift reagents (Mabry et al., 1970; Markham & Mabry, 1975) suggested the likely presence of 3,7-disubstituted flavonol glycoside with free hydroxyl groups at 5 and 4′-positions. Two intermediate spots were detected upon mild acid hy-

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Table 1
^{1}H-NMR data and HMBC results of compounds **3**, **2A** and **2B**[a]

No.	**3**		**2A**		**2B**	
	^{1}H-NMR	HMBC correlations	^{1}H-NMR	HMBC correlations	^{1}H-NMR	HMBC correlations
6	6.41 *d*, *J* = 2.4	95.87, 107.49	6037 *s*		6.37 *s*	
8	6.67 *d*, *J* = 2.4	100.55, 107.49	6.73 *s*		6.69 *s*	
2′	7.75 *d*, *J* = 9		8.03 *d*, *J* = 9.5		7.69 *d*, *J* = 8.8	
3′	6.92 *d*, *J* = 8	122.24	6.76 *d*, *J* = 8.8		6.73 *d*, *J* = 8.8	
5′	6.92 *d*, *J* = 8	122.24	6.76 *d*, *J* = 8.8		6.73 *d*, *J* = 8.8	
6′					7.69 *d*, *J* = 8.8	
Rha-3						
1″	5.45 *d*, *J* = 1.2	136.97, 82.6			5.22 *s*	
2″	4.21 *m*				3.42 *m*	
3″	3.82 *d*, *J* = 3.3				3.82 *m*	
4″	3.35 *m*				3.28 *m*	
5″	3.68 *m*				3.76 *m*	
6″	1.05 *d*, *J* = 6.6	82.6			0.8 *d*, *J* = 4.4	69.01, 76.95
Rha-7						
1‴	5.55 *d*, *J* = 1.2	163.43	5.51 *d*, *J* = 1.2		5.50 *s*	
2‴	4.00 *m*		3.40 *m*		3.25–3.8 *m*	
3‴	3.84 *d*, *J* = 3.3		3.62 *m*		3.25–3.8 *m*	
4‴	3.84 *t*		3.32 *m*		3.25–3.8 *m*	
5‴	3.60 *m*		3.08 *m*		3.25–3.8 *m*	
6‴	1.25 *d*, *J* = 6.5		1.10 *d*, *J* = 6.58		1.1 *d*, *J* = 5.66	
Xylose						
1⁗	4.30 *d*, *J* = 8.6	82.6				
2⁗	3.20 *m*					
3⁗	3.30 *m*					
4⁗	3.45 *m*					
5⁗	3.08 *m*					
glc. 3						
1″			5.42 *d*, *J* = 7.33			
2			3.18 *m*			
3″			3.20 *m*			
4″			3.08 *br*			
5″			3.82 *br*			
6			β-3.32 *br*, α-3.54 *br*			
Apiose						
1⁗					5.15 *d*, *J* = 2.2	73.55, 76.95
2⁗					3.94 *br.s*	
4⁗					3.53 *m*, 3.75 *d*, *J* = 9.6	
5⁗					3.33 *d*, *J* = 4.4 3.29, *br.m*	

[a] The solvent is DMSO-d_6 for **2A** and **2B** and CD_3OD for **3**. The chemical shifts are expressed in (ppm) and the coupling constant (*J*) is expressed in Hz/s. The multiplicities are represented by *s* for singlet, *d* for doublet, *t* for triplet, *m* for multiplet and *br* for broad.

drolysis of **2B** with 1 N HCl, before yielding the aglycone. This suggested the presence of three sugar moieties. Two sugars were detected and proved to be rhamnose and apiose by comparing their paper chromatography and GC of their TMS derivatives with the natural authentic samples. GC indicated that the ratio of rhamnose to apiose was 2 : 1. NMR of **2B** further confirmed the presence of two rhamnose (signals at δ 0.8 and δ 1.1 in ^{1}H-NMR and at δ 17.82 and δ 18.11 in ^{13}C-NMR for the two methyl groups) and one apiose residue. The carbon signal at δ 109.20 as well as the CH_2 signal (DEPT) resonated at δ 73.55 were assigned to the anomeric and C4⁗ of the apiose moiety, respectively, (Tables 1 and 2). The rest of the sugar carbons were assigned by comparison with the published data (Markham & Mahan Chari, 1982; Markham et al., 1978). The aglycone was proved to be kaempferol by direct co-chromatography with authentic sample, UV and ^{1}H-NMR. The mass spectrum (FAB^+) of **2B** showed the presence of fragments having *m*/*z* 733 calculated for M + Na, 711 for M + 1 corresponding to molecular formula $C_{32}H_{38}O_{18}$, 565 for (M + 1)-rha, 433 for (M + 1)-rha-api, and 287 for the aglycone M + 1. This is consistent with the presence of two rhamnose, one apiose and one kaempferol unit. The fragmentation sequence proved that one rhamnose and the apiose fragment must be terminal sugars (Crow, Tomer, Looker & Gross, 1986).

The ^{1}H-, ^{13}C-NMR and DEPT spectra (Tables 1 and 2) confirmed this structural hypothesis. The anomeric carbon atoms of the two rhamnose units resonate at δ 101.56 and δ 98.47. The chemically shifted signal at δ 101.56 was assigned to the rhamnose unit linked at C3, while the signal at δ 98.47 was assigned to the rhamnose at C7 of the aglycone (Agrawal & Bansal, 1989). An HMBC experiment (Table 1) revealed a correlation between the signal at δ 76.95 assigned to C4″ of the rhamnose at C3 of the aglycone and the anomeric proton of apiose resonated at δ 5.15 as well as to the signal at δ 0.8 assigned to protons of the methyl group of the rhamnose residue. The latter was assigned to H6 of rhamnose at position 3 of the aglycone (Chang, 1978). This suggests that apiose residue is attached to C4″ of the rhamnose moiety at position 3 of the aglycone. This was confirmed from the low-field shift of C4″ at δ 76.95 and from the high-field shift of H4″ at δ 3.28 (Agrawal & Bansal, 1989; Carotenuto, de Feo, Fattorosso, Lanzotti, Magno & Cicala, 1996; Kikuchi & Matsuda, 1996). Different protons of the sugar residues were assigned using the anomeric protons as starting points in the ^{1}H-NMR spectrum for analysis of the HHCOSY and CHSHF spectra. From these data, **2B** was identified as kaempferol-3-*O*-[4-β-D-apiofuranosyl]-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside which has been not reported before.

The striking similarity of IR, UV and MS FAB$^+$ between **2B** and **3** suggested a close similarity in their structure. The UV spectrum and its changes in the presence of diagnostic shift reagents (Mabry et al., 1970; Markham & Mabry, 1975) pointed to the presence of free hydroxyl groups at C5 and C4′ of a 3,7-disubstituted flavonoid glycoside framework.

Acid hydrolysis of **3** gave the same result as **2B** except for the presence of xylose sugar instead of apiose. The MS FAB$^+$ of **3** is in agreement with the suggested structure. Fragment *m*/*z* 711 (M + 1) calculated for $C_{32}H_{38}O_{18}$, *m*/*z* 565 (loss of one rhamnose), *m*/*z* 578 (loss of xylose) and *m*/*z* 287 accounted for M + 1 of the aglycone.

The NMR spectra of **3** (Tables 1 and 2) confirmed the previous conclusions. The chemical shift of the two anomeric carbon atoms of rhamnose residues unambiguously confirmed their linkage to C3 and C7 of the kaempferol residue (Agrawal & Bansal, 1989). As mentioned for **2B**, the xylose was deduced to be linked to C4″; rhamnose linked to C3 of kaempferol (Agrawal & Bansal, 1989; Carotenuto et al., 1996). The previous conclusion was confirmed from the HMBC experiment since the proton signals at δ 6.41 and δ 6.67 (assigned to 6 and 8 positions, respectively) are correlated with the carbon resonances at (δ 107.49, δ 95.87) and (δ 107.49, δ 100.55) assigned to (C10, C8) and (C10, C6), respectively. Also, protons at 3′ and 5′ (δ 6.92) were found to interact with C1′ (δ 122.24). The location of the rhamnose units at positions 3 and 7, with anomeric carbons resonating at δ 103.09 and 99.78, respectively, was confirmed from HMBC correlation between their respective anomeric protons and target carbon atoms. The anomeric proton at δ 5.45 (rha-3) was correlated with C3 (δ 136.97) and the proton at δ 5.55 (rha-7) was correlated with C7 (δ 163.43). These results con-

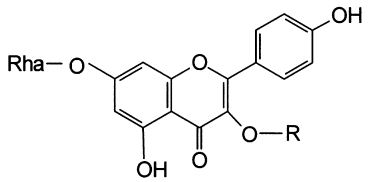
Table 2
^{13}C-NMR of compounds **3**, **2A** and **2B**[a]

Carbon	**3**	**2A**	**2B**
2	157.95 (*s*)	155.93	156.27
3	136.97 (*s*)	133.16	133.70
4	179.74 (*s*)	177.32	177.52
5	162.93 (*s*)	161.57	161.60
6	100.55 (*d*)	99.62	99.66
7	163.43 (*s*)	161.57	161.6
8	95.87 (*d*)	96.6	95.2
9	159.59 (*s*)	156.79	156.27
10	107.49 (*s*)	105.95	106.16
1′	122.24 (*s*)	118.71	123.41
2′	131.94 (*d*)	131.24	130.83
3′	116.60 (*d*)	116.16	117.00
4′	161.73 (*s*)	157.30	159.33
5′	116.60 (*d*)	116.60	117.00
6′	131.94 (*d*)	131.24	130.24
Rha-3			
1″	103.09 (*d*)		101.56
2″	73.57 (*d*)		70.70
3″	71.87 (*d*)		70.41
4″	82.60 (*d*)		76.95
5″	71.83 (*d*)		69.01
6″	17.68 (*q*)		17.82
Rha-7			
1‴	99.78 (*d*)	98.45	98.47
2‴	72.03 (*d*)	70.19	70.52
3‴	71.67 (*d*)	70.09	70.23
4‴	73.57 (*d*)	71.82	71.82
5‴	71.24 (*d*)	70.04	70.01
6‴	18.09 (*q*)	18.09	18.11
Xylose			
1⁗	107.67 (*d*)		
2⁗	75.20 (*d*)		
3⁗	77.73 (*d*)		
4⁗	70.93 (*d*)		
5⁗	67.06 (*t*)		
glc. 3			
1″		101.12	
2″		74.40	
3″		77.65	
4″		70.01	
5″		76.63	
6″		61.03	
Apiose			
1⁗			109.20 (*d*)
2⁗			76.24 (*d*)
3⁗			79.22 (*s*)
4⁗			73.55 (*t*)
5⁗			63.60 (*t*)

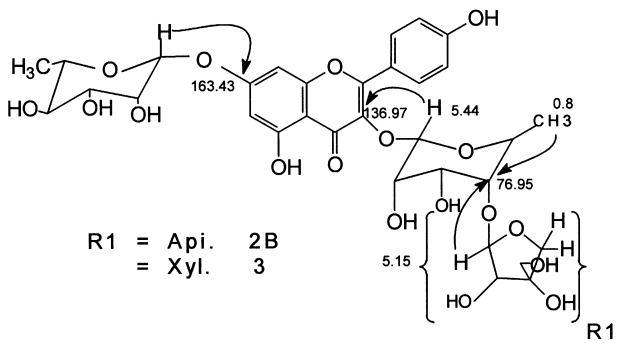
[a] The solvent is DMSO-d_6 for **2A** and **2B** and CD_3OD for **3**. The chemical shifts are expressed in (ppm). The multiplicities are represented by *s* for singlet, *d* for doublet, *t* for triplet and *q* for quartet.

firm the previous assignment based on the conclusion of Agrawal and Bansal (1989). The xylose moiety was deduced to be attached to C4″ of the rhamnose at position-3 from HMBC correlation of its anomeric proton (δ 4.3) with the rhamnose C4″ (δ 82.6) which, in turn, was correlated with the 6-methyl protons of rhamnose at position-3. From these data, **3** was identified as kaempferol-3-*O*-(4-β-D-xylopyranosyl)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside.

From the chemotaxonomic point of view, the genus *Chenopodium* contains both flavones and flavonols. Flavones are reported in *C. graveolens* (Mata, Navarrete, Alvarez, Pereda-Miranda, Delgado & Romo de Vivar, 1987), methoxylated flavones in *C. botrys* (de Pascual-T, Gonzalez, Vicente & Bellido, 1981), 3-*O*-substituted flavonol glycosides in *C. quinoa* and *C. ambrosioides* (Jain et al., 1990; de Simone, Dini, Pizza, Saturnino & Schettino, 1990). Concerning *C. murale*, this is the first report for all the discussed compounds. The presence of apiose in *C. murale* supports the previous reports of its presence in *C.quinoa* (de Simone et al., 1990) as well as in another genus in the family Chenopodiaceae; *Spinacia* (Aritomi, Komori & Kawasaki, 1986; Williams & Harborne, 1994).



R = Rha. 1
= Glu. 2A



R1 = Api. 2B
= Xyl. 3

3. Experimental

3.1. General

Mps uncorr., UV spectra were run in MeOH and IR spectra in KBr discs. NMR spectra were run at 600 MHz (^{1}H) and 150 MHz (^{13}C) in DMSO-d_6 (compound **2A** and **2B**) or in CD_3OD (compound **3**) using TMS as internal standard. MS were obtained by FAB^+ at 70 eV. TLC was performed using silica gel GF_{254} (Merck), EtOAc–MeOH–H_2O (100 : 15 : 10) mixture was used as solvent (A). Whatmann No. 1 paper was used in PC, 15% AcOH and EtOAc–Pyridine–H_2O (5 : 5 : 4) mixtures were used as solvents (B and C, respectively). $AlCl_3$ (+UV 366 nm) and aniline hydrogen phthalate spray reagents were used for detection.

Plant materials, extraction, chromatography, and preparation of fraction II (Gohar & Elmazar, 1997) Fraction II was subjected to repeated column chromatography (250 silica gel). Elution was done using EtOAc–MeOH–H_2O (100 : 10 : 5) mixture, 100 ml fractions were collected. Two groups of the resolved compounds were separately collected.

From the first group (900 ml), compound **1** was recovered (Gohar & Elmazar, 1997). The second group, following 1200 ml gave a residue (4.8 g) which was subjected to repeated column chromatography, using the same condition. From the first 1200 ml eluate, compound **2** was obtained (2.8 g). From the next 800 ml eluate, compound **3** was recovered by repeated recrystallization from MeOH (255 mg). RpC18 — TLC of **2** using 40% MeOH followed preparative reversed phase HPLC using 45% aqueous MeOH resulted in resolution of **2** into **2A** (137 mg) and **2B** (157 mg); Waters 600E-Millipore 6plepf504 attached to waters 486 Tunable Absorbence detector, column 7.8 × 300 mm, C18 prep., λ_{max} 345 nm, aufs 0.05, att., variable 512–1024, flow rate 4 ml/min, chart speed 0.25 cm/min.

3.2. Kaempferol-3-O-β-D-glucopyranoside-7-O-α-L-rhamnopyranoside *2A*

Pale yellow crystals, mp 254°C; $[\alpha]_D^{25} = -74^\circ$ (MeOH: *c* 0.25); FAB-MS (positive ion) *m*/*z* 595 M + 1; 617, M + Na; 433, (M + 1)-gluc; UV spectra, λ_{max} nm MeOH 210, 266, 347; + NaOMe 212, 266, 396; + $AlCl_3$ 212, 274, 301^{sh}, 350, 397; + $AlCl_3$–HCl 212, 274, 301^{sh}, 349, 397; + NaOAc 213, 266, 352; + NaOAc–H_3BO_3 213, 266, 352; IR. 3420 (OH), 2950 (C–H), 1650 (C=C aromatic), 1610 (C=O), 1130–1000 cm^{-1} (glycosidic linkage); NMR data (Tables 1 and 2).

3.3. Acid hydrolysis

An alcoholic solution (20 mg) was refluxed on boiling water bath with 1 N HCl. The solution was monitored by PC System B, time interval 5 min, for 1 h. The excess acid was precipitated with Ag_2O, the alcohol evaporated and the aglycone extracted with EtOAc

and recrystallized from methanol. The sugars in the aqueous solution were examined by PC (System C) and by GLC, and the aglycone was subjected to UV and ^{1}H-NMR analysis.

3.4. GLC analysis of sugars

The neutral aqueous hydrolysates were silylated with BSFTA/TMS for 15 min at room temperature in pyridine. Silylated samples were subjected to GLC analysis: column BP5-25 m, 0.25 mm id; column temperature 200–300°C; 5°C/min; 20 min; dect, temperature 300°C (Fid); helium.

3.5. Identification of aglycone (kaempferol)

Yellow needles, mp 280°C, UV λ_{max} nm: MeOH 265, 371; + NaOMe 263, 285, 359^{sh}, 451; + $AlCl_3$ 261, 300^{sh}, 364, 426; + $AlCl_3$–HCl 261, 300^{sh}, 346^{sh}, 427; + NaOAc 262, 322^{sh}, 384; + NaOAc–H_3BO_3 257, 314^{sh}, 369. ^{1}H-NMR δ 8.07, *d*, J = 8.69 Hz (H2′,6′); δ 6.89, *d*, J = 8.79 Hz (H3′,5′); δ 6.39, *d*, J = 1.46 Hz (H8); δ 6.17, *d*, J = 2.19 Hz (H6).

3.6. Kaempferol-3-O-[4-β-D-apiofuranosyl]-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside **2B**

Yellow crystals, mp 224°C; $[\alpha]_D^{25} = -181°$ (MeOH: *c* 0.15), FAB-MS (positive ion) *m*/*z* 733 (13.5) M + Na, 711 (10.18) M + 1, 565 (1), (M + 1)-rha, 433 (M + 1)-rha-api, 287 (2.5) M + 1 for aglycone; UV spectra λ_{max} nm: MeOH 210, 265, 343; + NaOMe 211, 265, 387 + $AlCl_3$ 212, 268, 398; + $AlCl_3$–HCl 210, 267, 343^{sh}, 397; + NaOAc 210, 265, 343; + NaOAc–H_3BO_3 212, 265, 344; IR 3420 (OH), 2950 (C–H), 1650 (C=C), 1610 (C=0), 1130–1000 cm^{-1} (glycosidic linkage); NMR data (Tables 1 and 2). Acid hydrolysis and identification of sugars and aglycone as compound **2A**.

3.7. Kaempferol-3-O-(4-β-D-xylopyranosyl)-α-L-rhamnopyranoside-7-O-α-L-rhamnoside **3**

Pale yellow crystals, mp 232°C; $[\alpha]_D^{25} = -154°$ (MeOH: *c* 0.114); FAB-MS (positive ion) *m*/*z* 711 (2.5) M + 1, 578 (10) (M + 1)-xyl, 565 (6.5) (M + 1)-rha, 287 M + 1 for aglycone; UV spectra λ_{max} nm MeOH 209, 265, 326+; + NaOMe 210, 247, 390; + $AlCl_3$ 210, 247, 301^{sh}, 349, 398; + $AlCl_3$–HCl 210, 275, 301^{sh}, 344, 397; + NaOAc 214, 248, 350; + NaOAc–H_3BO_3 212, 247, 344; IR. 3420 (OH), 2950 (C–H), 1650 (C=C), 1610 (C=O), 1130–1100 cm^{-1} (glycosidic linkage); NMR experiments (Tables 1 and 2). Acid hydrolysis and identification of sugars and aglycone as compound **2A**.

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