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Polyphenolic Metabolites of the Flowers of *Tamarix tetragyna*

Amani M. D. El-Mousallami[#], Sahar A. M. Hussein and Mahmoud A. M. Nawwar*

National Research Centre, El-Dokki, Cairo, Egypt,[#]Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt

Abstract – Phytochemical study of the constitutive polyphenolics of the flower aqueous alcohol extract of *Tamarix tetragyna* was carried out. The new sulphated flavonol, quercetin 3',4'-dimethyl ether 3-*O*-KSO_3 as well as the new natural galloyl glucose, 2-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose were isolated and characterised. The known sulphated flavonols, kaempferol 7,4-dimethyl ether 3,5-di-*O*-KSO_3, quercetin 7-methyl ether 3,3',4'-tri-*O*-KSO_3, quercetin 7,4'-dimethyl ether 3-*O*-KSO_3 and quercetin 3-*O*-KSO_3 and the known sulphated phenolics, isoferulic acid 3-*O*-KSO_3 and ellagic acid 4,4'-dimethyl ether 3-*O*-KSO_3 were also separated and identified. The structures were established by conventional methods of analysis and confirmed by ^{1}H-, ^{13}C-NMR and negative ESI-mass spectrometry. 2D-homonuclear chemical shift correlation NMR experiment was applied for the new natural galloylglucose.

Key words – *Tamarix tetragyna,* Tamaricaceae, quercetin 3',4'-dimethylether 3-potassium sulphate, 2-*O*-galloyl-(α/β)-$^4C_1$-glucose; NMR spectral analysis

Introduction

In Egypt, the genus *Tamarix* contains five species (Täckholm, 1974) which provide extracts used in folk medicine (Boulos, 1983), mainly as anti-inflammatory and antipyretic agents. The extracts contain a wide variety of phenolics, including lignans (Souleman *et al.*, 1991), phenolic glycerides (Souleman *et al.*, 1991; Barakat *et al.*, 1987) and sulphates (Souleman *et al.*, 1998), gallotannins (Nawwar *et al.*, 1994a), ellagitannins (Nawwar *et al.*, 1994b; Hussein, 1997) and flavonol glucuronoids (Nawwar *et al.*, 1984a) and flavonol sulphates (Barakat, 1998; Souleman, 1998) as well.

The present work reports isolation and structure elucidation of the new flavonol sulphate, quercetin 3'4'-dimethyl ether 3-*O*-SO_3K (**1**) and the new natural gallotannin, 2-*O*-galloyl-β-glucopyranose (**6**) from the flowers of *T. tetragyna*. In addition, the known compounds, kaempferol 7,4,-dimethyl ether 3,5-di-*O*-SO_3K (**2**), quercetin 7-methyl ether 3,3',4'-tri-*O*-SO_3K (**3**), quercetin 7,4'-dimethyl ether 3-*O*-SO_3 K (**4**), quercetin 3-*O*-SO_3K (**5**), isoferulic acid 3-*O*-SO_3 K (**7**) and ellagic acid 4,4'-dimethyl ether 3-*O*-SO_3 K (**8**) have been identified. It should be noted that this is only the second reported natural occurrence of compounds (**2**, **3**, **7** and **8**), which were previously characterized from the flowers of *T. amplexicaulis* (Souleman *et al.*, 1998; Souleman, 1998). Compound (**6**) is known as a synthetic product (Schmidt, 1961). Its positional isomers, 1-*O*-galloyl-β-glucose, 4-*O*-galloyl-β-glucose and 6-*O*-galloyl-β-glucose were recently characterized from *Macaranga tanarius* L. (Euphorbiaceae), (Lin *et al.*, 1990).

Results and Discussion

Preliminary screening of the aqueous ethanolic (1:3) extract of the flowers of *Tamarix tetragyna* Ehrenb. by 2D-PC indicated the presence of a complicated mixture of phenolic compounds (positive results with $FeCl_3$ spray reagent) among which compounds **1-5**, **7** and **8** have exhibited the characteristic features of anionic flavonoids and phenolics (dark purple, mauve or buff spots on PC under UV light, high mobility in water, low mobility in organic solvents and migration towards the anode on electrophoretic chromatograms). Compounds **1-8** were isolated from the extract by CC on Sephadex LH-20, using H_2O for elution, followed by repeated fractionation on the same adsorbent, using EtOH (95%) or *n*-BuOH saturated with H_2O as an eluent, thus affording pure samples of each. The known compounds **2-5**, **7** and **8** exhibited chromatographic, electrophoretic (Table 1), colour

*Author for correspondence.

Table 1. Chromatographic, electrophoretic and UV spectral data of *Tamarix tetragyna* sulphated compounds (**1-5 & 7,8**)

Compounds	Chromatographic and electrophoretic data				UV spectral data λ_{max} (nm)					
	R_{fs} (X100)				MeOH (a)	(a)+ NaOAc(b)	(b)+ H_3BO_3	(a)+$AlCl_3$	(a)+ NaOMe	(a)+HCl
	H_2O	HOAC (15%)	BAW	cm						
Quercetin 3',4'-dimethyl-ether 3-*O*-KSO_3 (**1**)	66	47	65	6.0	253,266, 345	273,319, 377	254,267*, 343	265,359, 424	276,342*, 389	254*,267, 366
Quercetin 3',4'-dimethyl ether (**1_a**)	00	08	79	00	254,266*, 366	253*,275, 382	255,266*, 367	268,362, 420	276,340, 396	255,305*, 366
Kaempferol 7,4'-dimethyl -ether 3,5-di-*O*-KSO_3 (**2**)	67	54	39	6.6	268,259*, 324,362*	268,333	267,342	267,295*, 323,360*	280,320, 380	264,264, 290,320, 363g
Quercetin 7-methyl ether-3,3',4'-tri-*O*-KSO_3 (**3**)	96	72	25	6.6	243*,267, 341	257*,267, 360	268,345	257*,276, 305, 365,402	257*,266, 340	253,267, 366
Quercetin 7,4'-dimethyl-ether 3-*O*-KSO_3 (**4**)	69	50	62	6.0	252,267, 290, 349	250,268, 350	255,268, 350	272,300, 350*,390	250,265*, 342	253,253, 267,368
Quercetin 3-*O*-KSO_3 (**5**)	75	48	34	6.1	255,267*, 303*,350	269,300*, 378	260,300*, 375	372,303*, 432	270,320, 400	256,267*, 368
Isoferulic acid 3-*O*-KSO_3(**7**)	76	68	74	5.2	292,307					
ellagic acid 4,4'-dimethyl-ether 3-*O*-KSO_3 (**8**)	66	52	43	5.0	246,255*, 344,384					

* : Shoulder.

Table 2. ^{1}H NMR chemical shifts (δppm) and coupling constants (J in Hz) of *Tamarix tetragyna* sulphated compounds (**1-5 & 7,8**)

Compounds	Protons No.				
	H-2	H-5	H-6	H-7	H-8
Quercetin 3',4'-dimethyl ether-3-*O*-SO_3K (1)			6.18(d,J=2.5)		6.42(d,J=2.5)
Quercetin 3',4'-dimethyl ether (1_a)			6.20(d,J=2.5)		6.50(d,J=2.5)
Kaemferol 7,4'-dimethyl ether-3,5-di-*O*-SO_3K (2)			6.35(d,J=2.5)		6.73(d,J=2.5)
Quercetin 7-methyl ether-3,3',4'-tri-*O*-SO_3K (3)			6.36(d,J=2.5)		6.72(d,J=2.5)
Quercetin 7,4'-dimethyl ether-3-*O*-SO_3K (4)			6.35(d,J=2.5)		6.74(d,J=2.5)
Quercetin 3-*O*-SO_3K (5)			6.17(d,J=2.5)		6.37(d,J=2.5)
Isoferulic acid 3-*O*-SO_3K (7)	7.64 (d,J=2.5)	77.00 (d,J=7.5)	7.32 (dd,J=2.5, 7.5)	7.45 (d,J=15)	6.18 (d.J=15)
Ellagic acid 4,4'-dimethyl ether-3-*O*-SO_3K (8)		7.64(s)			
	H-2'	H-3'	H-5'	H-6'	OMe
Quercetin 3',4'-dimethyl ether-3-*O*-SO_3K (1)	7.60(d,J=2.5)	7.15(d,J=8)	7.15(d,J=8)	7.75(dd,J=2.58)	3.85(s)
Quercetin 3',4'-dimethyl ether (1_a)	7.75(d,J=2.5)		7.15(d,J=8)	7.80(dd,J=2.58)	3.90(s)
Kaemferol 7,4'-dimethyl ether-3,5-di-*O*-SO_3K (2)	8.17(d,J=7.5)	7.02(d,J=7.5)	7.02(d,J=7.5)	8.17(d,J=7.5)	3.84(s),3.88(s)
Quercetin 7-methyl ether-3,3',4'-tri-*O*-SO_3K (3)	7.90 (d,J=2.5)		6.90 (d,J=7.5)	8.1 (dd,J=2.5,7.5)	3.86(s)
Quercetin 7,4'-dimethyl ether-3-*O*-SO_3K (4)	8.02 (d,J=2.5)		7.25 (d,J=7.5)	7.76 (dd,J=2.5,7.5)	3.84(s),3.88(s)
Quercetin 3-*O*-SO_3K (5)	7.55 (d.J=2.5)		7.60 (dd,J=2.5, 7.5)		
Isoferulic acid 3-*O*-SO_3K (7)					3.94(s),3.98(s)
Ellagic acid 4,4'-dimethyl ether-3-*O*-SO_3K (8)			7.60(s)		3.94(s),4.00(s)

Table 3. ^{13}C-NMR chemical shifts (δ) of *Tamarix tetragyna* sulphated compounds (**1-5** & **7,8**)

Carbom No.	Compounds							
	1	1_a	2	3	4	5	7	8
1								
2	156.3	146.4	156.1	156.2	156.6	156.2	126.9	107.7
3	133.1	136.3	132.7	132.0	133.1	132.6	114.2	143.6
4	177.2	176.1	176.9	177.7	177.2	177.0	146.7	128.9
5	160.6	160.8	158.1	160.9	160.6	160.9	150.2	154.6
6	99.8	98.4	101.6	98.0	97.5	98.8	112.5	107.9
7	165.0	164.1	165.3	165.2	164.9	164.2	121.4	113.1
8	94.8	93.8	92.3	92.1	91.7	93.9	144.6	158.6
9	156.9	156.4	158.1	156.2	156.5	156.2	114.7	
10	104.6	103.2	109.1	105.1	104.0	104.2	167.5	
1'	123.5	123.4	123.4	127.3	123,4	121.1		107.7
2'	111.8	111.0	130.2	117.1	114.8	115.5		141.1
3'	148.7	148.5	113.5	152.0	146.7	145.2		134.8
4'	150.9	150.5	160.7	149.6	149.4	148.8		147.7
5'	112.0	111.7	113.6	123.5	112.0	116.5		107.9
6'	112.0	121.6	130.2	121.2	119.9	121.9		111.8
7'	122.7							158.7
MeO	55.5&55.8	55.5&55.8	55.8&56.0	56.1	55.6&55.8		55.7	56.9&56.2

properties, UV spectral (Table 1) and hydrolysis data identical with those of kaemferol 7,4'-dimethyl ether 3-*O*-SO_3K, quercetin 7-methyl ether 3,3',4'-tri-*O*-SO_3K, quercetin 7,4'-dimethyl ether 3-*O*-SO_3K quercetin 3-*O*-SO_3K, isoferulic acid 3-*O*-SO_3K and ellagic acid 4,4-dimethyl ether 3-*O*-SO_3K, respectively. These structures were then confirmed by -ve ESI-MS, ^{1}H- (Table 2) and ^{13}C-NMR (Table 3) spectral analyses.

The new compound **1** was isolated as an off-white amorphous powder which showed chromatographic properties, an anionic characters in electrophoretic analysis and UV absorption maxima, in MeOH and with diagnostic shift reagents (Harborne *et al.*, 1975; *Mabry et al.*, 1969 and Barron, *et al.*, 1988), [a 7 nm shift with NaOAc, no shift with NaOAc/H_3BO_3, a small and stable shift with NaOMe and a 21 nm shift with HCl], consistent with a 3,3',4'-trisubstituted quercetin which should have an ionic substituent, mostly sulphate, at position 3. Mild acid hydrolysis of **1** with 0.05 N aq. HCl or 10 % aq. AcOH at 100° for 3 minutes or 10 minutes, respectively yielded no intermediates (coPC), but gave directly an aglycone (**1_a**), which deposited from the aq. acidic hydrolysate, on standing for 1 hr at room temp. It was identified, after being filtered off from the hydrolysate, to be quercetin 3',4'-dimethyl ether, through chromatographic (Table 1), electrophoretic, UV spectral (Table 1), ^{1}H- (Table 2) and ^{13}C-NMR (Table 3) analyses. The aq. HCl hydrolysate gave a heavy white precipitate with aq. $BaCl_2$ to prove the presence of sulphate group. Atomic absorption analysis confirmed that the sulphate radical existed in the molecule of **1** as potassium sulphate. This was further evidenced through the formation of a yellow precipitate on treating an aq. solution of **1** with aq. sodium cobaltinitrite (Feigel, 1958).

In addition, **1** exhibited a molecular anion peak [M -K]$^-$, in negative ESI-MS, at *m/z* 409. This and the above given data proved that compound **1** is quercetin 3',4'-dimethyl ether 3-*O*-KSO_3. This view was then supported through NMR spectroscopic analysis. The recorded ^{1}H-NMR (Table 2) spectra (room temp. DMSO-d_6) though revealed closely similar patterns of proton resonances for both **1** and its free aglycone, quercetin 3',4'-dimethyl ether, yet the ^{13}C NMR spectra proved sulphatation at C-3 of the parent compound **1**. This followed from the upfield shift (Δδ=3.2 ppm) of the C-3 carbon and from the accompanying lowfield shifts (Δδ=9.9 and 1.1 ppm) of the resonances of the *ortho* carbons C-2 and C-4, respectively, all on comparison with the corresponding resonances in the spectrum of the free aglycone. Consequently, compound **1** is quercetin 3',4'-dimethyl ether 3-*O*-KSO_3, which represents, to the best of our knowledge, a new natural product.

The new natural phenolic **6** was obtained as an amorphous powder which possesses galloyl ester-like characters (intense blue colour with $FeCl_3$, rosy red colour with KIO_3 (Gupta *et al.*, 1982) and UV

spectral maximum in MeOH at 273 nm). Negative ESI-mass spectral analysis established that **6** was a monogalloyl glucose of a M_r: 332 amu ([M-H]$^-$ at *m/z* 331). It yielded, on normal acid hydrolysis (2 N aq. HCl, 100°, 3 hrs) gallic acid (coPC, UV, ^{1}H and ^{13}C NMR spectral analysis) together with glucose (coPC), thus proving the identity of **6** as monogalloyl glucose. ^{1}H-NMR determined the site of attachment of the galloyl moiety to the glucose one in the molecule of **6**. The recorded spectrum (room temp., Acetone-d_6) revealed a free anomeric OH group. This unequivocally followed from the two doublets at δ 5.38 (J=2.5 Hz) and at 4.8 (J=8 Hz), attributable to the α- and β-anomeric glucose proton, respectively. The spectrum also showed two downfield glucose proton resonances at δ 4.7 (dd, J=2.5 Hz, and J=8 Hz) and at 4.85 (t, J=8 Hz) assignable to the H-2 glucose protons in both α- and β-anomers, respectively. The assignments were based on the observation that the doublet of doublets mode of splitting of the resonance located at 4.7 ppm is typical of an axial H-2 proton in an α-$^4C_1$-glucose core, being coupled to both the α-anomeric equatorial proton (J=2.5 Hz) and to the axial H-3 proton (J=8 Hz) in the same moiety. The lowfield position of the H-2 proton resonances, is obviously, due to galloylation of the geminal OH glucose group. These assignments were confirmed by a homonuclear chemical shift correlation (COSY) experiment, whereby cross peakes, in the recorded spectrum, have correlated the glucose proton resonances in the expected sequence. Besides, the values of the coupling constants given above indicate that the glucose core of **6** is adopting a $^4C_1$-conformation. Also, the resonances of the equivalent H-2 and H-6 protons of the galloyl moiety, in each anomer has revealed themselves as two distinct sharp singlets at δ 7.15 and 7.2. Consequently compound **6** is monogalloyl-(α/β)-$^4C_1$-glucose. Final proof of structure **6** was then achieved through ^{13}C-NMR spectral analysis. The recorded spectrum contained, essentially double resonances for most of the glucose and galloyl carbons. Assignments of these resonances were made, mostly by comparison with the ^{13}C-NMR data, reported for similar galloyl glucoses (Nawwar *et al.*, 1984b), as well as by consideration of the known α- and β-effect caused by esterifying the sugar hydroxyl groups. The α- and β- anomeric carbon resonances were readily identified from their characteristic chemical shift values (δ 90.9, C-α and 96.2, C-β), while the two most upfield resonances at δ 62.6 and 62.4 were attributed to the free methylenic carbons in both anomers. Attachment of the galloyl moiety to the glucose carbon C-2 was evidenced by the β-upfield shifts (see Experimental) recognized for the vicinal anomeric and C-3 carbons as well (all in comparison with the chemical shifts of the resonances of the corresponding carbons in the spectrum of unsubstituted α/β glucopyranose). In both anomers, C-2 was found to resonate downfield (α-effect) at δ ppm 75.9 (C-2α) and at 76.6 (C-2β). Other resonances in this spectrum exhibited chemical shift values which were in accordance with the proposed structure of **6.**

Furthermore, the measured chemical shift values of the glucose carbon resonances proved that this moiety existed in the pyranose form, thus confirming the final structure of **6** to be 2-mono-*O*-galloyl(α/β)-$^4C_1$-glucopyranose, a new galloylglucose which has not been reported before to occur in nature.

Experimental

General – For NMR analysis, a JEOL EX-270 NMR spectrometer was used with superconducting magnet from Oxford and 5 mm Dual probehead for ^{1}H- and ^{13}C-analyses. ^{1}H NMR resonances were measured relative to TMS and ^{13}C resonances to DMSO-d_6 or $(CD_3)_2CO$-d_6 and converted to the TMS scale by adding 39.5 or 29.8. Typical conditions: spectral width = 4000 Hz for ^{1}H and 15000 Hz for ^{13}C, 32 K data points and a flip angle of 45°. The UV spectra were measured in MeOH and with diagnostic shift reagents, using Shimadzu UV-240 spectrometer. Negative ESI-MS: measured by a SSQ Finnigan MAT 4600 quadrupole mass spectrometer. Electrophoretic analysis: On Whatman paper No. 3MM, in buffer soln. of pH 2.2 (H_2O-AcOH-HCOOH, 200:7.5:2.5), under 50 v/cm, for 2 hrs, on an Elphor Vario 1 (Bender and Hobein, München, Germany). PC was carried out on Whatman No. 1 paper using solvent systems (1) H_2O; (2) 15 % AcOH-H_2O (15:85); (3) BAW (*n*-BuOH-AcOH-H_2O, 4:1:5, top layer); (4) C_6H_6-*n*-BuOH-pyridine-H_2O (1:5:3:3, top layer). Solvents 3 and 4 were used for sugar analysis.

Plant material – Fresh flowers of *Tamarix tetragyna* Ehrenb. were collected from the marshy habitats around Qaroun lake, El-fayoum, Egypt, in January and authenticated by Dr. L. Boulos, Professor of Botany, NRC, Cairo, Egypt. A voucher specimen has been deposited at the herbarium of the NRC.

Isolation and identification – Fresh flowers (1.5 kg) of *T. tetragyna* were exhaustively extracted (300.4 g) with EtOH-H_2O (3:1). a concd. soln. of the extract (40.8 g in 25 ml MeOH) was applied to a Sephadex LH-20 column and eluted by H_2O to yield 8 major frs (I-VIII). Compounds **1** (151 mg), **2** (173 mg), **3** (78 mg), **4** (102 mg) and **5** (65 mg) were isolated from fr. V by CC over Sephadex LH-20, using *n*-BuOH saturated with H_2O for elution then purifying the crude sample on columns containing the same adsorbent, using EtOH (95%) as an eluent. Compound **6** (162 mg) was separated from fr. VI through column fractionation over Sephadex LH-20 and EtOH (95%) for elution. Compound **7** (59 mg) was isolated from fr. VII by CC over Sephadex LH-20, using *n*-BuOH saturated with H_2O as an eluent and compound **8** (73 mg) was separated from fr. VIII by applying the same CC technique.

Quercetin 3',4'-dimethyl ether 3-*O*-SO_3K (1). R_fs and electrophoretic mobility: Table 1. UV data: Table 1. M_r=448, ESI-MS (ng. ion): *m/z*: 409 [M-K]$^-$. **1** yielded directly, quercetin 3',4'-dimethyl ether on mild acid hydrolysis either with 0.05 N HCl or with 10% aq. AcOH (14 mg were refluxed with 2 ml aq. 0.05 N HCl, 100° for 3 min.; 17 mg were refluxed with 2.5 ml aq. AcOH, 100° for 10 min.). Quercetin 3',4'-dimethyl ether: R_fs: Table 1. UV data: Table 1. ^{1}H-NMR (DMSO-d_6, room temp.): Table 2. ^{13}C-NMR: Table 3. ^{1}H-NMR of **1**: Table 2. ^{13}C-NMR of **1**: Table 3.

2-Mono-O-galloyl-(α/β)-$^4C_1$-glucopyranose (6). R_fs: 68 (1), 77 (2), 35(3). UV data: λ_{max}(nm): 273. M_r 332, ESI-MS: negative ion: *m/z*: 331 [M-H]$^-$. On complete acid hydrolysis [22 mg in 15 ml aq. 2 N HCl, 100°, 3 hr] compound **6** yielded glucose (coPC) and gallic acid. Gallic acid: R_fs: 53 (1), 68(2), 78 (3). UV data: λ_{max} (nm): 272. ^{1}H-NMR (acetone-d_6, room temp.): δ (ppm): 6.9 (s, H-2 & H-6). ^{13}C NMR: δ (ppm) 120.6 (C-1), 108.8 (C-2 & C-6), 145.5 (C-3 & C-5), 138.1 (C-4), 167.7 (C-7). ^{1}H-NMR of **6**: δ (ppm): α-glucose moiety: 5.38 (d, J=2.5 Hz, H-1), 4.7 (dd, J=8 Hz and 2.5 Hz, H-2), 4.1 (t, J=8 Hz, H-3), 3.55 (t, J=8 Hz, H-4), 3.7-3.9 (m, H-5 & H-6, hidden by other sugar proton resonances); β-glucose moiety: δ (ppm) 4.8 (d, J=8 Hz, H-1), 4.85 (t, J=8 Hz, H-2), 3.7-3.9 (m, H-3, H-4, H-5 & H-6, hidden by other sugar proton resonances); galloyl moiety: 7.15 (s) and 7.2 (s). ^{13}C-NMR of **6** : δ (ppm): α-glucose moiety: 90.9 (C-1), 75.9 (C-2), 71.9 (C-3), 71.7 (C-4), 72.5 (C-5), 62.2 (C-6); β-glucose moiety: 96.2 (C-1), 76.6 (C-2), 75.3 (C-3), 71.5 (C-4), 77.5 (C-5), 62.4 (C-6); galloyl moieties: 121.9, 122.2 (C-1), 109.9, 110.1 (C-2 & C-6), 145.9, 146.1 (C-3 & C-5), 138.7, 138.8 (C-4), 166.7, 166.8 (C-7).

Known Compounds (2-5, 7 & 8). R_fs: Table 1. UV data: Table 1. ^{1}H-NMR: Table 2. ^{13}C-NMR: Table 3.

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