Natural Product Sciences
4(4) : 215 ~ 220 (1998)

Sulphated Flavonols of the Flowers of *Tamarix amplexicaulis*

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Abstract – A new flavonol 3,5-di-*O*-KSO_3 : kaempferol 7,4'-dimethyl ether 3,5-*O*-KSO_3, was isolated and identified from the flowers of *Tamarix amplexicaulis*. The known compounds quercetin 3-mono-*O*-KSO_3, kaempferol 4'-methyl ether 3-mono-*O*-KSO_3, kaempferol 7,4'-dimethyl ether 3-*O*-KSO_3, quercetin 7,4'-dimethyl ether 3-mono-*O*-KSO_3, kaempferol 3-*O*-glucuronide and quercetin 3-*O*-glucuronide were also separated and identified. Structures were established by conventional methods, including electrophoretic analysis, and confirmed by negative FAB-MS, ^{1}H- and ^{13}C-NMR.

Key words – *Tamarix amplexicaulis*, Tamaricaceae, flowers, flavonol potassuim sulphate, kaempferol 7,4'-dimethyl ether 3,5-di-*O*-KSO_3, negative FAB-MS, NMR.

Introduction

In a continuing search among Egyptian Tamaricaceous plant for novel phenolics with possible biological activity (Barakat *et al.*, 1996, Hussein 1997 and Nawwar *et al.*, 1994), the aqueous ethanolic flower extract of *Tamarix amplexicaulis*, Ehrenb, was found, in the present study, to contain the novel flavonol 3,5-di-potassuim sulphate, kaempferol 7,4'-dimethyl ether 3,5-di-*O*-KSO_3 (**7**). The known compounds kaempferol 3-*O*-*β*-glucupyranuronide (**1**), quercetin-*O*-*β*-glucupyranuronide (**2**), kaempferol 4'-methylether 3-*O*-KSO_3 (**3**), quercetin 3-*O*-KSO_3 (**4**), kaempferol 7,4'-dimethyl ether 3-*O*-KSO_3 (**5**) and quercetin 7,4'-dimethyl ether 3-*O*-KSO_3 (**6**) were also characterized.

It should be noted, however, that this is the second reported natural occurrance of a flavone, including flavone 3-ol, in which the 5-hydroxyl group is sulphated (Barron *et al.*, 1988 and Saleh *et al.*, 1975). The first report was about the isolation, from the leaves of *Tamarix aphylla*, of a rhamnetin 3,5,4'-trisulphate-3'-glucuronide (Saleh *et al.*, 1975).

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Experimental

General

For NMR analysis, A JEOL EX-270 NMR spectrometer, 270 MHz for ^{1}H-NMR and 67.5 MHz for ^{13}C-NMR, was used with superconducting magnet from Oxford and 5 mm Dual probehead for ^{1}H and ^{13}C-analysis. 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 taken in MeOH using Shimadzu UV-240 spectrometer, FAB-MS (negative mode): measured by MM7070E-mass spectrometer (VG analytical) and EI-MS by Varian Mat 112-ET spectrometer. PC was carried out on Whatman No.1 paper using solvent systems [1] H_2O, [2] HOAc-H_2O (3 : 47), [3] *n*-BuOH-HOAc-H_2O (4 : 1 : 5, top layer), [4] C_6H_6-*n*-BuOH-H_2O-pyridine (1 : 5 : 3 : 3, top layer). Solvent [3] was used for

prep. PC on Whatman No. 3MM paper and solvents [3] and [4] for sugar analysis.

Plant Material

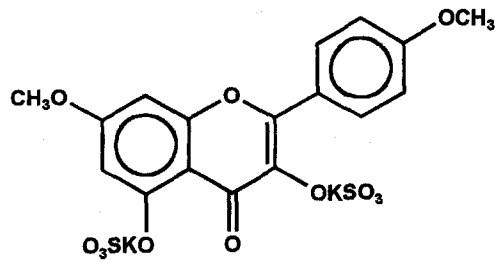
A sample of *T. amplexicaulis* flowers, collected from a mature tree, growing in the marshy habitate at the Mediterranean coast, 15 km east of El- Kantara city, in Sinai peninsula, Egypt, during March 1996, was authenticated by Dr. N. El-Hadeady, Prof. of Botany, Faculty of Science, Cairo University. A voucher specimen is deposited at the herbarium of the NRC.

Extraction, isolation and identification

An aqu. EtOH extract (1:3) of the fresh flowers, concentrated *in vacuo*, was applied to a Sephadex LH-20 CC and eluted by *n*-BuOH saturated with water. Eluents of the ten successive fractions (I-X) (collected by inspecting the column under UV light) were individually dried *in vacuo* and examined by 2D-PC. Compound **1** (168 mg) and **2** (328 mg) were isolated in the pure state from fr. VI, through refractionation over Sephadex LH-20 column, using H_2O as an eluent. Compound **3** (112 mg) was purified from fr. VII through successive CC over Sephadex LH-20 using, H_2O and then *n*-BuOH saturated with H_2O. Compound **4** (76 mg) was separated pure from fr. VIII through Sephadex LH-20 CC, using *n*-BuOH saturated with H_2O for elution. Compound **5** (201 mg) and **6** (68 mg) separated from fr. IX through Sephadex LH-20 CC, using *n*-BuOH saturated with H_2O for elution. Compound **7** (110 mg) was isolated pure from the last major column fr. X through repeated CC over Sephadex LH-20, using H_2O as an eluent.

kaempferol 7,4'-dimethyl ether 3,5-di-*O*-KSO_3 (7) – R_fS: Table 1. Electrophoretic mobility (cm): in buffer of pH 2.2, H_2O-HOAc-HCOOH, 200-7.5-2.5, 2hr, 50 v/cm: Table 1. UV. (MeOH, MeOH+NaOAc, MeOH+NaOAc+H_3BO_3, MeOH+$ALCl_3$, MeOH+NaOMe and MeOH+HCl) λ_{max}: Table 1. negative FAB-MS: m/z 511 $[M-K]^-$ & 473 $[M-2K+H]^-$, Mr 550 amu. Compound **7** yielded kaempferol 7,4'-dimethyl ether 3-KSO_3 and kaempferol 7,4'-dimethyl ether on mild acid hydrolysis either with 10% aqueous AcOH or with aqueous 0.05 N HCl (62 mg were refluxed with 2.5 ml aqu. AcOH, 100° for 15 min., the reaction was traced through PC analysis with sampling rate 3 min.; 14 mg were refluxed with 2 ml aqu. 0.05 N HCl, 100° for 5 min.). The released kaempferol 7,4'-dimethyl ether 3-O-KSO_3 and kaempferol 7, 4'-dimethyl ether: R_fS: Table 1: Electrophoretic mobility (cm): Table 1; UV (MeOH and with diagnostic shift reagents) λ_{max}: Table 1; negative FAB-MS: m/z 393 $[M-K]^-$, Mr 342 and m/z 313 $[M-H]^-$, Mr 314, respectively. ^{1}H-NMR (DMSO-d_6, room temp.) of **7**: m/z δ 6.35 (d, J=2.5 Hz, H-6), 6.73 (d, J =2.5 Hz, H-8), 7.02 (d, J=7.5 Hz, H-3' and H-5'), 8.17 (d, J=7.5 Hz, H-2' and H-6'), 3.84 (s, OMe). ^{13}C-NMR of **7**: Table 2.

Known compounds: kaempferol 3-*O*-β-glucupyranuronide (1) – R_fS: Table 1. UV (MeOH and with shift reagents) λ_{max}: Table 1. Complete acid hydrolysis (18 mg, 5 ml aqu. 2 N HCl, 100°, 3 hr.) yielded kaempferol and glucuronic acid (CoPC). negative FAB-MS: m/z 461 $[M-H]^-$, Mr 462. ^{1}H-NMR (DMSO-d_6-room temp.) : δ 6.17 (d, J=2.5 Hz, H-6), 6.38 (d, J=2.5 Hz, H-8), 6.84 (d, J=7.5 Hz, H-3' and h-5'), 7.97 (d, J=7.5 Hz, H-2' and H-6'), 5.42 (d, J=8 Hz, H-1"), 3.15-3.85 (m, sugar protons overlapped with OH protons). ^{13}C-NMR: Table 2.



Compound 7

Quercetin-3-*O*-β-glucupyranuronide (2) – R_fS: Table 1, UV (MeOH and with shift reagents) λ_{max}: Table 1. Complete acid hydrolysis (22 mg, 5 ml aqu. 2 N HCl, 100°, 3 hr) yielded quercetin and glucuronic acid (CoPC). negative FAB-MS: m/z 477 $[M-H]^-$, Mr 478. ^{1}H-NMR: δ 6.22 (d, *J*=2.5 Hz, H-6), 6.4 (d, *J*=2.5 Hz, H-8), 6.84 (d, *J*=7.5, H-5'), 7.48 (d, *J*=2.5 Hz, H-2'), 7.57 (dd, *J*=7.5 Hz and *J*=2.5 Hz, H-6'), 5.4 (d, *J*=8 Hz, H-1"), 3.2-395 (m, sugar proton overlapped with OH protons. ^{13}C-NMR: Table 2.

kaempferol 4'-methyl ether 3-*O*-KSO$_3$ (3) – R_fS: Table 1. Electrophoretic mobility (cm) : Table 1. UV (MeOH and with shift reagents) λ_{max}: Table 1. Mild acid hydrolysis (25 mg, 5 ml aqu. 10% AcOH, 100°, 15 min.) yielded kaempferol 4'-methyl ether (kaempferide, CoPC). negative FAB-MS: m/z 379 $[M-K]^-$, Mr 418. ^{1}H-NMR: δ 6.20 (d, *J*=2.5 Hz, H-6), 6.44 (d, *J*=2.5 Hz, H-8), 7.02 (d, *J*=7.5 Hz, H-3' and H-5'), 8.18 (d, *J*=7.5 Hz, H-2' and H-6'), 3.84 (s, OCH_3). ^{13}C-NMR: Table 2.

Quercetin 3-*O*-KSO$_3$ (4) – R_fS: Table 1. Electrophoretic mobility (cm): Table 1. UV (MeOH and with shift reagents) λ_{max}: Table 1. Mild acid hydrolysis (22 mg, 5 ml aqu. 10% AcOH, 100°, 15 min.) yielded quercetin (CoPC), negative FAB-MS: m/z 381 $[M-K]^-$ Mr 420. ^{1}H-NMR: δ 6.17 (d, *J*=2.5 Hz, H-6), 6.37 (d, *J*=2.5 Hz, H-8), 6.8 (d, *J*=7.5 Hz, H-5'), 7.55 (d, *J*=2.5 Hz, H-2'), 7.6 (dd, *J*=7.5 Hz and 2.5 Hz, H-6'). ^{13}C-NMR: Table 2.

kaempferol 7,4'-dimethyl ether 3-*O*-KSO$_3$ (5) – R_fS: Table 1. Electrophoretic mobility (cm): Table 1. UV (MeOH and with shift reagents) λ_{max}: Table 1. Mild acid hydrolysis (27 mg, 5 ml aqu. 10% AcOH, 100°, 15 min.) yielded kaempferol 7,4'-dimethyl ether (CoPC). negative FAB-MS: m/z 393 $[M-K]^-$, Mr 432. ^{1}H-NMR: δ 6.34 (d, *J*=2.5 Hz, H-6), 6.72 (d, *J*=2.5 Hz, H-8), 7.02 (d, *J*=7.5 Hz, H-3' and H-5'), 8.22 (d, *J*=7.5 Hz, H-2' and H-6'), 3.84 (s, OCH_3), 3.86 (s, OCH_3). ^{13}C-NMR: Table 2.

Quercetin 7,4'-dimethyl ether 3-*O*-KSO$_3$ (6) – R_fS: Table 1 Electrophoretic mobility (cm): Table 1. UV (MeOH and with shift

Table 1. Chromatographic, electrophoretic and UV spectral data of compounds (**1-7**)

Compound	Chromatographic data. R_fs (×100)			Electrophoretic mobility (cm)						
	H_2O	HOAc	BAW		MeOH (a)	(a)+NaOAc(b)	(b)+H_3BO_3	(a)+$AlCl_3$	(a)+NaOMe	(a)+HCl
1-Kaempferol-3-*O*-glucuronide	73	40	52	–	268, 350	273, 298*, 373	268, 293*, 352	276, 386	281, 415	–
2-Quercetin-3-*O*-glucuronide	70	38	49	–	256, 265*, 292*, 357	271, 320*, 366	270, 292*, 376	271, 350, 394	272, 320*, 402	–
3-Kaempferide-3-*O*-KSO$_3$.	75	54	46	6.0	255*, 266, 342	256*, 267, 346	256, 346	272, 327*, 387	270, 350	253, 267*, 365
4-Quercetin-3-*O*-KSO$_3$.	75	48	34	6.1	255, 267*, 303*, 350	269, 300*, 378	260, 300*, 375	372, 303*, 432	270, 320, 400	256, 267*, 368
5-Kaempferol-7,4'-dimethylether-3-*O*-KSO$_3$.	72	52	65	6.0	286, 340	268, 345	268, 348	272, 295*, 385	272, 325*, 392	253, 266, 322*, 362
6-Quercetin-7,4'-dimethylether-3-*O*-KSO$_3$.	69	50	62	6.0	252, 267, 290, 349	250, 270, 350	255, 268, 350	272, 300, 350*, 390	250, 265*, 342	253, 267*, 268
7-Kaempferol-7,4'-dimethylether-3,5-di-*O*-KSO$_3$.	67	54	39	6.6	268, 295*, 324, 362*	268, 333	267, 342	267, 295*, 323, 360*	280, 320, 380	264, 290, 320, 363

*: inflection

reagents) λ_{max}: Table 1. Mild acid hydrolysis (26 mg, 5 ml aqu. 10% AcOH, 100°, 15 min.) yielded Quercetin 7,4'-dimethyl ether (CoPC). negative FAB-MS: m/z 409 $[M-K]^-$, Mr 448. ^{1}H-NMR: δ 6.35 (d, J=2.5 Hz, H-6), 6.74 (d, J=2.5 Hz, H-8), 7.25 (d, J=7.5 Hz, H-5'), 7.76 (dd, J=7.5 Hz and J=2.5 Hz, H-6'), 8.02 (d, J =2.5 Hz, H-2'), 3.84 (s, OCH_3), 3.88 (s, OCH_3). ^{13}C-NMR: Table 2.

Results and Discussion

The fresh flowers of *Tamarix amplexicaulis* Ehrenb (Tamaricaceae), a bushy tree of fleshy, very small scale-like leaves and pink flowers, growing wild in Egypt, were exhaustively extracted with aqueous ethanol (1 : 3). Compounds **1-7** were isolated from the dried extract by successive CC on Sephadex LH-20, using *n*-BuOH saturated with H_2O or H_2O only as solvent for elution. The known compounds **1-6** showed chromatographic, electrophoretic, UV absorption and results of hydrolytic procedures identical to those of kaempferol 3-*O*-β-glucuronide (Nawwar *et al.*, 1984); quercetin 3-*O*-β-glucuronide (Nawwar *et al.*, 1984); kaempferide 3-*O*-KSO_3 (Souleman, 1989); quercetin 3-*O*-KSO_3 (Barron *et al.*, 1986); kaempferol 7,4'-dimethyl ether 3-*O*-KSO_3 (Nawwar *et al.*, 1984) and quercetin 7, 4'-dimethyl ether 3-*O*-KSO_3 (Barron *et al.*, 1988), respectively. These structures were confirmed by atomic absorption, FAB-MS (negative mode), ^{1}H- and ^{13}C-NMR spectral

Table 2. ^{13}C-NMR Chemical shifts (ppm) of compounds **1-7** and their related aglycones

Carbon No.	Quercetin*	Quercetin-3-*O*-glucuronide (**2**)	Quercetin-3-*O*-KSO_3 (**4**)	Quercetin-7,4'-dimethyl-ether-*O*-KSO_3 (**6**)	Kaempferol*	Kaempferol-3-*O*-glucuronide (**1**)	Kaempferide	Kaempferide**-*O*-KSO_3 (**3**)	Kaempferol*-7,4'-dimethyl-ether	Kaempferol-7,4'-dimethyl-ether-3-*O*-KSO_3 (**5**)	Kaempferol-7,4'-dimethyl-ether-3,5-di-*O*-KSO_3 (**7**)
2	146.9	156.4	156.2	156.6	146.8	156.3	146.1	155.4	146.7	156.0	15.1
3	135.5	132.2	132.6	133.1	135.7	133.1	136.0	132.8	136.3	132.8	132.7
4	175.8	177.3	177.0	177.2	175.9	177.0	176.0	177.1	176.1	177.9	176.1
5	160.7	161.3	160.9	160.6	160.7	161.1	160.3	159.8	160.7	161.2	158.1
6	98.2	98.9	98.8	97.5	98.2	98.9	98.2	98.0	97.6	97.6	101.6
7	163.9	164.4	164.2	164.9	163.9	164.6	164.2	163.2	165.0	165.0	165.3
8	93.3	93.7	93.9	91.7	93.5	94.0	93.2	93.0	92.2	92.2	92.3
9	156.2	156.4	156.2	156.5	156.2	156.3	156.1	155.9	156.3	156.3	158.1
10	103.1	103.9	104.2	104.0	103.0	104.2	103.1	104.2	104.1	105.2	109.1
1'	122.1	120.9	121.1	123.4	121.7	120.9	123.2	123.1	123.2	122.7	123.4
2'	115.3	115.3	115.5	114.8	129.5	131.2	129.5	131.1	129.5	131.0	130.2
3'	145.0	145.0	145.2	146.7	115.5	115.4	114.0	113.8	114.2	113.6	113.5
4'	147.6	148.7	148.8	149.4	159.2	160.5	160.7	161.0	160.2	161.0	160.7
5'	115.6	116.2	116.5	112.0	115.5	115.4	114.0	113.8	114.2	113.6	113.5
6`	120.0	121.8	121.9	119.9	129.5	131.2	129.5	131.1	129.5	131.0	130.2
1"		101.2				101.9					
2"		71.5				71.6,74.1					
3"		73.9				76.1					
4"		76.0				76.2					
5"		76.0				170.2					
6"		170.1									
OMe				55.8, 55.6			56.0	56.2	55.3, 55.9	56.0, 55.3	55.3, 55.0

*: data from Nawwar *et al.*, 1984.

analysis.

Compound **7** was isolated as an off-white amorphous powder with chromatographic properties (dull buff spot on PC under UV light which turns yellow when fumed with ammonia of high R_f- in H_2O), electrophoretic mobility (towards the anode, 6.6 cm, in buffer soln. of pH 2.2, H_2O-HOAc-HCOOH, 200-7.5-2.5, 2hr, 50 v/cm) and UV spectra (in MeOH and with diagnostic shift reagents, Table 1) which suggest its structure to be an anionic flavonol. Mild acid hydrolysis (aqueous 10% AcOH, 100°, 15 minutes) of **7** gave one intermediate which was separated through preparative PC on Whatman paper No.3 MM, using BAW (*n*-BuOH-HOAc-H_2O, 4 :1:5, top layer) as solvent. The aglycone released during hydrolysis was, also separated during the same preparative procedures. The isolated intermediate exhibited chromatographic properties (dark purple spot on PC under UV light which turned yellow when fumed with ammonia, high R_f- in H_2O), electrophoretic mobility (6 cm, under the above described conditions), UV absorption maxima (Table 1), and an anion peak in negative FAB- MS at m/z 393 $[M-K]^-$, corresponding to Mr of 432, which proved its identity as kaempferol 7,4'-dimethylether 3-sulphate. The isolated parent aglycone was found to be kaempferol 7,4'-dimethyl ether (CoPC, UV spectra and negative FAB- MS: m/z 313 [M-H], Mr 314). Mild acid hydrolysis of **7** with aqueous 0.05 N HCl, 100° for 5 minutes gave the same results. However, the aqueous HCl hydrolysate gave a white precipitate with aqueous $BaCl_2$, thus proving the presence of sulphate which exist in the molecule of **7** as potassium sulphate as deduced from the results of atomic absorption analysis of an aqueous solution of **7** and from the formation of a yellow precipitate on treating this solution with aqueous sodium cobaltinitrite (Feigel, 1956). Compound **7** exhibited a molecular anion peaks, in FAB-MS, at 511 $[M-K]^-$ and at 473 $[M-2K+H]^-$, corresponding to a molecular weight of 550 amu. Consequently, **7** is, therefore kaempferol 7,4'-dimethyl ether 3,5-di-*O*-KSO_3. Confirmation of this achieved structure was received from NMR spectral analysis. The recorded ^{1}H-NMR spectrum of **7** (DMSO-d_6, room temp.) was quite similar to those of the intermediate, kaempferol 7,4'-dimethyl ether 3-*O*-mono-KSO_3 and of the aglycone itself (see experimental). However, ^{13}C-NMR spectral analysis provided a confirmation of the deduced structure as fellows: The chemical shift values of the carbon resonances in the spectrum of **7** though are similar to those of the corresponding resonances in the spectra of the intermediate, kaempferol 7,4'-dimethyl ether 3-*O*-KSO_3 (Table 2) yet a distinction can be made since the resonances of the C-5, C-10 and C-6 of **7** are shifted in comparison with the related signals in the spectrum of the intermediate. This changes are obviously due to substitution with additional sulphate (Nawwar *et al.*, 1981) at C-5, thus causing an upfield shift ($\Delta\delta$=3.1 ppm) to its resonance and an accompanying downfield shifts ($\Delta\delta$=3.9 and 4.0 ppm, respectively) of the ortho-carbons, C-10 and C-6, respectively. The absence, in this spectrum of other ^{13}C resonances, apart from those of the flavonol moiety, supported the presence of an inorganic (sulphate) substitutents. Hence, the structure of compound **7** was finally confirmed to be kaempferol 7,4'-dimethyl ether 3,5-di-*O*-KSO_3, which represents a new natural sulphated flavonoid.

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(Accepted August 7, 1998)