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Contribution to the Phytochemical Study of Egyptian Tamaricaceous Plants

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Abstract – A novel flavonol trisulphate, quercetin 7-methyl ether 3,3',4'-tri-O-KSO_3 was isolated from the fresh leaves of *Tamarix amplexicaulis* (Tamaricaceae) along with the known flavonol mono sulphates, quercetin 3-*O*-KSO_3 and quercetin 4'-methyl ether 3-*O*-KSO_3. Structures were achieved through conventional analytical methods, including electrophoretic analysis and confirmed by FAB-MS and NMR spectroscopy.

Key words – *Tamarix amplexicaulis*, Tamaricaceae, leaves, flavonol trisulphate, rhamnetin 3,3',4'-potassium trisulphate, FAB-MS, ^{1}H-NMR, ^{13}C-NMR.

Introduction

Egyptian *Tamarix* plants commonly known as 'Tarfa' belong to definite five species, (Täckholm, 1974) and provide extracts which are used in traditional medicine mainly as anti-inflammatory and antipyretic agents (Barakat *et al.*, 1996). The plants contain a wide variety of phenolics, including lignans (Souleman *et al.*, 1991), phenolic glycerides (Barakat *et al.*, 1987 and Souleman *et al.*, 1991), ellagitannins (Nawwar *et al.*, 1982), gallotannins (Nawwar *et al.*, 1984), and flavonol sulphates (Hussein, 1993) as well. As a part of the continuing study on the phenolics of biologically active extracts derived from Egyptian *Tamarix* plants, the isolation and characterization of the novel natural product, rhamnetin 3,3',4'-tri-*O*-KSO_3, from the leaves of *Tamarix amplexicaulis* are reported in the present study. The isolation and elucidation of the known compounds quercetin 3-*O*-KSO_3 and tamarixetin 3-*O*-KSO_3 were also described herein.

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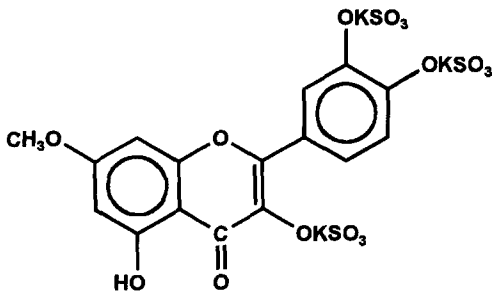
Results and Discussion

Preliminary screening of the aqueous ethanolic (1 : 3) extract of the leaves of *T. amplexicaulis* by 2D-PC indicated the presence of a complicated mixture of phenolic components (positive results with $FeCl_3$ spray reagent), among which compounds **1-3** have exhibited the characteristic features of anionic flavonols (dark purple spots on PC, which turned yellow on fuming with ammonia and high R_f-values in H_2O). Compounds **1-3** were isolated from the aqueous ethanolic extract by CC on Sephadex LH-20 using *n*-BuOH saturated with H_2O for elution followed by refractionation on the same adsorbent using H_2O as an eluent, thus affording individual pure samples of **1-3**. Compound **1** was isolated for the first time from natural source (Barron *et al.*, 1988). The remaining compounds **2** and **3** are known and gave chromatographic properties, electrophoretic mobility (on Whatman No. 3MM paper, in buffer soln. of pH 2.2, H_2O-HOAc-HCOOH, 200-7.5-2.5, 2 hr, 50 v/cm) and UV spectral data (in MeOH and with diagnostic shift reagents, Table 1)



Compund 1

identical with those reported for quercetin 3-*O*-KSO_3 (**2**) and quercetin 4'-methyl ether 3-*O*-KSO_3 (**3**). Confirmation of these structures was received through FAB-MS (negative mode), ^{1}H-NMR (see experimental) and ^{13}C-NMR (Table 2).

The novel compounds **1** was isolated as an off-white amorphous powder and showed chromatographic properties (dark purple spot on PC, with exceptionally high R_f in H_2O and low R_f in BAW), an anionic feature in electrophoretic analysis and UV absorption maxima, in MeOH and with shift reagents (no shift with NaOAc or NaOAc+ H_3BO_3, and a large shift of 24 nm with HCl) which suggested its structure to be an oligosulphated flavonol (Barron *et al.*, 1988). Mild acid hydrolysis of **1** with 0.05 N HCl, at 100° for 5 minutes yielded three different intermediates (CoPC). The hydrolysate gave a heavy white precipitate with aqueous $BaCl_2$ to prove the presence of sulphate radical. Atomic absorption analysis of an aqueous solution of **1** proved that the sulphate radical is present as potassium sulphate as was further confirmed through the formation of yellow precipitate on treating the aqueous solution of **1** with sodium cobaltinitrite (Feigel, 1956). Mild acid hydrolysis of **1** with 10% aqueous HOAc for 15 minutes at 100° and tracing the reaction every 3 minutes proved the formation of three distinct intermediates (**1a, 1b** and **1c**) in addition to a flavonol aglycone which was precipitated from the cooled hydrolysate, filtered on and identified to be quercetin 7-methyl ether, or rhamnetin [CoPC, UV

Table 1. Chromatographic, electrophoretic, and UV spectral data of compounds **1-3**

Compounds	Chromatographic data R_fs (×100)				UV spectral data λ_{max} (nm)					
	H_2O	HOAc	BAW		MeOH (a)	(a)+ NaOAc	(b)+ H_3BO_3	(a)+ $AlCl_3$	(a)+ NaOMe	(a)+ HCl
Rhamnetin-3,3',4'-tri-*O*-KSO_3 (**1**)	96	72	25	6.5	243*, 267, 341	257*, 267, 360	268, 345	257*, 276, 305, 365*, 402	257*, 266, 340	253, 267, 366
Rhamnetin-3',4'-di-*O*-KSO_3 (intermediate I)	78	53	30	6.2	253, 267*, 293*, 327*, 363	256, 377, 410	260, 372	270, 305*, 360*, 432	266, 405	253, 268*, 366
Rhamnetin-3-*O*-KSO_3 (**1a**)	59	50	60	6.0	268, 302*, 348	266, 370, 415*	266, 380	273, 302*, 340*, 430	245, 270, 392	266, 366
Rhamnetin-3'-*O*-KSO_3 (**1b**)	55	48	70	6.0	268, 365	267, 360, 398*	267, 366	265, 430	272, 435	267, 366
Rhamnetin-4'-*O*-KSO_3 (**1c**)	53	47	70	6.0	250, 266*, 360*	252, 266*, 365	253, 266*, 360	263, 300*, 345, 420	252, 356*, 412	266, 365
Rhamnetin	0	0	81	–	257, 266*, 370	267, 375	267, 380	265, 356*, 427	270, 410	257, 370
Quercetin-3-*O*-KSO_3 (**2**)	75	48	34	6.1	255, 267*, 303*, 350	269, 300*, 378	260, 300*, 375	372, 303*, 432	270, 320, 400	256, 267*, 368
Tamarixetin-3-*O*-KSO_3 (**3**)	71	52	61	6.0	253, 267, 290*, 348	274, 365	254, 267, 350	268*, 274, 300*, 360*	269, 320, 398	254, 268*, 390*

Table 2. ^{13}C-NMR chemical shifts (ppm) of compounds **1-3** and their related derivatives

Carbon No.	Quercetin	Quercetin 3-*O*-KSO_3 (**2**)	Tamarixetin	Tamarixetin 3-*O*-KSO_3 (**3**)	Rhamnetin	Rhamnetin 3',4'-di-*O*-KSO_3	Rhamnetin 3,3',4'-tri-*O*-KSO_3 (**1**)
2	146.9	156.2	146.2	156.8	147.3	147.2	156.2
3	135.5	132.6	136.0	132.3	136.3	137.0	132.0
4	175.8	177.0	175.9	176.6	175.2	176.9	177.7
5	160.7	160.9	160.8	161.1	160.8	161.5	160.9
6	98.2	98.8	98.0	100.3	97.6	98.3	98.0
7	163.9	164.2	163.1	164.6	165.6	166.3	165.2
8	93.3	93.9	93.3	94.4	91.9	92.6	92.1
9	156.2	156.2	156.2	156.8	156.7	157.4	156.2
10	103.1	104.2	103.0	104.8	104.3	105.0	105.1
1'	122.1	121.1	123.2	123.4	122.8	126.6	127.3
2'	115.3	115.5	114.8	115.3	115.2	116.9	117.1
3'	145.0	145.2	146.0	145.9	145.1	151.6	152.0
4'	147.6	148.8	149.1	149.6	147.8	140.8	140.6
5'	115.6	116.5	111.5	111.8	115.6	122.7	123.5
6'	120.0	121.9	119.4	121.1	120.6	120.6	121.2
OMe	–	–	56.1	55.6	55.7	55.8	56.1

spectra, (Table 1), EI-MS and ^{1}H-NMR]. Preparative PC, on Whatman No. 3MM paper, using *n*-BuOH as solvent system, of the hydrolysate afforded individual samples of **1a, 1b** and **1c**. They were identified through chromatographic, electrophoretic and UV spectral analysis to be rhamnetin 3-O-KSO_3 (dark purple spot on PC under UV light, which turned yellow when fumed with ammonia), rhamnetin 3'-*O*-KSO_3 (yellow spot on PC turning light yellow with ammonia) and rhamnetin 4'-*O*-KSO_3, (yellow spot on PC turning yellow with ammonia), respectively. Mild acid hydrolysis of **1** with 1% methanolic HOAc at room temp for 96 hr, accompanied by daily paper chromatographic analysis of the reaction, yielded one intermediate I, which was separated through prep. PC, using BAW as solvent system and identified to be rhamnetin 3',4'-di-*O*-KSO_3 through chromatographic (yellow spot on PC, under UV light which turned yellow when fumed with ammonia), electrophoretic mobility and UV spectral data (Table 1). Compound **1** exhibited a molecular anion peak, in negative FAB-MS at m/z 631 $[M-K]^-$, 593 $[M-2K+H]^-$, and at 555 $[M-3K+2H]^-$, corresponding to a molecular weight Mr of 670 amu. This and the above given data proved that compound **1** is rhamnetin 3,3',4'-tri-*O*-KSO_3. The recorded ^{1}H-NMR spectrum (DMSO-d_6, room temp.) of **1** supported the achieved structure and revealed a pattern of proton resonances (see experimental) which could be attributed to a 3,3',4'-tri-*O*-substituted rhamnetin (downfield shift of $\Delta\delta$=0.26 and 0.56 ppm for the H-2' and H-6' proton resonances, respectively, all in comparison with the corresponding signals in the spectrum of rhamnetin itself). The ^{13}C-NMR spectral analysis of **1** finally confirmed its deduced structure as follows: Methyl etherification at C-7 of the flavonol aglycone was evidenced from the lowfield shift of the resonance of C-7 ($\Delta\delta$=1.3 ppm) and from the accompanying upfield shifts of the C-8 and C-6 resonances ($\Delta\delta$=1.2 and 0.2 ppm, respectively). Taking into account that the effect of sulphation on the chemical shifts of carbon resonances is the same as that of glycosidation or acetylation (Nawwar *et al.*, 1981). The presence of sulphate substituent at C-3 followed from the upfield shift of the C-3 resonance and the lowfield shift of the ortho carbon resonances C-2 and C-4 (see experimental). Sulphation at C-3'

and C-4' was concluded by comparing the chemical shifts calculated for the B-ring carbon resonances of a rhamnetin 3',4'-di-*O*-sulphate with the corresponding resonances in the recorded spectrum of compound **1**. The comparison showed that both calculated and recorded shifts are in good agreement. Other signals in this spectrum possess chemical shift values (Table 2) which were in accordance with the achieved structure, thus finally confirming compound **1** to be rhamnetin 3,3',4'-tri-*O*-KSO_3. This is the first reported natural occurrence of this compound.

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 using Shimadzu UV-240 spectrometer. For FAB-MS (negative mode), a MM 7070 E mass spectrometer (VG analytical) has been used. 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 together with solvent [4] for sugar analysis.

Plant Material

Samples of *T. amplexicaulis* leaves, were collected from a mature tree, growing in the marshy habitats near the Mediterranean coast, 15 km east of El-kantara city in Sinai peninsula, Egypt, in March 1996 and authenticated by Dr. Nabiel El-Hadidi, Prof. of Botany, Faculty of Science, Cairo University.

Extraction, isolation and identification

An aqu. EtOH extract (3:1) of the collected fresh leaves sample (2 kg), concentrated *in vacuo* was applied to a polyamide 6S CC (Riedel-De Häen AG, Seelze Hannover, Germany) and eluted by H_2O followed by H_2O-EtOH mixts of decreasing polarities to yield 13 fractions (I-XIII) which were individually subjected to 2D-PC. The last fraction XIII contained mainly compounds **1-3**, which were individually separated through repeated Sephadex LH-20 CC fractionation, thus yielding pure 192 mg sample of **1**, 102 mg sample of **2** and 168 mg sample of **3**.

Quercetin 7-methyl ether 3,3',4'-tri-*O*-KSO_3 (1) – R_fs: Table 1. Electrophoretic mobility (cm): Table 1. UV (MeOH, MeOH+NaOAc, MeOH+NaOAc+H_3BO_3, MeOH+$AlCl_3$, MeOH+NaOMe and MeOH+HCl) λ_{max} (nm): Table 1. Negative FAB-MS: m/z 631 $[M-K]^-$, 593 $[M-2K+H]^-$ and 555 $[M-3K+2H]^-$, Mr 670 amu. Mild acid hydrolysis of **1** (14 mg, refluxed with 5 ml aqu. 0.05 N HCl, 100°, for 5 minutes) yielded three distinct intermediates (CoPC). The hydrolysate gave a heavy white precipitate with aqu. $BaCl_2$. Mild hydrolysis with aqu. AcOH (53 mg, refluxed with 5 ml aqu. 10% AcOH, 100°, for 15 minutes, paper chromatographic analysis each 3 minutes for the mixture) yielded rhamnetin and three intermediates, **1a, 1b, 1c**. Rhamnetin: light yellow precipitate: R_fs: Table 1. UV (MeOH and with diagnostic shift reagents) λ_{max} (nm): Table 1; EI-MS: m/z 316 $[M]^+$, Mr 316 amu. ^{1}H-NMR (DMSO-d_6, room temp.): δ ppm 6.4 (d, J=2.5 Hz, H-6), 6.82 (d, J=2.5 Hz, H-8), 6.9 (d, J=7.5 Hz, H-5'), 7.60 (dd, J=2.5 Hz and J=7.5 Hz, H-6'), 7.68 (d, J=2.5 Hz, H-2'), 3.85 (s, OCH_3). Pure samples of **1a, 1b** and **1c**, separated by Prep. PC: R_fs: Table 1; Electrophoretic mobility (cm) Table 1; UV (MeOH and with diagnostic shift reagents) λ_{max}: Table 1; Mild acid hydrolysis with methanolic HOAc (34 mg in 5 ml MeOH:HOAc, 99:1, room temp,

96 hr) yielded one intermediate I. Prep. PC afforded a pure sample (12 mg) of the formed intermediate I: R_fs: Table 1; electrophoretic mobility (cm): Table 1; (MeOH and with shift reagents): Table 1; ^{1}H-NMR (DMSO-d_6, room temp.) of the intermediate I: δ ppm 6.24 (d, J=2.5 Hz, H-6), 6.6 (d, J=2.5 Hz, H-8), 6.94 (d, J=7.5 Hz, H-5'), 7.9 (dd, J=7.5 Hz and J=2.5 Hz, H-6'), 8.1 (d, J=2.5 Hz, H-2'), 3.8 (s, OCH_3); ^{13}C-NMR of I: Table 2. ^{1}H-NMR (DMSO- d_6, room temp.) of **1**: δ ppm 6.36 (d, J=2.5 Hz, H-6), 6.72 (d, J= 2.5 Hz, H-8), 6.9 (d, J=7.5 Hz, H-5'), 7.9 (d, J=2.5 Hz, H-2'). 8.1 (dd, J=7.5 Hz and J=2.5 Hz, H-6'), 3.86 (s, OCH_3). ^{13}C-NMR of **1**: Table 2.

Known compounds: quercetin 3-*O*-KSO$_3$ (2) – R_fs: Table 1. Electrophoretic mobility (cm): Table 1. UV (MeOH and with shift reagents) λ_{max} (nm): Table 1. -ve FAB-MS: m/z 381 $[M-K]^-$ 420 amu. ^{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 J=2.5 Hz, H-6'). ^{13}C-NMR: Table 2.

Quercetin 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} (nm): Table 1. -ve FAB-MS: m/z 395 $[M-K]^-$, Mr 434 amu. ^{1}H-NMR: δ 6.2 (d, J=2.5 Hz, H-6), 6.4 (d, J=2.5 Hz, H-8), 7.15 (d, J =7.5 Hz, H-5'), 7.62 (m, H-2' and H-6'), 3.83 (s, OCH_3). ^{13}C-NMR: Table 2.

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