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Constitutive flavonoids of the flowers of *Tamarix tetragyna*

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Abstract – A phytochemical investigation of the aqueous ethanolic flower extract of *Tamarix tetragyna* led to the isolation and characterization of the hitherto unknown conjugates, kaempheride 3,7-dipotassium sulphate and kaempferol 3,4'-dipotassium sulphate as well. Twelve known flavonol compounds, including kaempheride 3-potassium sulphate and kaempheride 3-*O*-β-glucuronide were also isolated and identified. ^{1}H- and ^{13}C-NMR spectra for the known kaempheride derivatives have been recorded and assigned for the first time. Structures of all compounds were established by conventional methods of analysis and confirmed by ^{1}H-, ^{13}C-NMR and mass spectral analysis.
Key words – *Tamarix tetragyna*, Tamericaceae, flavonol sulphates, Kaempferol 3,4'-dipotassium sulphate, Kaempheride 3,7-dipotassium sulphate.

Introduction

In Egypt, the leaves and young branches of *Tamarix* plants, (as a medicinal plants), are cooked for oedema of spleen and mixed with ginger for uterus affections, while the bark, when boiled in water with vineger is used as lotion against lice (Bulas, 1983). Whilst almost all parts of the Egyptian Tamarix plants, *Tamarix nilotica*, *T. aphylla* and *T. tetragyna* have been comprehensively studied for their phenolic (Nawwar *et al.*, 1984a, b and Heba *et al.*, 1996), only a single phytochemical investigation have been carried out on the wood of the latter (Sahar, 1997). In the present study, we report the investigation of the constitutive flavonoids of *T. tetragyna* flowers and describe the isolation and structure elucidation of the new sulphated flavonoids kaempheride 3,7-dipotassium sulphate **2**, and kaempferol 3,4'-dipotassium sulphate **3**. The present study describe also the isolation and characterization of the known flavonoids, kaempferol 3-*O*-α-rhamnoside 7-*O*-β-glucoside **1**; kaempherol 3-*O*-α-rhamnoside **4**; kaempheride 3-potassium sulphate **5**; kaempherol 3-*O*-β-glucuronide **6**; quercetin 3-*O*-β-glucuronide **7**; tamarixetin 3-potassium sulphate **8**; kaempheride 3-*O*-β-glucuronide **9**; 7,4'-dimethoxy-kaempherol 3-potassium sulphate **10**; kaempheride **11**; tamarixetin **12**; quercetin **13** and kaempferol **14**. ^{1}H- and ^{13}C-NMR of compounds **5** and **9** were recorded and assigned for the first time. The presence of four sulphated flavonols in the investigated *T. tetragyna* flower extract is thus not all surprising in view of the fact that *Tamarix* plants are growing in marshy habitats and that the flavonoid ester formation is associated with the adaptability of plants to their environment (Harborne, 1971; McClure, 1970).

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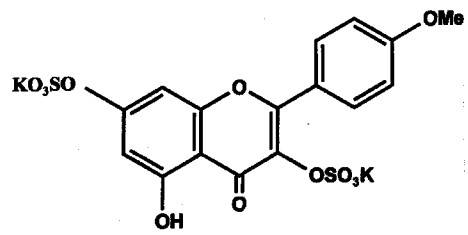
Results and Discussion

Aqueous ethanolic flower extract of *T. tetragyna* was shown by 2D-PC screening to contain a complicated mixture of phenolics (positive responses towards $FeCl_3$ spray reagent), and flavonols glycosides (colour on PC without and with ammonia vapour). Compounds **1-14** were isolated by standard methods (CC on polyamide and Sephadex LH-20 and prep. PC). The known compounds (**1, 4-14**) gave chromatographic, UV spectral, FAB-MS, hydrolytic and ^{1}H-NMR spectral data typical for kaempferol 3-*O*-α-rhamnoside 7-*O*-β-glucoside **1** (Pawlowska, 1980); kaempferol 3-*O*-α-rhamnoside **4** (King and Acheson, 1950); kaempferide 3-potassium sulphate **5** (Soileman, 1983); kaempferol 3-*O*-β-glucuronide **6** and quercetin 3-*O*-β-glucuronide **7** (Nawwar *et al*, 1984, 1970); tamarixetin 3-potassium sulphate **8** (Soileman, 1983); kaempferide 3-*O*-β-glucuronide **9** (Chauhan *et al.*, 1979); 7,4'-dimethoxy kaempferol 3-potassium sulphate **10** (Nawwar *et al.*, 1984); kaempferide **11**; tamarixetin **12** (Soileman, 1983); quercetin **13** and kaempferol **14** (Nawwar *et al.*,

1984). The ^{1}H- and ^{13}C-NMR spectra of compounds **5** and **9** were recorded and assigned for the first time. The ^{1}H-NMR spectrum (DMSO-d_6, room temp.) of **5** was closely similar to that of its flavonol moiety (see Experimental), thus proving the absence of any other protonated moieties except the aglycone. ^{13}C-NMR of both **5** and its aglycone **11** (table 1) provided a confirmation of the achieved structure and showed similarities in the chemical shift values of resonances of similar carbons in both spectra, but a distinction can be made since the resonances of the C-3 and C-2 of **5** are quite different from those of the corresponding carbons in the aglycone **11**. This change in chemical shifts is obviously due to substitution with the sulphate residue at C-3. The ^{1}H-NMR spectrum of **9** (DMSO-d_6, room temp.) exhibited an anomeric sugar proton resonance appearing as a doublet of J=8 Hz at δ 5.35, in addition to the typical proton resonances pattern of the flavonol kaempferide (see Experimental). From the ^{13}C-NMR spectrum of **9**, the presence of a glucuronic acid moiety followed from the carboxyl carbon resonance at δ 169.9, the β-anomeric carbon resonace at δ 101.8 and from the remaining four carbon resonances in the sugar region of this spectrum (Table 1). This moiety must be attached to the kaempferide hydroxyl number 3 because the resonance of the corresponding carbon C-3, was shifted upfield and ortho-carbons, C-2 and C-4 were shifted downfield (Table 1). The β-configuration of the glucuronide moiety was derived from the C-1 chemical shift at δ 101.8, while the pyranose form of this moiety was deduced from the chemical shift values of sugar carbon resonances.



Compound **2**.

The new flavonoid **2**, isolated as an amorphous white powder of chromatographic properties and UV spectral data consistent with 3,5,7-trisubstituted kaempferol derivatives (dark purple spot on PC under UV light, turning dull yellow on prolong exposure to ammonia vapour, high R_f values in aqueous solvents, low R_f values in organic solvents, no NaOAc shift and an unstable NaOMe spectrum on UV spectral analysis). Compound **2** showed cationic electrophoretic properties and gave a yellow ppt. When treating its aqueous solution with aqueous sodium cobaltynitrite. Besides, it exhibited a molecular ion peak $[M-K]^-$ at *m/z*: 497, corresponding to a Mr of 536 in negative FAB-MS analysis. On normal acid hydrolysis, **2** yielded 4'-methoxykaempferol as the only organic hydrolysis product (CoPC, UV spectral ^{1}H- and ^{13}C-NMR data). However the hydrolysate gave a white ppt. When treated

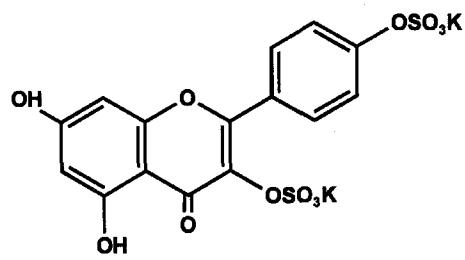
Table 1. ^{13}C chemical shifts (ppm) of the isolated *Tamarix tetragyna* flavonoids

Carbon No.	Kaempferol	Kaempferide	**2**	**3**	**5**	**9***
2	146.8	146.1	155.5	155.3	155.4	156.4
3	135.7	136.0	132.6	132.0	131.8	133.0
4	175.9	176.0	177.2	177.0	177.1	177.2
5	160.7	160.3	161.0	160.0	159.8	160.1
6	98.2	98.2	98.8	98.0	98.0	98.9
7	163.9	164.2	161.3	163.1	163.2	164.3
8	93.5	93.2	93.8	93.0	93.0	93.9
9	156.2	156.1	156.6	156.1	155.9	156.4
10	103.0	103.1	103.1	104.1	104.2	103.9
1'	121.7	123.2	122.9	123.1	123.1	120.6
2'	129.5	129.5	130.5	131.0	131.1	130.9
3'	115.5	114.0	113.9	113.6	113.8	115.2
4'	159.2	160.7	161.2	157.3	161.0	161.2
5'	115.5	114.0	113.9	113.6	113.8	115.2
6'	129.5	129.5	130.5	131.0	131.1	130.9
MeO		55.2	55.5	--	55.4	55.8

Chemical shifts of the β-glucopyranuronide moiety of **9**: δ: 101.8 (C-1), 71.4 (C-2), 74.3 (C-3), 76.52 (C-4), 76.7 (C-5), 169.9 (C=O).

with aqueous $BaCl_2$. On controlled acid hydrolysis with 10% aqueous HOAc, compound **2** yielded kaempferide 7-monopotassium sulphate (yellow spot on PC, under UV light, no shift with NaOAc and stable NaOMe spectrum on UV spectral analysis, cationic electrophoretic mobility and ^{1}H-NMR data, see Experimental). This and the above given data proved the structure of **2** to be kaempferide 3,7-dipotassium sulphate. Confirmation of this structure was then received through ^{1}H- and ^{13}C-NMR analysis. In the recorded ^{1}H-NMR spectrum, substitution of the kaempferide moiety at position 7, followed from the downfield shift of the resonances of the H-6 (δ 0.18) and that of the H-8 protons (δ 0.2), all in comparison with the resonances of the corresponding protons in the spectrum of kaempferide. The recorded ^{13}C-NMR spectrum, exhibited, as expected, the kaempferide pattern of carbon resonances only, whereby a 13 distinct resonances, in addition to a methoxyl one (δ 55.5) have been recognized. Sulphatation at positions 3 and 7, which is known to be equal in its effect on resonances chemical shifts, to acetylation and glycosidation (Nawwar and Buddrus, 1981), followed from the upfield of the C-3 and C-7 resonances and the accompanying downfield shifts of their ortho related carbons, C-2 & C-4, C-6 & C-8, respectively, thus finally confirming the structure of **2** to be kaempferid 3,7-dipotassium sulphate.

The new natural product **3**, obtained as an amorphous white powder of chromatographic properties and UV spectral data similar to those reported for 3,4'-di-O-substituted kaempferol, (dark purple spot on PC, under UV light, of relatively high R_f values in aqueous solvents and exhibiting unstable NaOMe spectrum on UV spectral analysis). It showed cationic characters and migrated towards the cathode on electrophoretic analysis. Its aqueous solution gave a yellow ppt. When treated with aqueous sodium cobaltinitrite, thus proving the presence of potassium ion (Feigel, 1956). On normal acid hydrolysis of **3** (1.5 N aqueous HCl, 100°, 10 min.), only kaempferol (CoPC, UV and ^{1}H-NMR spectral data) was yielded, while on controlled acid hydrolysis (10% aqueous AcOH, 100°, 3 min.), it yielded an intermediate **3a** which appeared as a dull yellow spot on PC, under UV light and migrated towards the cathode on electrophoretic analysis. The UV spectral and FAB-MS analytical data ([M-k]$^-$: *m/z* 365, corresponding to Mr of 404 mu) of **3a** proved its



Compound **3**.

identity as kaempferol 4'-mono-potassium sulphate. Consequently compound **3** is kaempferol 3,4'-dipotassium sulphate. Support for this conclusion was then received through negative FAB-MS of **3** which revealed a molecular ion peak at m/z 483, corresponding to Mr of 522 mu. This structure was then confirmed by ^{1}H-NMR analysis. The received spectrum (DMSO-d_6, room temp.) showed two meta coupled aromatic proton resonances recognized as two doublets (J = 2.5 Hz) at δ 6.12 and 6.37, assignable to H-6 and H-8 in a 7-unsubstituted kaempferol derivative. In this spectrum, the chemical shift values of the remaining two ortho coupled aromatic doublets (J = 7.5 Hz), recognized at δ 6.92 (H-3' & H-5') and at 8.2 (H-2' & H-6') reflect substitution at the 4'-OH group of the kaempferol moiety in **3.** Finally, the recorded ^{13}C-NMR spectrum confirmed its structure as kaempferol 3,4'-dipotassium sulphate, which represents to the best of our knowledge a new natural product.

Experimental

^{1}H-NMR chemical shifts were measured relative to TMS and ^{13}C-NMR chemical shifts relative to DMSO-d_6 and converted to the TMS scale by adding 39.5. Typical conditions: spectral width = 4000 or 2700 Hz for ^{1}H and 17500 or 13500 for ^{13}C, 32 K data points and a flip angle of 45°. FAB-MS were measured on MM 7070 E spectrometer (VG analytical) and ESI-MS spectra were measured on a SSQ Finnigan MAT 4600 quadrupol mass spectrometer equipped with a home-built ES-ion source (ISAS Dortmund, Germany). PC was carried out on Whatman No. 1 paper, using solvent systems: (1) H_2O; (2) HOAc-6 (HOAc-H_2O, 3:47); (3) HOAc-15 (HOAc-H_2O, 3:17); (4) BAW (*n*-BuOH-HOAc-H_2O, 4:1:5, upper layer); (5) C_6H_6-*n*-BuOH-H_2O-pyridine (1:5:3:3, upper layer). Solvents 2 and 4 were used for prep. PC on Whatman No. 3 MM paper, solvents 4 and 5

for sugar analysis.

Plant material – Fresh flowers were collected from a mature *Tamarix tetragyna* Ehronb shrub up to 2 m height, growing wild in the marshy habitats around Qarun lake, El-Fayum district, upper Egypt, in April 1995 and authenticated by Dr. Nabiel El-Hadiedy, Professor of Botany, faculty of Science, Cairo University.

Isolation and identification – Fresh flowers were exhaustively extracted with EtOH-H_2O (3:1). The concd. Extract was applied to a polyamide 6S CC (Riedel-De Haen AG, Seelze Hannover, Germany) and eluted by H_2O followed by H_2O-EtOH mixts. of decreasing polarities to yield **6** major fractions (I-V), compound **1** was isolated from fr. II by applying a combination of polyamide CC, using a mixture of MeOH-toluene-H_2O (60:38:2), as solvent and prep. PC using BAW as solvent, compounds **2 & 3** were isolated from fr. III by prep. PC, using BAW as solvent, compounds **4 & 5** were isolated from fr. IV by applying a combination of cellulose CC, using EtOH-H_2O (1:1) for elution and prep. PC, using BAW as solvent, compounds **6-10** were isoalted from fr. V by applying a combination of polyamide CC, using a mixture of MeOH-toluene-H_2O (60:38:2) for elution and prep. PC using BAW and HOAc-6 as solvents and compounds **11-14** were isolated from fr. VI also by polyamide CC, using EtOAc saturated with water for elution and the subsequent crystallization of the desorbed crude materials.

Kaempferide 3-potassium sulphate 5: R_fS (x 100): 48(1), 44(3), 37(4). UV data: λ_{max}^{MeOH}(nm): 252 inflection, 264, 350; +NaOAc:257 inflection, 272, 375; +H_3BO_3: 253 inflection, 265, 352; +$AlCl_3$: 255 inflection, 274, 354, 395; +NaOMe: 270, 400. Migrated distance on electrophoretic analysis (buffer soln. of pH 2, 0.75M HCO_2H, 50 v/cm, 30°, 90 min): 6.6 cm. FAB-MS: negative ion: 379 $[M-K]^-$, Mr: 418. ^{1}H-NMR: δ: 6.13 (d, J = 2,5 Hz, H-6); 6.36 (d, J = 2.5 Hz, H-8); 7.04 (d, J = 8 Hz, H-3 & H-5'); 8.1 (d, J = 8 Hz, H-2 & H-6'); 3.8 (s, OMe). ^{13}C-NMR: see Table 1.

Kaempferide 3-O-β-glucopyranuronide 9: R_fS (x100): 70(1), 42(3), 45(4). UV data λ_{max}^{MeOH}(nm): 267, 345; +NaOAc: 274, 366; +H_3BO_3: 272, 360; +$AlCl_3$: 275, 382; +NaOMe: 278, 400. β-glucuronidase enzymic hydrolysis: 12 mg of **9** were incubated together with 0.5 ml of β-glucuronidase in acetate buffer (pH = 5.1) at 37° for 24 hr. CoPC confirmed the release of kaempferide. ^{1}H-NMR: δ: kaempferide moiety: 6.27 (d, J = 2.5 Hz, H-6); 6.4 (d, J = 2.5 Hz, H-8); 6.86 (d, J = 8 Hz, H-3' and H-5); 8.03 (d, J = 8 Hz, H-2 and H-6); 3.8 (s, OMe, hidden by water and hydroxyl proton signal); β-glucuronide moiety: 5. 35 (d, J = 8 Hz, H-1, β-glucuronide); 4.9 (OH proton humb, removed on addition of a ½ drop of TFA); 3.1-3.9 (m, glucuronide proton signals). ^{13}C NMR: see Table 1.

Kaempferide 3,7-dipotassium sulphate 2: R_fS (x100): 50(1), 44(3), 34(4). UV data: λ_{max}^{MeOH} (nm): 267, 345; +NaOAc: 266, 370; +H_3BO_3: 257 inflection, 267, 355; +$AlCl_3$: 272, 358, 392; +NaOMe: 272, 402. Migrated distance on electrophoretic analysis: 10.0 cm. FAB-MS: negative ion: 497 $[M-K]^-$, Mr: 536. On normal acid hydrolysis [8 mg in 5 ml aq. 1.5N HCl, 100°, 10 min], **2** yielded kaempferide (CoPC, UV spectral, ^{1}H-NMR, Table 1). On controlled acid hydrolysis [10% aq. HOAc, 100°, 10 min], it yielded kaempferide 7-mono-potassium sulphate: R_fS (x100): 6(1), 11(3), 67(4); UV data λ_{max}^{MeOH}(nm): 269, 360; +NaOAc: 269, 357, 370; +H_3BO_3: 270, 360; +$AlCl_3$: 272, 395; +NaOMe: 270, 403. Distance migrated on electrophoretic analysis: 6.8 cm; ^{1}H-NMR; δ: 6.34 (d, J = 2.5 Hz, H-6); 6.5 (d, J = 2.5 Hz, H-8); 7.0 (d, J = 8 Hz, H-3 and H-5); 8.1 (d, J = 8 Hz, H-2 and H-6); 3.78 (s, OMe). ^{1}H NMR of **2**: δ: 6.36 (d, J = 2.5 Hz, H-6); 6.5 (d, J = 2.5 Hz, H-8); 6.7 (d, J = 8 Hz, H-3 and H-5); 8.12 (d, J = 8 Hz, H-2 and H-6); ^{13}C- NMR: Table 1.

Kaempferol 3,4'-di-potassium sulphate 3: R_fS (x100); 52(1), 47(3), 32(4). UV data λ_{max}^{MeOH}(nm): 255 inflection, 265, 350; +NaOAc: 256, 271, 376; +H_3BO_3: 255 inflection, 262, 350; +$AlCl_3$: 255 inflection, 272, 355, 392; +NaOMe: 270, 405. Migrated distance on electrophoretic analysis: 10.2 cm. FAB-MS: negative ion: 483 $[M-K]^-$, Mr, 522. On normal acid hydrolysis [22 mg in 10 ml aq. 1.5N HCl, 100°, 10 min], **3** yield kaempferol (CoPC, UV spectral and ^{1}H-NMR). On controlled acid hydrolysis [10% aq. HOAc, 100°, 3 min] it yielded kaempferol-4-monopotassium sulphate **3a**: R_fS (x100): 9(1), 18(3), 56(4). UV data λ_{max}^{MeOH}(nm): 267, 368; +NaOAc: 273, 305, 378; +H_3BO_3: 252 inflection, 267, 366; +$AlCl_3$: 252 inflection, 271, 350, 422; +NaOMe: 280, 411. Migrated distance on electrophoretic analysis: 6.9 cm. FAB.MS: negative ion: 365 $[M-K]^-$, Mr: 404. ^{1}H NMR of **3**: δ: 6.12 (d, J = 2.5 Hz, H-6); 6.37 (d, J = 2.5 Hz, H-8); 6.92 (d, J = 7.5 Hz, H-3 and H-5); 8.20 (d, J = 7.5 Hz, H-2' and H-6); ^{13}C-NMR: see Table 1.

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