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Flavonoid and methoxyellagic acid sodium sulphates from *Frankenia laevis* L.

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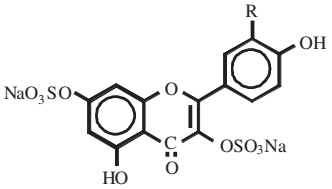
Two new flavonol di-sodium sulphates and an ellagic acid methyl ether mono-sodium sulphate have been found in the aqueous alcohol whole plant extract of *Frankenia laevis* L. They have been identified as the 3,7-di-sodium sulphate of kaempferol, the 3,7-di-sodium sulphates of quercetin and the 4′-mono-sodium sulphate of ellagic acid-3-methyl ether. Also, five known compounds have been isolated and characterized from the same extract. Establishment of all structures has been achieved mainly, by ESI-MS and NMR.

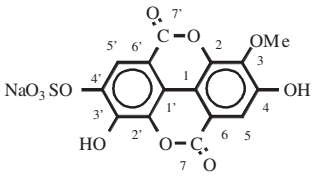
1. Introduction

Six different taxa of the genus *Frankenia,* including *F. laevis* L., previously proved to contain flavonoid mono-potassium sulphates (Harborne 1975). In the same study, one of these taxa, namely, *F. pulevrulenta,* was investigated briefly and proved to contain the 7-potassium sulphate of kaempferol and isorhamnetin, together with the 7-potassium sulphate-3-glucuronide of kaempferol, quercetin and isorhamnetin. The phenolic compounds of *F. laevis* L. were not subjected to any comprehensive investigation before. The present paper deals with the isolation and structural elucidation of eight phenolics, **1**–**8**, including two new flavonoid anions **1** and **2,** a new mono-methoxy ellagic acid anion **3** and five known compounds, namely, gallic acid **4**, gallic acid-3-methyl ether **5**, ellagic acid **6**, ellagic acid-3-methyl ether **7** and ellagic acid-3,3′-dimethyl ether **8**, from the aqueous alcohol (25%) whole plant extract of *F. laevis*. The plant is a low scabrid-puberulent shrub of 10–30 cm height which represents, together with *F. pulverulenta,* the only Frankeniaceae plants growing wild in Egypt.



Kaempferol 3,7-disodium sulphate 1 : R = H
Quercetin 3,7-disodium sulphate 2 : R = OH

The flavonoid sulphates act as enzymes inhibitors (Haraguchi et al. 1996), allergens (Sallusto et al. 2000), antioxidants (Yagi et al. 1994) and regulators of auxin transport (Faulkner and Rubery 1992). The potassium salts of kaempferol and quercetin-3,7-disulphate have been isolated once before, the former from *Reaumuria mucronata* (Tamaricaceae) and the latter from *Flaveria bidentis* (Asteraceae). Their structures were determined on the basis of chromatographic, electrophoretic, UV spectral and hydrolytic evidences (Nawwar et al. 1977; Varin et al. 1987).



Ellagic acid 3-monomethyl ether-4' -sodium sulphate 3

2. Investigations, results and discussion

Compounds **1**–**8** were isolated from an aqueous ethanolic (25% EtOH) whole plant extract of *F. laevis* by a combination of repeated Sephadex LH-20 column and prep. paper chromatography (PPC). The known compounds **4**–**8** gave chromatographic, UV, ESI-MS, ^{1}H and ^{13}C NMR data identical with those reported for gallic acid (Nawwar et al. 1984a), gallic acid-3-methyl ether (Souleman et al. 1998), ellagic acid (Nawwar and Souleman 1984b) ellagic acid-3-methyl ether (Takahashi et al. 1977) and ellagic acid 3,3′-dimethyl ether (Nawwar et al. 1982), resp.

Compound **1** is an off-white amorphous powder that exhibits chromatographic properties, an anionic character on electrophoretic analysis and UV maxima in MeOH and after addition of diagnostic shift reagents (Table 1), (Harborne and Williams 1975; Mabry et al. 1970) which suggested it to be an anionic flavonol derivative, most probably a 3,7-disubstituted kaempferol (El-Mousallamy et al. 2002). On mild acid hydrolysis, compound **1** yielded two intermediates, $\mathbf{1_a}$ and $\mathbf{1_b}$ which were separated by PPC. Their chromatographic and electrophoretic properties, UV absorption (Table 1) and results of mild acid hydrolysis are closely similar to those of 7-*O*- and 3-*O*-substituted kaempferol. All hydrolysates gave a precipitate with aque-

Table 1: Chromatographic, electrophoretic and UV spectral data of compounds 1–3 and their hydrolysis products

Compound	Chromatographic properties			Electrophoretic mobility	UV Spectral data λ_{maz} nm, in MeOH					
	R_f (X 100)				MeOH	(a) + NaOAc	(b) + H_3BO_3	(a) + $AlCl_3$	(a) + NaOMe	(a) + HCl
	H_2O	HOAC	BAW		(a)	(b)				
Kaempferol-3,7-di-sodium sulphate (**1**)	80	69	15	6.3	269, 345	267, 360	269, 344	272, 300#, 342, 392	266, 385	267, 363
Kaempferol-7-sodium sulphate (**1a**)	30	22	45	4.2	263, 360	254, 352	262, 360	274, 342, 415	253, 398	265.378
Kaempferol-3-sodium sulphate (**1b**)	37	28	50	5.0	267, 337	272, 345	266, 337	272, 302, 348, 395	270, 395	266, 360
Quercetin-3,7-di-sodium sulphate (**2**)	77	66	14	6.2	257, 267#, 300, 350	267, 374	268, 310, 432#	272, 357, 400#	272, 415	255, 266#, 367
Quercetin-7-di-sodium sulphate (**2a**)	28	18	42	4.2	252, 266#, 365	250, 375	255, 382	264, 356#, 425	258, 410	263, 380
Quercetin-3-sodium sulphate (**2b**)	35	26	42	4.9	255, 267#, 303#, 350	269, 378	260, 375	272, 300#, 432,	270, 400	267, 373
Ellagic acid-3-methyl ether-4′-sodium sulphate (**3**)	65	55	32	5.0	248, 342# 359					
Ellagic acid-3-methyl ether (**3a**)	00	09	71		250, 346# 363					

ous barium chloride, thus proving the presence of sulphate groups in the intermediates **1a** and **1b** and the parent compound **1**. The aqueous solution of **1** failed to give any precipitate with sodium hexanitrocobaltate (potassium ion), but the solid substance gave the typical sodium flame test specific for sodium ions, thus proving that the sulphate increments of **1** are existing as sodium salt. All these data are in accordance with **1** to be kaempferol 3,7-disodium sulphate. On negative ESI-MS analysis **1** exhibited a molecular ion $[M-Na]^-$ at $m/z = 467$, and ions at $m/z = 387$, 365 and 285, attributable to $[M-SO_3Na]^-$, $[387+H-Na]^-$ and $[387+H-SO_3Na]^-$ ions, respectively, while on positive ESI-MS analysis, it exhibited a molecular ion $[M+H]^+$ at $m/z = 491$, together with an $[M+23]^+$ ion at $m/z = 513$, corresponding to $C_{15}H_8O_{12}S_2Na_2$, (490). The ^1H NMR spectrum of **1** (DMSO-d_6, room temp.) proved an *O*-substitution located at C-7 of the kaempferol moiety. This followed from the lowfield location of the two resonances at δ 6.45 (d, J = 2 Hz) and 6.81 (d, J = 2 Hz) attributable to the 6-H and 8-H kaempferol protons, resp. The spectrum showed also two doublets at δ 6.98 and 7.89, both (J = 8 Hz) assignable to the equivalent 3′-H and 5′-H and 2′-H and 6′-H. In the ^{13}C spectrum of **1** (Table 2) most of the chemical shift values were similar to those reported for 3,7-di-*O*-glycosylated kaempherol (El-Mousallamy et al. 2002; Agrawal 1989). The attachment of sulphate substituents to C-7 and C-3 of the aglycone moiety followed from the upfield shift of the resonances of these carbons and the downfield shift of the resonances of their *ortho*- and *para*-located carbons (see Experimental, all in comparison with the corresponding resonances of free kaempferol). For similar shifts Barron and Ibrahim (1988); Nawwar and Buddrus (1981). Other resonances in this spectrum exhibited chemical shifts

Table 2: ^{13}C NMR data of compounds 1–3

Carbon No.	1	Kaempferol+	**2**	Quercetin+	**3**	**3a**
	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm), multiplicity and J value (Hz)	δ (ppm), multiplicity and J value (Hz)
1					111.8 (d 6.1)	112.0 (d 6)
2	155.5	146.8	155.4	146.9	141 (s)	140.9 (s)
3	132.9	135.7	130.0	135.5	140.2 (m)	140.5 (m)
4	177.2	175.9	177.0	175.8	153.0 (s)	152.8
5	160.8	160.7	160.2	160.7	114.4 (d 166)	111.7 (d 165)
6	98.4	98.2	97.1	98.2	112.0 (s)	112.3 (s)
7	161.0	163.9	160.3	163.9	158.3 (6.9)	158.5 (d 6.0)
8	93.0	93.5	92.6	93.3		
9	157.8	156.2	158.0	156.2		
10	105.7	103.0	105.8	103.1		
1′	120.9	121.7	121.5	121.1	116.2 (d 6.2)	112.5 (d 6.2)
2′	130.2	129.5	115.7	115.3	114.0 (s)	136.03 (s)
3′	115.9	115.5	145.3	145.0	146.0 (d 6.9)	140.5 (m)
4′	159.6	159.2	148.7	147.6	138.0 (s)	153.0 (s)
5′	115.9	115.5	115.9	115.6	117.5 (d 165)	111.6 (d 164)
6′	130.2	129.5	122.1	120.0	114.2 (s)	107.9 (s)
7′					160.8 (d 4.5)	159.1 (s)
OMe					61.1 (q 146)	61.0 (q 146)

+ Data from Nawwar et al. (1984c)

which were in close agreement to the achieved structure of **1** as kaempferol 3,7-di-sodium sulphate, a natural product which has not reported before to occur in nature.

Compound **2**, an off-white amorphous powder, shows chromatographic properties, electrophoretic mobility, UV data (Table 1) and results of mild acid hydrolysis which led to its preliminary identification as the quercetin analogue of compound **1**. Negative ESI-MS analysis of **2** exhibited a molecular ion $[M-Na]^-$ at $m/z = 483$, accompanied by three ions at $m/z = 403$, 381 and 301, attributable to an $[M-SO_3Na]^-$, $[403+H-Na]^-$ and $[403 + H\text{-}SO_3Na]^-$ ions, respectively. However, on positive ESI-MS analysis, **2** exhibited a molecular ion $[M + H]^+$ at $m/z = 507$ and $[M + 23]^+$ ion at $m/z = 529$ corresponding to $C_{15}H_8O_{13}S_2Na_2$ (506). These data, together with the results of ^1H and ^{13}C NMR analysis (Table 2) confirm **2** to be quercetin 3,7-di-sodium sulphate, which has not been reported before as a natural product.

Compound **3**, a light brown powder, exhibits chromatographic characteristics, results of colour reactions, electrophoretic mobility and UV absorptions, similar to those of anionic ellagic acid-dimethyl ether (Hussein 1997, Table 1). It gave the sodium flame test. Mild acid hydrolysis of **3** afforded the aglycone $\mathbf{3_a}$ (paper chromatography), which precipitated from the hydrolysate on cooling. The aqueous filtrate gave the precipitate with barium chloride. Chromatographic behaviour, colour properties and UV absorptions (Table 1) suggested $\mathbf{3_a}$ to be most probably an ellagic acid monomethyl ether derivative. Negative ESI-MS analysis exhibited $[M-H]^-$ at $m/z = 315$ and a significant ion at $m/z = 301$, corresponds to $[M-CH_3]^-$. The ^1H NMR spectrum of $\mathbf{3_a}$ (DMSO-d_6, room temp.) revealed two aromatic proton singlets at δ 7.48 and 7.45 ppm and a methoxyl proton singlet at δ 4.1 ppm. The proton decoupled ^{13}C NMR spectrum disclosed well separated 15 carbon resonances, the most upfield (δ 61.2 ppm) was assigned to the methoxyl function. This location indicats that the methoxyl group is attached to a ring carbon which has adjacent oxygenated ring carbons on both sides (Hussein et al. 1997). Consequently, $\mathbf{3_a}$ is identified to be ellagic acid 3-monomethyl ether. This was further confirmed through assignment of the remaing carbon resonances (Table 2). The assignments were greatly supported by comparison with the ^{13}C NMR data reported previously, for both ellagic acid (Nawwar et al. 1994) and ellagic acid 3,3′-dimethyl ether (Nawwar et al. 1982). On negative ESI-MS analysis, **3** exhibited a molecular ions $[M-H]^-$ and $[M-Na]^-$ at $m/z = 417$ and 395, resp., while on positive ESI-MS it exhibited a molecular ion at $[M+Na]^+$ at 441, corresponding to $C_{15}H_7O_{11}Sna$ (418), which was confirmed by sulphur analysis (found: S, 7.42%; calculated: S, 7.65%). The ^1H NMR spectrum of **3** (DMSO-d_6, room temp.) revealed two aromatic singlets at δ 7.84 and 7.45 ppm. The downfield shift of the former resonance (in comparison with the corresponding resonance in the spectrum of $\mathbf{3_a}$), is due to sulphatation of the hydroxyl group *ortho* to the proton resonating at 7.84 ppm. Therefore, the sulphate moiety is either attached at C-4 or C-4′ of the ellagic acid-3-methyl ether moiety of **3**. The spectrum revealed also a singlet at δ 4.05 ppm, attributed to OMe-group at C-3. The ^{13}C NMR spectral data of **3** could be assigned only by measuring the 2-D HMQC and HMBC spectra. In the HMQC spectrum, the lowfield proton resonance at δ 7.84 ppm was correlated to the aromatic carbon resonating at δ 117.5 ppm, while in the HMBC spectrum, cross-peaks correlate the same proton to the carbons resonating at δ 114.2, 138.0, 146.04 and 116.2 ppm. These correlations confirmed that the five carbons involved belong to the same aromatic ring in the molecule of **3**, and that this ring bears the sodium sulphate substituent. The above mentioned chemical shift values were best interpreted as follows: δ ppm 117.5 (C-5′), 116.2 (C-1′), 146.04 (C-3′), 138.0 (C-4′) and 114.2 (C-6′). The HMBC spectrum revealed also a correlation between the lowfield proton at δ 7.84 ppm and the carbon resonating at δ ppm 160.8, assignable to C-7′. Assignment of the remaining carbon resonances in the molecule of **3** (Table 2) has been achieved also on the same basis and were confirmed by measurements of ^1H-^{13}C coupling constants from a gated decoupled spectrum, thus proving the structure of compound **3** to be ellagic acid 3-monomethyl ether-4′-sodium sulphate. This is a new phenolic compound which has not been reported to occur in nature.

3. Experimental

3.1. Instruments and materials

1 H NMR spectra were measured by a Jeol-YH-300 NMR spectrometer, at 300 MHz. ^1H chemical shifts were measured relative to TMS and ^{13}C NMR chemical shifts to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 4 KHz for ^1H and 19 KHz for ^{13}C, 32 K data points and a flip angle of 45°. ESI-MS spectra were measured on SSQ Finnigan MAT 4600 quadrupol mass spectrometer (Institut für Chemie, Humboldt Universität, Berlin). Paper chromatography analysis was carried out on Whatman no. 1 paper, using solvent systems: (1) H_2O; (2) 6% HOAc; (3) BAW (*n*-BuOH-HOAc-H_2O, 4 : 1 : 5, upper layer); (4) C_6H_6-*n*-BuOH-H_2O-pyridine (1 : 5 : 3 : 3, upper layer). Solvents 1 and 3 were used for ppc on Whatman no. 3MM.

3.2. Plant material

Fresh shrubs of *Frankenia laevis* L. were collected from North Sinai, near El-Areish city, Egypt, during april 2000 and authenticated by Dr. M. El-Gibali, National Research Centre (NRC), Cairo, Egypt. A voucher specimen is deposited at the NRC.

3.3. Extraction, isolation and purification

The ground dried plant samples were extracted with EtOH-H_2O (25% EtOH) under reflux on a boiling water-bath (3 kg, three extractions each with 5 L for 8 h). The concentrated extract was applied to a Sephadex LH-20 column (125 × 5 cm) and eluted with water followed by H_2O-MeOH mixtures of decreasing polarities to yield nine fractions. Compounds **1**–**3** were isolated from the 20% MeOH fraction by repeated Sephadex LH-20 column fractionation, using water saturated with *n*-butanol, which led to the successive desorption of these compounds. Preparative paper chromatography (ppc) of the crude materials of (**1, 2** and **3**) using BAW as solvent afforded pure samples of **1**, 134 mg; **2**, 102 mg and **3**, 119 mg.

3.4. New natural products

3.4.1. Kaempferol-3,7-di-sodium sulphate (**1**)

R_f-values: Table 1. Electrophoretic mobility (cm): Table 1. UV spectral data: (MeOH) λ_{max}: Table 1. Mild acid hydroloysis (22 mg of **1**, heated with aqueous 0.1 H Cl (5 ml), at 100 °C, 2 min) yielded two intermediates ($\mathbf{1_a}$ and $\mathbf{1_b}$), separated by ppc (Whatman 3MM, Solvent: H_2O); $\mathbf{1_a}$: R_f-values: Table 1; UV spectral data: (MeOH) λ_{max}: Table 1; Mild acid hydrolysis by 0.1 N aqueous HCl yielded kaempferol (comparative paper chromatography, Co-PC); $\mathbf{1_b}$: R_f-values: Table 1; UV spectral data: (MeOH) λ_{max}: Table 1; Mild acid hydrolysis by 0.1 N aqueous HCl yielded kaempferol (Co-PC). The hydrolysate of **1** + $BaCl_2 \rightarrow BaSO_4$. For ESI-MS and ^1H NMR see section 2. ^{13}C NMR: Table 2

3.4.2. Quercetin-3,7-di-sodium sulphate (**2**)

R_f-values: Table 1. Electrophoretic mobility (cm): Table 1. UV spectral data: (MeOH) λ_{max}: Table 1. Mild acid hydroloysis (19 mg of **2**, heated with aqueous 0.1 N HCl (5 ml), at 100 °C, 2 min) yielded two intermediates ($\mathbf{2_a}$ and $\mathbf{2_b}$), separated by ppc (Whatman 3MM, Solvent: H_2O); $\mathbf{2_a}$: R_f-values: Table 1; UV spectral data: (MeOH) λ_{max}: Table 1; Mild acid hydrolysis by 0.1 N aqueous HCl yielded quercetin (Co-PC). $\mathbf{2_b}$: R_f-values: Table 1; UV spectral data: (MeOH) λ_{max}: Table 1; Mild acid hydrolysis by 0.1 N aqueous HCl yielded quercetin (Co-PC). The hydrolysate of **2** + $BaCl_2 \rightarrow BaSO_4$. For ESI-MS and ^1H NMR see section 2. ^{13}C NMR:

3.4.3. Ellagic acid-3-methyl ether-4′-sodium sulphate (**3**)

R_f-values: Table 1. Electrophoretic analysis: mobility: Table 1. UV (MeOH) λ_{max}: Table 1. Mild acid hydrolysis (33 mg of **3** heated with 10 ml aqueous 0.1 N HCl at 100 °C for 2 min, followed by filtration of the precipitated $\mathbf{3_a}$. Analytical data of $\mathbf{3_a}$: R_f-values: Table 1; (MeOH) UV λ_{max}: Table 1; For ESI-MS and ^{1}H NMR see section 2. ^{13}C chemical shifts, multiplicities and coupling constants: Table 2.

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