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Phenolic sodium sulphates of *Frankenia laevis* L.

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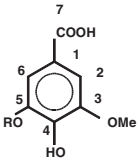
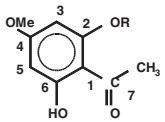
Four new phenolic anionic conjugates have been isolated from the whole plant aqueous alcohol extract of *Frankenia laevis* L. Their structures were established, mainly on the basis of ESI-MS, 1D and 2D NMR spectroscopic evidence, as gallic acid-3-methyl ether-5-sodium sulphate, acetophenone-4-methyl ether-2-sodium sulphate, ellagic acid-3,3′-dimethyl ether-4,4′-di-sodium sulphate and ellagic acid-3-methyl ether-4-sodium sulphate.

1. Introduction

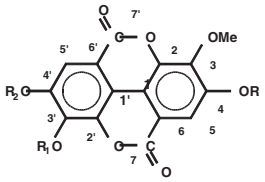
In continuation of the phytochemical investigations of the constitutive phenolics in folk medicinal plants, growing wild in the saline sandy soil along the Mediterranean coastal-strip of Egypt (Hussein 1997; Nawwar et al. 1994a; Nawwar and Hussein 1994; Souleman et al. 1998), I am describing the isolation and structural elucidation, of new phenolic sodium sulphates (**1**–**4**) from the aqueous alcohol extract of *Frankenia laevis*, gallic acid-3-methyl ether-5-sodium sulphate (**1**), acetophenone-4-methyl ether-2-sodium sulphate (**2**), ellagic acid-3,3′-dimethyl ether-4,4′-di-sodium sulphate (**3**) and ellagic acid-3-methyl ether-4-sodium sulphate (**4**). The plant is a low scabrid-puberulent shrub of 10–30 cm height which has not been subjected before to any phytochemical investigations for its phenolic constituents. It represents, besides *F. pulverulenta,* the only Frankeniaceae plant growing wild in Egypt. *F. pulverulenta* was previously shown to contain the 7-potassium sulphates of kaempferol, isorhamnetin, quercetin-3-*O*-glucuronide, kaempherol-3-*O*-glucuronide and isorhamnetin-3-*O*-glucuronide (Harborne 1975). The presence of sodium in the isolated compounds **1**–**4** was confirmed by flame atomic absorption and by ESI-MS analysis in both negative and positive modes. Compounds **1**–**4**, therefore, represent the first occurrence of phenolic sodium sulphates in nature. Generally, phenolic sulphates are of rare occurrence in nature and of the few of these conjugates which have been previously characterized, the 5-potassium sulphate of gallic acid-3-methyl ether, the 3-potassium sulphate of ellagic acid-4,4′-dimethyl ether, the 4-potassium sulphate of ellagic acid-3,3′-dimethyl ether and the 3-potassium sulphate of isoferulic acid, were isolated from different Egyptian *Tamarix* species (Tamaricaceae) (Hussein 1997; Nawwar and Hussein 1994). In addition, the 4,4′-dipotassium sulphate of ellagic acid-3,3′-dimethyl ether has been reported previously as a synthetic product (Satoshi et al. 1990).



Compound (1): R=SO_3 Na
Compound (1_a): R=H



Compound (2): R=SO_3 Na
Compound (2_a): R=H



Compound (3): R_1=OMe, R, R_2=SO_3Na
Compound (3_a): R_1=OMe, R, R_2=H
Compound (3_b): R_1=OMe, R_2=H, R=SO_3Na
Compound (4):R_1, R_2=H, R=SO_3Na
Compound (4_a): R, R_1, R_2=H

It should be mentioned also that derivatives of ellagic acid sulphates have previously been reported to possess potent inhibitory activity against aldose reductase. The activity of these derivatives was even higher than that recorded for ellagic acid itself, most probably due to the present sulphate group(s) (Satoshi et al. 1990).

2. Investigations, results and discussion

Preliminary screening of the aqueous ethanolic (25%) whole plant extract of *Frankenia laevis* by electrophoretic and paper chromatographic analysis indicated the presence of several phenolics of anionic character. The extract was fractionated over Sephadex LH-20, using water/methanol mixtures of decreasing polarities. Repeated Sephadex LH-

Table 1: Chromatographic, electrophoretic and UV spectral data of compounds 1–4 and their hydrolysis products

Compound	Chromatographic properties R_f-(X 100)			Electro-phoretic mobility	UV Spectral data λ_{maz} nm, in MeOH
	H_2O	HOAC	BAW		
Gallic acid-3-methyl ether-5-sodium sulphate (**1**)	95	85	50	6.4	266, 295 (inflection)
Gallic acid-3-methyl ether (**1a**)	52	54	85		272
Acetophenone-4-methyl ether-2-sodium sulphate (**2**)	92	83	55	6.2	230, 265, 307 (Shoulder)
Acetophenone-4-methyl ether (**2a**)	52	56	59		227, 269, 310 (Shoulder)
Ellagic acid-3,3′-dimethyl ether-4,4′-di-sodium sulphate (**3**)	68	58	39	5.7	260, 290 (inflection), 353, 370 (inflection)
Ellagic acid-3,3′-dimethyl ether (**3a**)	00	09	82		251, 362 (shoulder), 375
Ellagic acid-3,3′-dimethyl ether-4-sodium sulphate (**3b**)	62	55	41	5.2	262, 290 (inflection), 355, 372 (inflection)
Ellagic acid-3-methyl ether-4-sodium sulphate (**4**)	60	52	38	5.2	248, 342 (inflection), 359
Ellagic acid-3-methyl ether (**4a**)	00	09	71		250, 346 (inflection), 363

20 column fractionation of the 10% aqueous methanol fraction, followed by preparative paper chromatography afforded pure samples of compounds **1**–**4**.

Compound **1** isolated as an amorphous white powder which appeared as an intense blue spot on paper chromatograms, under short UV light (R_f-value Table 1) and migrated towards the anode on paper electrophoretic analysis. Besides, it turned dirty blue when sprayed with $FeCl_3$ and dark purple when sprayed with aniline/xylose spray reagent specific for carboxylic acids (Smith 1960). Compound **1** gave an intense UV absorption maximum, in MeOH at 266 nm (Table 1) and yielded on mild acid hydrolysis a product (**1a**) identified to be gallic acid-3-methyl ether (Co-PC, UV absorption maxima, ^1H and ^{13}C NMR). The hydrolysate gave a heavy white precipitate with aqueous $BaCl_2$, thus proving the presence of a sulphate group. The aqueous solution of the compound failed to give any precipitate with sodium cobaltylnitrite, specific for the potassium ion. However, the presence of a sodium ion in this solution was confirmed by flame atomic absorption (sodium spectral line detected at 589 nm). Compound **1** showed, in its negative ESI-MS spectrum, a molecular ion $[M - H]^-$ at $m/z = 285$, and two significant peaks at $m/z = 263$ and 183, consistent with $[M - Na]^-$ and $[M - SO_3Na]^-$ ions, respectively. On positive ESI-MS, **1** exhibited molecular ions $[M + H]^+$ and $[M + Na]^+$, at $m/z = 287$ and 309, respectively. In addition, it gave, on elemental analysis a carbon content of 32.12% and a hydrogen content of 3,01%, corresponding to the molecular formula $C_8H_8O_8Sna$ (calculated element percent: C 33.56, H 2.79%). This and the above given analytical data suggested that compound **1** is a 3-monomethoxy gallic acid which bears a sodium sulphate substituent. In the ^{13}C NMR spectrum of (**1**), the carbon resonances were assigned by applying the substituent additive rules on the ^{13}C NMR data of gallic acid-3-methyl ether (Nawwar and

Table 2: ^{13}C chemical shifts (δ ppm), multiplicities and coupling constants (Hz) of compounds 1–4 and their hydrolysis products

Carbon No.	**1**	**1a**	**2**	**2a**	**3**	**3a**	**3b**	**4**	**4a**
1	119.6 (t 2)	120.9	108.4 (t 7.5)	105.7 (t 7.8)	115.1 (d 7)	111.8 (d 6.1)	115.0 (d 7.2)	114.4 (d 7)	112.0 (d 6)
2	108.9 (dd 165, 7.5)	105.2	156.8 (d 1.7)	164.9 (d 1.8)	141.0 (s)	141.1 (s)	140.8 (s)	141.6 (s)	140.9 (s)
3	149.1 (d 2.9)	148.2	100.8 (dd 165.5, 7.5)	93.9 (dd 165, 7)	143.3 (m)	140.2 (m)	143.6 (m)	142.8 (m)	140.5 (m)
4	141.6 (t 7.4)	139.5	165.3 (m)	166.5 (m)	153.2 (s)	153.0 (s)	153.1 (s)	152.o (s)	152.8 (s)
5	144.1 (d 3.1)	145.5	97.0 (dd 165.5, 7.5)	93.9 (dd 165, 7)	113.2 (d 167)	111.4 (d 166)	113.2 (d 166)	112.4 (d 167)	111.7 (d 165)
6	117.4 (dd 165, 7.5)	111.2	165.2 (d 1.5)	164.9 (d 1.8)	113.0 (s)	112.0 (s)	113.2 (s)	112.9 (s)	112.3 (s)
7	166.7 (t 4.4)	167.1	204.8 (q 1.5)	203.97 (q 1.4)	158.5 (s)	158.3 (s)	158.7 (s)	159.0 (s)	158.5 (s)
CH_3			32.9 (q 144)	33.4 (q 143.2)					
1′					115.1 (d 7.5)	111.8 (d 6.1)	111.4 (d 6)3	112.3 (d 7)	112.5 (d 6.5)
2′					141.0 (s)	141.1 (s)	140.8 (s)	136.3 (s)	136.03 (s)
3′					143.3 (m)	140.2 (m)	140.4 (m)	140.1 (m)	140.5 (m)
4′					153.0 (s)	153.0 (s)	153.2 (s)	153.2 (s)	153.0 (s)
5′					113.2 (d 167)	111.4 (d 166)	111.8 (d 167)	111.3 (d 166)	111.6 (d 164)
6′					113.0 (s)	112.0 (s)	112.5 (s)	107.7 (s)	107.9 (s)
7′					158.5 (s)	158.3 (s)	158.7 (s)	159.3 (s)	159.1 (s)
OMe							61.3 (q 146) 61.7 (q 146)	61.1 (q 146)	60.0 (q 146)

Hussein 1994). Unambiguous assignments were achieved by measuring the $^{1}H-^{13}C$ coupling constants from a gated decoupled ^{13}C spectrum of **1**. The ^{1}H NMR spectrum showed resonances for two aromatic *meta* coupled protons ($J = 2.5$ Hz) at δ 7.2 and 7.6 ppm, assignable to H-2 and H-6, respectively. The third singlet recognized in this spectrum at δ 3.8 was assigned to the methoxyl protons. The ^{1}H decoupled ^{13}C spectrum showed all of the 8 expected carbon resonances (Table 2) from which the lowfield one, at δ 166.7 ppm was assigned to the carboxyl carbon as was confirmed by its splitting, in the gated decoupled spectrum (t, $^{3}J = 4.4$ Hz). The resonances for the protonated carbons C-2, at δ 108.9 and C-6, at 117.4 ppm, were identified by their one-bond couplings ($^{1}J = 165$ Hz and 165.5 HZ, respectively) and the additional three bond-couplings ($^{3}J = 7.5$ Hz and 7 Hz, respectively). A three bond-coupling was also found for C-4 (t, $^{3}J = 7.4$ Hz) at δ 141.6 ppm. The remaining carbons C-3 at δ 149.1 and C-5 at δ 144.1 ppm, each exhibited a doublet due to a two-bond coupling ($^{2}J = 2.9$ Hz and 3.1 Hz, respectively). These data confirmed the identity of compound **1** as gallic acid-3-methyl ether-5-sodium sulphate, which has not been reported before to occur in nature.

Compound **2** was isolated as a light brown amorphous powder of chromatographic and elctrophoretic properties (Table 1) which reflect anionic characters. It showed UV spectral maxima in MeOH at 227, 269 and 310 nm (Table 1), which shifted, on addition of NaOMe to 248 and 328 (intense) nm. Mild acid hydrolysis of **1** gave a hydrolysis product **2a**, with no ionic characters. This product exhibited UV spectral maxima, in MeOH at 228, 274, 312 (shoulder) nm (Table 1), which were shifted to 246, 329 nm, on addition of NaOMe. On negative ESI-MS, it exhibited a significant ion at $m/z = 166$, consistent with an $[M - H\text{-}15]^-$ and a molecular ion $[M - H]^-$ at $m/z = 181$, corresponding to a molecular mass of 182. The ^{1}H NMR Spectrum of **2a** showed only resonances at δ 6.7 (s, H-3 and H-5), 3.7 (s, OMe-3) and at 2.68 ppm (s, Me-8). Ambiguity about the structure of **2a** was unraveled by measuring and assigning proton decoupled, gated decoupled and a DEPT ^{13}C NMR spectra (DMSO-d_6, room temperature). The proton decoupled spectrum disclosed distinct 7 resonances (Table 2), two of which appeared with intensity, approximately double of those of the other. The two most upfield resonances at δ 33.4 and 56.2 ppm which appeared in the gated decoupled spectrum as a quartet ($J = 142$ Hz and 147 Hz, respectively) were assignable to an aliphatic methyl and to a methoxyl group, respectively. In this spectrum, another resonance was recognized at δ 204.0 ppm as a quartet of coupling constant 1.4 HZ, thus proving a coupling through two bonds (^{2}J). This coupling and chemical shift value were consistent with a carbonyl carbon in an aryl-alkyl ketone (Kalinowski et al. 1984). The intense resonance at δ 93.9 ppm (dd, $J = 165$ Hz and 7 Hz) was attributed to the two equivalent protonated carbons (C-3 and C-5), while the intense resonance at δ 165.0 ppm (d, $J = 1.8$ Hz) was assigned to the two equivalent oxygenated carbons (C-2 and C-6). The location of the remaining resonances, in this spectrum, at δ 105.7 (t, $^{3}J = 7.8$ Hz) and at 166.5 ppm (m), when incorporated with the above given ^{13}C NMR data proved a 2,6-dihydroxy-acetophenone-4-methyl ether structure for product **2a**, a conclusion which was further confirmed by a DEPT experiment.

The aqueous hydrolysate of the parent compound **2** gave a white precipitate with aqueous $BaCl_2$ to prove the presence of a sulphate radical. The presence of sodium ions in the hydrolysate was confirmed by flame atomic absorption. On negative ESI-MS analysis, **2** exhibited a molecular ion $[M - H]^-$ at $m/z = 283$, accompanied by an ion at 261, attributable to an $[M - Na]^-$ ion, while on positive ESI-MS analysis, it exhibited a molecular ion at $m/z = 285$, corresponding to a molecular mass of 284 and a molecular formula of $C_9H_9O_7SNa$, as was confirmed by elemental analysis (Found: C: 37.81%, H: 3,3%; Calculated: C: 38.02%, H: 3.16%). This and the above given data led to the conclusion that compound **2** is a phloroacetophenone-4-methyl ether-2-sodium sulphate. In the ^{1}H spectrum of **2**, the presence of a substituent at the C-2 position followed from the chemical shifts and mode of splitting of the resonances of protons H-2 and H-6, which appeared as two distinct *meta* coupled doublets ($J = 2.5$ Hz) at δ 6.6 and 6.2 ppm, respectively, due to the losses of symmetry in the molecule of **2** by the introduction of the sulphate radical at the C-2 position. The remaining two resonances at δ 2.6 (s) and 3.8 (s) are obviously due to the aliphatic methyl and methoxyl groups present in the molecule. The absence of symmetry in the molecule of **2** was also recognized in its ^{13}C spectrum, which revealed 9 distinct carbon resonances (Table 2). Applying the sulphate substituent additive rules (Nawwar and Buddrus 1981), the position of the sulphate group at C-2 was deduced from the upfield shift of the C-2 resonance and the large downfield shift of the adjacent C-3 resonance (all in comparison with the corresponding resonances in the spectrum of **2a**). Assignments of the remaining carbon resonances (see Experimental) were confirmed by measurement of a gated decoupled and a DEPT spectra. These and the above given analytical data finally confirmed the identity of compound **2** as phloroacetophenone-4-methyl ether-2-sodium sulphate, a new natural phenolic anionic conjugates, which is reported here for the first time.

Compound **3**, an amorphous yellow powder which showed chromatographic properties and UV spectral maxima, in MeOH (Table 1) closely similar to those of ellagic acid derivatives (Nawwar and Souleman 1984). It exhibited anionic characters and yielded on mild acid hydrolysis by aqueous 0.1 N HCl, at 100 °C for 2 min, an aglycone **3a**. This aglycone was found to be identical, through its chromatographic properties, non-ionic behaviour, UV absorption (Table 1), results of ESI-MS, ^{1}H and ^{13}C NMR spectral analysis with ellagic acid-3,3′-dimethyl ether (Nawwar et al. 1982). The aqueous hydrolysate gave a heavy white precipitate with aqueous $BaCl_2$ and proved to contain sodium ions by flame atomic absorption. Compound **3** when refluxed with aqueous 10% acetic acid, at 100 °C for 2 min was hydrolysed to the aglycone **3a** and an intermediate **3b**. Each was separated pure by applying a combination of Sephadex LH-20 column fractionation and preparative paper chrmomatography. The off-white powder of **3b**, thus obtained exhibited analytical data (R_f-values, UV absorption, ^{1}H and ^{13}C spectral data) identical with those reported for ellagic acid-3,3′-di-methyl ether-4-sulphate (Nawwar et al. 1982). However, a distinction was made by the recognition that the intermediate **3b** contained sodium, instead of potassium ions. This view was confirmed by the results of its ESI-MS analysis (positive mode: $[M + H]^+$ and $[M + Na]^+$ at $m/z = 433$ and 455, respectively and negative mode: $[M - H]^-$ and $[M - Na]^-$ at $m/z = 431$ and 409), thus proving a molecular mass of 432 for which. On ESI-MS (positive mode) of the parent compound **3**, a molecular ion $[M + H]^+$ was recognized at $m/z = 535$. However, the spectrum obtained through nega-

tive ESI-MS analysis was more informative and revealed ions at m/z = 533, 511, 431 and 329, attributable to $[M-H]^-$, $[M-Na]^-$, $[M-NaSO_3]^-$ and $[M+H-2(NaSO^3)]^-$, corresponding to a molecular mass of 534 and a molecular formula of $C_{16}H_8O_{14}S_2Na_2$, as was confirmed through elemental analysis (Found: C : 35.66% H: 1.72%, Calculated: C : 35.96% H: 1.47%). Consequently, compound **3** is ellagic acid-3,3′-dimethyl ether-di-sodium sulphate. The received ^{1}H NMR spectrum of **3**, (DMSO-d_6, room temperature) was very simple and revealed only, two singlets at δ 7.9 and 4.1 ppm. The former singlet was assigned to the two equivalent protons H-5 and H-5′ in the symmetrical molecule of ellagic acid-3,3-dimethyl ether-4,4′-di-sodium sulphate, while the latter was attributed to the protons of the two equivalent methoxyl groups OMe-3 and OMe-3′. Symmetrical substitution of the 3,3′-dimethoxy ellagic acid moiety in the molecule of **3** by sodium sulphate moieties was further deduced from the pattern of carbon resonances recoginzed in its ^{13}C NMR spectrum. This pattern consisted only from 8 distinct resonances (Table 2). The chemical shifts and multiplicities (measured from a gated decoupled spectrum) of these resonances, were closely similar to those calculated by applying the substituent additive rules on the ^{13}C NMR data of the aglycone **3a**, thus finally confirming the identity of compound **3** as ellagic acid-3,3′-di-methyl ether-4,4′-di-sodium sulphate. This compound is a new natural product which was not reported previously to occur in nature.

Compound **4** obtained as an amorphous off-white powder which exhibited chromatographic properties, results of colour reactions, electrophoretic mobility and UV absorption data closely similar to those of compound **3** (Table 1). The aqueous solution of compound **4** was proved to contain sodium ion by flame atomic absorption. On mild acid hydrolysis, either by queous 0.1 N HCl, or 10% aqueous acetic acid, at 100 °C for 2 min, compound **4** hydrolysed directly to an aglycone **4a**, without producing any intermediate. The aqueous hydrolysate left after separation of the aglycone **4a**, gave a white precipitate with aqueous $BaCl_2$. Chromatographic behaviour, colour properties and UV absorption spectrum (Table 1), when compared with those of ellagic acid (Nawwar et al. 1994b) and of ellagic acid-3,3′-dimethyl ether **3a** (Nawwar et al. 1982), suggested that **4a**, is most probably, an ellagic acid monomethyl ether derivative. This view was then supported by negative ESI-MS analysis. The received spectrum exhibited a molecular ion $[M-H]^-$ at m/z = 315, corresponding to a molecular mass of 316 and a significant ion at m/z = 301, corresponding to $[M\text{-}CH_3]^-$. The ^{1}H NMR spectrum of **4a** revealed, as expected, two aromatic proton singlets, at δ 7.48 and 7.53 ppm and a methoxyl proton singlet at δ 4.04 ppm. The proton decoupled ^{13}C NMR spectrum of **4a** disclosed well separated 15 carbon resonances, the most upfield of which was recognized at δ 61.0 ppm and was assigned to the existing methoxyl function. This comparatively lowfield location of the resonance of the methoxyl group indicated that it is attached to an aromatic ring carbon which has an adjacent oxygenated ring carbons on both side (Hussein et al. 1997). This finding proved that the aglycone **4a** is ellagic acid-3-mono-methyl ether. Assignments of the remaing carbon resonances (Table 2) were greatly aided by the ^{13}C NMR data reported previously, for both ellagic acid (Nawwar et al. 1994b) and for ellagic acid-3,3′-di-methyl ether (Nawwar et al. 1982) and were found to be in close agreement with the chemical shift values calculated for ellagic acid-3-methyl ether, by applying substituent additive rules on the ^{13}C NMR data of ellagic acid. Consequently, the parent compound **4** is ellagic acid-3-methyl ether-mono-sodium sulphate.

On negative ESI-MS analysis, the parent salt **4** exhibited molecular ions $[M-H]^-$ and $[M-Na]^-$ at m/z = 417 and 395, respectively, while on positive ESI-MS it exhibited a molecular ion at $[M+Na]^+$ at 441, corresponding to a molecular mass of 418 and a molecular formula of $C_{15}H_7O_{11}SNa$, as was confirmed by elemental analysis (Found: C: 42.82% and H: 2.03%; Calculated: C: 43.06% and H: 1.67%).

To find out the site of attachment of the sulphate moiety to the ellagic acid-3-mono-methyl ether moiety in the molecule of **4**, NMR spectral analysis was then performed. The received ^{1}H NMR spectrum revealed two resonances, in the aromatic region at δ 7.86 and 7.56 ppm. Downfield shift of the former resonance [in comparison with the corresponding resonance, in the spectrum of **4a**] is certainly due to sulphataition of a hydroxyl group *ortho-* to the proton possessing this resonance. Therefore, the site of attachment of the sulphate moiety is either at the C-4 or the C-4′ position of the ellagic acid-3-methyl ether moiety of **4**. In addition, the spectrum also, revealed a singlet proton resonance at δ 4.0 ppm, attributed to OMe-3.

Establishment of the final structure of **4** was achieved through a comprehensive ^{13}C NMR spectral analysis. Unambiguous assignments could be accomplished only by measuring the 2-D HMQC and HMBC spectra. In the HMQC spectrum, the aromatic proton which possesses the downfield resonance at δ 7.86 ppm was found to be correlated to the aromatic carbon resonating at δ 112.4 ppm. In the HMBC spectrum, cross-peaks correlating the same proton to the carbons which resonate at δ 114.4, 142.8, 152.0 and 112.9 ppm were recognized. These correlations confirmed that the above determined 5 carbons belong to the same aromatic ring and that this ring bears the sodium sulphate substituent. When comparing these chemical shift values with those measured for compounds **3** and **3b** and when taking into consideration that the sulphate substituent is sited at position *ortho* to the protonated carbon, the above determined chemical shift values were, therefore assigned as follows: δ ppm 112.4 (C-5), 114.4 (C-1), 142.8 (C-3), 152.0 (C-4) and 112.9 (C-6). The HMQC and HMBC spectra also led to the unambiguous assignments (Table 2) of the remaining carbon resonances in the ^{13}C decoupled spectrum of **4**. All assignments were confirmed by measurements of ^{1}H-^{13}C coupling constants from a gated decoupled spectrum. These assigned data were best interpreted in terms of an ellagic acid-3-mono-methyl ether-4-sodium sulphate structure. Sulphatation of the the 4′-OH group, instead of the 4-OH, would result in a set of carbon chemical shifts which would deviate markedly, from the measured values. Consequently, compound **4** was identified to be ellagic acid-3-mono-methyl ether-4-sodium sulphate It represents a new phenolic compound which has not been reported previously to occur in nature.

3. Experimental

3.1. Instruments and materials

^{1}H NMR spectra were measured at 300 MHz. ^{1}H 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 = 4000 Hz for ^{1}H and 19000 Hz for ^{13}C, 32 K data points and a flip angle of 45°. Flame atomic absorption analysis was performed on a Varian Spectra-AA 220 instrument, lamp current: 5 ma, fuel: acetyline, oxidant: air, slit width: 0.5 nm. ESI-MS spectra were measured on SSQ

Finnigan MAT 4600 quadrupole mass spectrometer (Institut für Chemie, Humboldt- Universität, Berlin). Paper chromatographic 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 preparative paper chromatography on Whatman no. 3MM. Solvents 3 and 4 were used for sugar analysis.

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 Center (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 (1 : 3) under reflux over 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 inte) and eluted with water followed by H_2O−MeOH mixtures of decreasing polarities to yield a phenolic fraction (paper chromatography), eluted by H_2O−MeOH (90 : 10). Compounds **1**–**4** were isolated, from the 10% fraction by applying repeated Sephadex LH-20 column fractionation, using water for elution, which led to the successive desorption of these compounds. Preparative paper chromatography of the crude materials of **1** and **2**, using H_2O as solvent, and of the crude materials of **3** and **4**, using BAW as solvent, afforded pure samples [(**1**, 134 mg); (**2**, 102 mg); (**3**, 119 mg) and (**4**, 88 mg)] of each.

3.4. New natural products

3.4.1. Gallic acid-3-methyl ether-5-sodium sulphate (**1**)

R_f-values: Table 1. Electrophoretic analysis: on Whatman no 3MM paper, buffer solution of pH 2, H_2O−HCOOH−CH_3COOH (89 : 8.5 : 2.5), 2 h, 50 v/cm: Table 1. UV λ_{max}^{MeOH} nm: Table 1. Mild acid hydrolysis (43 mg of **1** refluxed with 10 ml aqueous 0.1 N HCl, at 100 °C, for 2 min, followed by extraction of the cold hydrolysate by ethyl acetate, washing by distilled water, filteration through anh. Na_2SO_4 and dryness of the washed dried ethyl acetate layer in vacuum), yielded gallic acid-3-methyl ether $\mathbf{1_a}$; analytical data of $\mathbf{1_a}$: R_f-values: Table 1; UV λ_{max}^{MeOH} nm: Table 1; ^{1}H NMR: δ ppm: 7.15 (d, J = 2.5 HZ, H-6); 7.24 (d, J = 2.5 Hz, H-2), 3.8 (s, OMe-3); ^{13}C NMR: Table 2. Flame atomic absorption of the hydrolysate: sodium line at 589 nm. ESI-MS of **1**: negative molecular ions: m/z = 263 $[M - Na]^-$ and 285 $[M - H]^-$ and a daughter ion: m/z 183 $[m\text{-}NaSO_3]^-$; positive molecular ions: m/z 309 $[M + Na]^+$ and 287 $[M + H]^+$, M_r = 286. ^{1}H NMR of **1**: δ ppm: 7.6 9d, J = 2.5 HZ, H-6); 7.2 (d, J = 2.5 Hz, H-2); 3.8 (s, OMe-3). ^{13}C chemical shifts, multiplicities and coupling constants Table 2.

3.4.2. Phloroacetophenone-4-methylether-2-sodium sulphate (**2**)

R_f-values: Table 1. Electrophoretic analysis: mobility: Table 1. UV λ_{max}^{MeOH} nm: Table 1. Mild acid hydrolysis (37 mg of **1** refluxed with 10 ml aqueous 0.1 N HCl, at 100 °C, for 2 min, followed by extraction of the cold hydrolysate by ethyl acetate, and proceeding as usual) yielded gallic acid-3-methyl ether **2a**; Flame atomic absorption of the hydrolysate: sodium line at 589 nm. Also, mild acid hydrolysis with aqueous acetic acid (7 mg of **2** were heated, under reflux, with 0.5 ml aqueous 10% CH_3COOH, over a boiling water bath, for 2 min) yielded **2a**. Analytical data of **2a**: R_f-values: Table 1; UV λ_{max}^{MeOH} nm: Table 1; ESI-MS: negative molecular ion: m/z = 181 $[M - H]^-$ and a daughter ion: m/z = 166 $[M - H\text{-}15]^-$; ^{1}H NMR: δ ppm: 6.7 (s, 2 H, H-3 & H-5); 3.7 (s, 3H, OMe-4), 2.68 (s, Me−C=O); ^{13}C chemical shifts, multiplicities and coupling constants: Table 2. ESI-MS of **2**: negative molecular ions: m/z = 261 $[M - Na]^-$ and 283 $[M - H]^-$; positive molecular ion: m/z 285 $[M + H]^+$, M_r = 284. ^{1}H NMR of **2**: δ ppm: 6.6 (d, J = 2.5 HZ, H-3); 6.2 (d, J = 2.5 Hz, H-5); 3.8 (s, OMe-4); 2.6 (s, Me−C=O). ^{13}C chemical shifts, multiplicities and coupling constants: Table 2.

3.4.3. Ellagic acid-3,3′-dimethyl ether-4,4′-di-sodium sulphate (**3**)

R_f-values: Table 1. Electrophoretic analysis: mobility: Table 1. UV λ_{max}^{MeOH} nm: Table 1. Mild acid hydrolysis (48 mg of **3** refluxed with 10 ml aqueous 0.1 N HCl, at 100 °C, for 2 min, followed by filteration of the formed precipitate **3a**; Flame atomic absorption of the aqueous hydrolysate: sodium line at 589 nm. Analytical data of **3a**: R_f-values: Table 1; UV λ_{max}^{MeOH} nm: Table 1; ESI-MS: negative molecular ion: m/z = 329 $[M - H]^-$ and a daughter ion: m/z = 314 $[M - H\text{-}15]^-$; ^{1}H NMR: δ ppm: 7.5 (s, 2 H, H-5 and H-5′); 4.08 (s, 3 H, OMe-3 and O-Me-3′); ^{13}C chemical shifts, multiblicities and coupling constants: Table 2. Also, mild acid hydrolysis by aqueous acetic acid (52 mg of **3** were heated, under reflux, with 5 ml aqueous 10% CH_3COOH, over a boiling water bath, for 2 min), followed by Sephadex LH-20 column fractionation, using H_2O as an eluent, and preparative paper chromatography, using BAW, as solvent afforded pure sample of intermediate **3b**. Analytical data of **3b**: R_f-values: Table 1; UV λ_{max}^{MeOH} nm: Table 1; ESI-MS: negative molecular ions: m/z = 431 $[M - H]^-$ and 409 $[M - Na]^-$; positive molecular ions: m/z = 433 $[M + H]^+$ and 455 $[M+Na]^+$; ^{1}H NMR: δ ppm: 7.78 (s, 1 H, H-5); 7.6 (s, i H, H-5′); 4.18 (s, 3 H, OMe-3); 4.10 (s, ih, O-Me-3′); ^{13}C chemical shifts, multiblicities and coupling constants: Table 2. ESI-MS of **3**: negative molecular ions: m/z = 511 $[M - Na]^-$ and 533 $[M - H]^-$ and daughter ions: m/z = 431 $[M - NaSO_3]^-$ 329 $\{M + H\text{-}2(NaSO_3)\}^-$; positive molecular ion: m/z 535 $[M + H]^+$, M_r = 534. ^{1}H NMR of **3**: δ ppm: 7.9 (s, 2 H, H-5 and H-5′); 4.1 (s, 6 H, OMe-4 and OMe-4′). ^{13}C chemical shifts, multiblicities and coupling constants: Table 2.

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

R_f-values: Table 1. Electrophoretic analysis: mobility: Table 1. UV λ_{max}^{MeOH} nm: Table 1. Flame atomic absorptionof an aqueous solution of **4**: sodium line at 589 nm. Mild acid hydrolysis (41 mg of **4** refluxed with 10 ml aqueous 0.1 N HCl, at 100 °C, for 2 min, followed by filtration of the formed precipitate **4a**; Flame atomic absorption of the aqueous hydrolysate: sodium line at 589 nm. Analytical data of **4a**: R_f-values: Table 1; UV λ_{max}^{MeOH} nm: Table 1; ESI-MS: negative molecular ion: m/z = 315 $[M - H]^-$ and a daughter ion: m/z = 301 $[M - H\text{-}15]^-$; ^{1}H NMR: δ ppm: 7.53 (s, 1 H, H-5); 7.48 (s. 1 H, H-5′); 4.04 (s, 3 H, OMe-3); ^{13}C chemical shifts, multiplicities and coupling constants: Table 2. Also, mild acid hydrolysis by aqueous acetic acid (5 mg of **4** were heated, under reflux, with 5 ml aqueous 10% CH_3COOH, over a boiling water bath, for 2 min), afforded 4_a, (Co-PC). ESI-MS of **4**: negative molecular ions: m/z = 395 $[M - Na]^-$ and 417 $[M - H]^-$; positive molecular ion: m/z = 535 $[M + Na]^+$, M_r = 441. ^{1}H NMR of **4**: δ ppm: 7.86 (s, 1 H, H-5); 7.56 (s, 1 H, H-5′); 4.0 (s, 3 H, OMe-4). ^{13}C chemical shifts, multiplicities and coupling constants: Table 2.

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