A New Ferulic Acid Ester and Other Constituents from *Tamarix nilotica* Leaves

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Phytochemical investigation of the leaves of *Tamarix nilotica* (Tamaricaceae) has led to isolation of methyl ferulate 3-*O*-sulphate (1) for the first time from natural sources. In addition, coniferyl alcohol 4-*O*-sulphate (2), kaempferol 4′-methyl ether (3), tamarixetin (4) and quercetin 3-*O*-β-D-glucupyranuronide (5) were isolated from the *n*-butanol soluble fraction of the extract. The pentacyclic triterpenoid, 3α-(3″,4″-dihydroxy-*trans*-cinnamoyloxy)-D-friedoolean-14-en-28-oic acid (6) was isolated from the *n*-hexane soluble fraction of the extract. The structures of these compounds were determined on the basis of spectroscopic analyses including 2 dimensional NMR. Compounds 3, 4 and 6 exhibited 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity with IC_{50} values of 35.2, 37.0 and 21.2 μM, respectively.

Key words *Tamarix nilotica*; Tamaricaceae; ferulic acid; flavonoid; triterpene; antioxidant

Tamarix nilotica (Tamaricaceae) has been used in traditional Egyptian medicine as antiseptic agent. The polyphenolic compounds and flavonoids of *T. nilotica* have been previously investigated.[1—5] In our efforts to discover new drugs from medicinal plants, antioxidant activity of a selection of commonly occurring wild plants growing in Beni-Sueif governorate, Upper Egypt, has been tested.[6] The *in vitro* antioxidant assays used were 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, superoxide anion scavenging activity and iron chelating activity. Extracts prepared from the leaves and flowers of *T. nilotica* have shown the highest antioxidant activity in the three kinds of assay. In this report, phytochemical investigation on the leaves of *T. nilotica* was carried out. A new derivative of ferulic acid (**1**) and five known compounds **2**—**6** were isolated. The structures of the isolated compounds were elucidated on the basis of spectroscopic data and their DPPH radical scavenging activity were evaluated.

Results and Discussion

Compound **1** was isolated from the *n*-butanol soluble fraction of the extract as a white amorphous solid. The UV spectrum showed absorptions at 205, 230, 299 and 314 nm. The

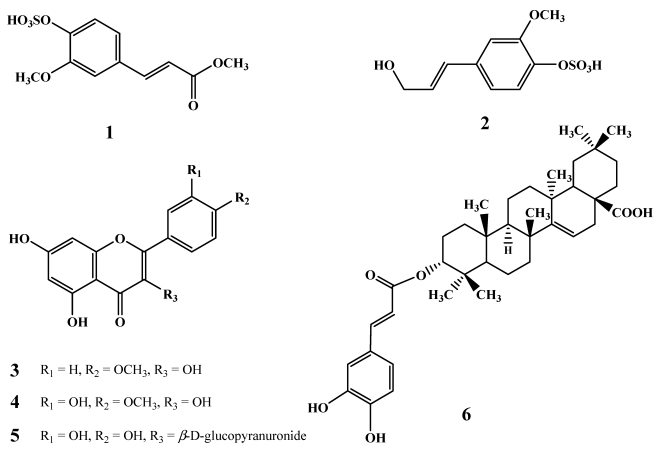

Fig. 1. Chemical Structures of Compounds **1**—**6**

^{1}H- and ^{13}C-NMR spectra (including distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC), correlation spectroscopy (COSY) and nuclear Overhauser effect spectroscopy (NOESY) experiments) have shown that compound **1** is a *trans*-ferulic acid derivative. Two 1H doublets at δ 7.60 and 6.37 (J=15.9 Hz) represented the *trans*-substituted olefinic H-1′ and H-2′. The ^{1}H-NMR signals of two 3H singlets at δ 3.86 and 3.75 represented two methoxy methyls. The singlet signal at δ 3.86, that showed a correlation with signal at δ_C 52.0 in the HMQC spectrum, has shown a correlation with the carboxyl carbon signal at δ_C 169.4 in the HMBC spectrum. Therefore, the signal at δ_C 52.0 is assigned to the methoxy carbon esterifying the carboxyl group. The other signal at δ_C 56.7 is assigned to the methoxy carbon attached to C-3 on the aromatic ring. This latter signal showed a HMQC correlation with the signal at δ 3.75 in the ^{1}H-NMR spectrum. A correlation in the HMBC spectrum between the signals at δ 3.75 and δ_C 155.2 confirms the assignment of the latter signal to C-3. The ^{1}H-NMR spectrum has shown signals for three coupled aromatic protons. The coupling constants of these signals indicated that two of these protons are in *ortho* positions while the third one is in *meta* position to one of the former protons. The broad singlet signal at δ 7.73 was assigned to proton attached to C-2. This signal showed a correlation in the HMQC spectrum with carbon signal at δ_C 122.6. Therefore, this signal was assigned to C-2. The signal at δ_C 155.2 (C-3) showed a HMBC correlations with δ 3.75 (methoxy group attached to C-3) and δ 7.73. This confirms the assignment of the latter signal to the proton attached to C-2. The HMBC correlation between signals at δ 7.73 and δ_C 128.4 confirms the assignment of this signal to C-1. The HMBC spectrum showed a correlation between signals at δ 7.73 and δ_C 143.2. The DEPT-135 spectrum indicated that the latter signal is assigned to a quaternary carbon. Therefore, this signal was assigned to C-4. The HMBC correlation between the proton signal at δ 7.04 (J=8.2 Hz) and δ_C 143.2 confirms the assignment of this signal to proton attached to C-5. The broad doublet signal at δ 7.35 was assigned to proton at-

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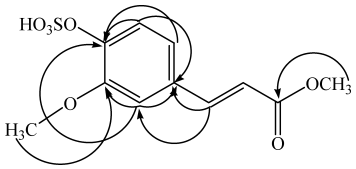

Fig. 2. Important HMBC Correlations of Compound **1**

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compound **1** in MeOH-d_4 (δ ppm)

Position	^{1}H (mult., J in Hz)	^{13}C
1	—	128.4
2	7.73 (1H, br s)	122.6
3	—	155.2
4	—	143.2
5	7.04 (1H, d, J=8.2)	114.0
6	7.35 (1H, br d, J=8.2)	127.7
1′	7.60 (1H, d, J=15.9)	116.6
2′	6.37 (1H, d, J=15.9)	145.8
3′	—	169.4
3′-$COOCH_3$	3.86 (3H, s)	52.0
3-OCH_3	3.75 (3H, s)	56.7

tached to C-6. The HMQC correlation between signals at δ 7.35 and δ_C 127.7 confirms the assignment of the latter signal to C-6. The doublet signal at δ 7.60 (J=15.9 Hz) showed HMBC correlation with δ_C 128.4 (C-1) and 122.6 (C-2). Therefore, the signal at δ 7.60 was assigned to proton attached to C-1′. The doublet signal at δ 6.37 (J=15.9 Hz) was assigned to proton attached to C-2′. Important HMBC correlations for compound **1** are shown in Fig. 2. A white precipitate was formed when $BaCl_2$ was added to the product obtained by acid hydrolysis of compound **1**, confirming the presence of a sulfate moiety. According to the earlier assignment, the sulfate moiety must esterify the hydroxyl group attached to C-4 on the aromatic ring. The HR-FAB mass spectrum has shown a *quasi*-molecular ion at *m/z* 287.0224 $[M-H]^-$ (Calcd for $C_{11}H_{11}O_7S$: 287.0225); in accordance with the sulfate ester of methyl ferulate. The ^{1}H- and ^{13}C-NMR chemical shifts and multiplicities of each protonated carbon are listed in Table 1.

Coniferyl alcohol sulfate (**2**) (Fig. 1) was isolated from the *n*-butanol soluble fraction of the methanol extract. This cinnamyl alcohol sulfate was first isolated from the stem bark and aerial tissues of *T. gallica*, *T. africana* and *T. bobeana*.[7] In the referenced study, other cinnamyl alcohol sulfates were detected in small amounts in these plants but they have not been fully identified. Compounds **3**, **4** and **5** (Fig. 1) were identified as kaempferol 4′-methyl ether, tamarixetin and quercetin 3-*O*-β-D-glucupyranuronide, respectively, by comparison of their spectral data with literature values.[3] Kaempferol 4′-methyl ether (**4**) was not reported earlier from *T. nilotica*.

Compound **6** (Fig. 1) was isolated as colorless amorphous solid from the *n*-hexane soluble fraction of the methanol extract. Spectroscopic data for compound **6** was compared with the literature values.[8,9] This compound was identified to be 3α-(3″,4″-dihydroxy-*trans*-cinnamoyloxy)-D-friedoolean-14-en-28-oic acid. This compound is first time isolated from this plant species.

Table 2. DPPH Radical Scavenging Activity of Compounds **1**—**6**

Compound	IC_{50} (μM)
1	>100
2	>100
3	35.2±2.1
4	37.0±1.5
5	>100
6	21.2±3.4
α-Tocopherol	19.4±1.1

Compounds **1**—**6** were tested for their *in vitro* antioxidant activity using DPPH radical scavenging assay (Table 2). Compounds **3**, **4** and **6** exhibited DPPH radical scavenging activity with inhibitory concentration (IC_{50}) values of 35.2, 37.0 and 21.2 μM, respectively. The antioxidant effect of the plant extract (IC_{50}=16.3 μM) is higher than the averaged sum of the effects of the isolated compounds (IC_{50}=31.1 μM) suggesting a synergistic action for the compounds present in the leaves of *T. nilotica*.

Experimental

General Experimental Procedures ^{1}H-NMR spectra were recorded in CD_3OD on a Bruker AM-500 spectrophotometer, while the ^{13}C-NMR spectra were recorded at 125 MHz on the same instrument in the same solvent. Chemical shifts are given relative to residual solvent signals. IR spectra were recorded on JASCO-320-A spectrometer. Mass spectra (electron ionization (EI)-MS and HR-EI-MS) were measured in an electron impact mode on Finnigan MAT 12 or MAT 312 spectrometers and ions are given in *m/z* (%). FAB-MS was measured on Jeol HX 110 mass spectrometers. TLC was carried out with pre-coated silica gel G-25-UV_{254} plates and detection was carried out by spraying with ceric sulphate solution.

Plant Material The leaves of *T. nilotica* were collected from Beni-Sueif governorate, Egypt, in October 2007. Plants were identified by Dr. Mohamed Abdelhalim, Department of Plant Systematics, Agricultural Research Center, Egypt. A voucher specimen was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Sueif University, Egypt.

Extraction and Isolation The dried and finely ground leaves of *T. nilotica* (2.1 kg) were macerated in 80% MeOH+H_2O (×2, 3 l) for one week at room temperature. The resulting extract was filtered and concentrated. The freeze-dried extract (34 g) was suspended in water (500 ml) and then successively partitioned with *n*-hexane, $CHCl_3$, EtOAc and *n*-butanol. The *n*-butanol-soluble fraction (9.2 g) was separated by polyamide column chromatography (400 g) using a gradient of MeOH–H_2O (H_2O, 1 l; 10—100% MeOH in H_2O, each 1 l) to yield eleven fractions according to the TLC profiles. Fraction 11 (120 mg) obtained from the 100% MeOH fraction was chromatographed over silica gel column (10 g) using a gradient of $CHCl_3$/MeOH (9.9 : 0.1—9.7 : 0.3—9.5 : 0.5—9.3 : 0.7—9 : 1—8.5 : 1.5, each 10 ml) to afford compound **3** (23 mg) and compound **4** (13 mg). Fraction 10 (480 mg) obtained from the 80% MeOH polyamide column was chromatographed over silica gel column (50 g) using a gradient of $CHCl_3$/MeOH/acetic acid (7.5 : 2.5 : 0.1—7 : 3 : 0.1—6.5 : 3.5 : 0.1—6 : 4 : 0.1—5.5 : 4.5 : 0.1—5 : 5 : 0.1—4.5 : 5.5 : 0.1—4 : 6 : 0.1, each 100 ml) to yield eight fractions. Fractions 5 and 6, that showed intensified yellow fluorescence with $AlCl_3$ and greenish yellow color with $FeCl_3$ spray reagents, were combined (105 mg) and re-chromatographed over sephadex LH20 column (10 g) using MeOH to afford 22 fractions. Fractions 7 and 8 were combined (23 mg) and purified by preparative TLC using pre-coated silica plates with solvent system ethyl acetate/acetic acid/formic acid/H_2O (11 : 1.1 : 1.1 : 3) to afford compound **5** (11 mg). Fraction 2 (280 mg) obtained from the 20% MeOH polyamide column was chromatographed over sephadex LH20 column (10 g) using MeOH to afford 15 fractions. Fraction 4 (65 mg) was rechromatographed over silica gel column (6 g) using a gradient of CH_2Cl_2/MeOH (8 : 2—6 : 4, each 5 ml) to afford 20 fractions. Fractions 12 and 13 were combined (32 mg) and purified by preparative TLC using pre-coated silica plates with solvent system ethyl acetate/acetic acid/formic acid/H_2O (11 : 1.1 : 1.1 : 3) to afford compound **2** (6 mg) and compound **1** (11 mg).

The *n*-hexane extract (1.5 g) was chromatographed over silica gel column (50 g) using a gradient of CH_2Cl_2/MeOH (10 : 0—9 : 1—8 : 2—6 : 4—4 : 6, each 100 ml) to yield 10 fractions. Fraction 6 (120 mg) obtained from the 6 : 4 fraction of the first column of the hexane fraction, was re-chromatographed over silica gel column (12 g) using a gradient of CH_2Cl_2/MeOH (20 : 0—19 : 1—18 : 2—17 : 3, each 10 ml) to afford 8 fractions. Fraction 5 eluted with 17 : 3 was re-chromatographed over silica gel column (5 g) using a gradient of *n*-hexane/EtOAc (80 : 20—70 : 30—60 : 40—50 : 50, each 5 ml) to afford compound **6** (17 mg).

(*E*)-Methyl 3-(3-Methoxy-4-(sulfoxy)phenyl)acrylate (**1**): White amorphous powder; UV: λ_{max} (MeOH) nm (log ε): 314 (1.88), 299 (1.91), 230 (1.88), 205 (1.97); FAB-MS (neg. mode) *m/z*: $[M-H]^-$ 287 (90%); HR-FAB-MS (neg. mode) *m/z*: $[M-H]^-$ 287.0224 (Calcd for $C_{11}H_{11}O_7S$: 287.0225); for ^{1}H- and ^{13}C-NMR spectral data see Table 1.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity The DPPH radical-scavenging activity was measured using a method described previously.[10] Briefly, 10 μl of each sample dissolved in DMSO was prepared in 96-well plates, and then 190 μl of 200 μM ethanolic DPPH solution was added. The mixture was incubated at room temperature for 30 min, and the absorbance of the reaction mixture was measured at 517 nm.

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