3-*O*-*trans*-Caffeoylisomyricadiol: A New Triterpenoid from *Tamarix nilotica* Growing in Saudi Arabia

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A detailed chemical study of the aerial parts of *Tamarix nilotica* (Tamaricaceae) from Saudi Arabia led to the isolation of a new pentacyclic triterpenoid, 3-*O*-*trans*-caffeoylisomyricadiol, in addition to nine known compounds. The structures of all isolated compounds were unambiguously elucidated by 1D, 2D NMR, and mass spectrometry. In the radical scavenging (DPPH) assay, 3-*O*-*trans*-caffeoylisomyricadiol exhibited potent antioxidant activity with an IC_{50} value of 3.56 μM, while that for quercetin (standard antioxidant) was 5.72 μM.

Key words: Tamarix nilotica, 3-*O*-*trans*-Caffeoylisomyricadiol, Antioxidant Activity

Introduction

Tamaricaceae is a small family including four genera and 120 species (Trease and Evans, 2002). The genera *Reaumuria* and *Tamarix* are also represented in Saudi Arabia where they contribute to the main floral elements due to their successful adaptation to arid environments (Chaudhary, 2001).

Extracts of *Tamarix* species have been widely used in traditional medicine in Asia and Africa mainly for their antiseptic, astringent, diaphoretic and diuretic properties (Bulos, 1983). In Egypt, the leaves and young branches of *Tamarix* plants are cooked in order to treat oedema of the spleen and are mixed with ginger for treatment of uterus affections, while its bark, when boiled in water with vinegar, is used as lotion against lice (Bulos, 1983). In Dhofar (southern Oman), dried leaves are boiled in water and the solution is given to women to ease prolonged or difficult labor; dried leaves are also applied on sores and wounds to prevent inflammations or infections whereas in Saudi Arabia, the leaves of a *Tamarix* species are wrapped on the head to relieve headache and fever (Ghazafar, 1994).

Aerial parts of *Tamarix* have been reported to contain lipophilic methylated flavonoids including kaempferol-7,4'-dimethyl ether (El Sissi *et al*., 1973), rhamnocitrin and isorhamnetin (Sultanova *et al*., 2004), tamarixetin (Sultanova *et al*., 2001), and its 3-sulfate derivative (Barberan *et al*., 1990). Flavonoid glycosides have been isolated from the flowers of *Tamarix nilotica*, namely kaempferol-3-*O*-*β*-D-glucuronide 6"-ethyl ester, both methyl and ethyl esters of quercetin-3-*O*-*β*-D-glucuronic acid, and kaempferol-7,4'-dimethyl ether 3-*O*-sulfate (Nawwar *et al*., 1984a, b). In addition, *Tamarix* spp. are known to contain monomeric and dimeric phenolic compounds including gallic acid, ellagic acid, its 3,3'-dimethyl ether, isoferulic acid, ferulaldehyde, nilocitin, and niloticol (Nawwar *et al*., 1982, 1984a, b; Barakat *et al*., 1987).

Tamarix nilotica (Ehrenb.) Bunge from Saudi Arabia, however, has not been phytochemically investigated so far. Our study of the aerial parts of *T. nilotica* led to the isolation of a new triterpenoid, 3-*O*-*trans*-caffeoylisomyricadiol as a major

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constituent, in addition to nine known polyphenolic constituents including mainly flavonoids, hydroxycinnamic acid derivatives, and β-sitosterol. Structure elucidation of the new compound and antioxidant activity of the phenolic constituents are reported.

Results and Discussion

The ethanolic extract of aerial parts of *Tamarix nilotica* was separated by column chromatography and semipreparative HPLC to yield ten polyphenolic constituents, **2** and **4**–**11**, in addition to the new triterpenoid **1**. Compound **1** was isolated as a colourless amorphous solid. The UV spectrum showed absorption bands at 216, 244, 255, 305, and 372 nm. HRFTMS (ion peak at *m/z* 605.4191 $[M+H]^-$, Δ +1.0 from calcd. *m/z* 605.4201) revealed the molecular formula $C_{39}H_{57}O_5$. In the ^{1}H NMR spectrum seven singlets representing tertiary methyl groups were observed at δ_H 0.80 (CH_3-23; δ_C 27.7), δ_H 0.89 (CH_3-24; δ_C 21.5), δ_H 0.93 (CH_3-25; δ_C 14.9), δ_H 0.85 (CH_3-26; δ_C 28.2), δ_H 1.05 (CH_3-27; δ_C 25.9), δ_H 0.92 (CH_3-29; δ_C 21.5), and δ_H 0.91 ppm (CH_3-30; δ_C 33.5) (Table I). An olefinic proton appeared as a double doublet at δ_H 5.42 ppm (1H, dd, J = 7.7 Hz, J = 2.9 Hz, H-15; δ_C 115.9). The presence of the CH_2OH moiety at C-17 was indicated by the quaternary carbon atom at δ_C 38.7 ppm which was assigned to C-17. This was corroborated from the HMBC spectrum which displayed correlations of the two protons at δ_H 2.98 ppm (1H, dd, J = 10.4 Hz, J = 5.4 Hz, H-28$_A$) and δ_H 2.86 ppm (1H, dd, J = 10.4 Hz, J = 2.1 Hz, H-28$_B$) with carbon resonances at δ_C 30.2, δ_C 44.1, and δ_C 27.3 ppm assigned for C-16, C-18, and C-22, respectively. Moreover, protons H-28$_A$ and H-28$_B$ showed clear correlations in ^{1}H-^{1}H COSY and HMQC spectra (Fig. 1) to the same carbon resonance at δ_C 63.2 ppm that was ascribed to C-28. The chemical shifts, multiplicity, and coupling constants indicated compound **1** to

Table I. ^{1}H and ^{13}C NMR data of **1** in DMSO-d_6 (500 MHz).

Position	δ_H (mult., J [Hz])	δ_C (mult.)	Position	δ_H (mult., J [Hz])	δ_C (mult.)
1	1.32 (2H, m)	32.6 (CH_2)	22	A) 1.50 (1H, m) B) 1.00 (1H, m)	27.3 (CH_2)
2	A) 1.90 (1H, m) B) 1.50 (1H, m)	22.3 (CH_2)	23	0.80 (3H, s)	27.7 (CH_3)
3	4.60 (1H, t, 2.5)	76.9 (CH)	24	0.89 (3H, s)	21.5 (CH_3)
4		36.3 (C)	25	0.93 (3H, s)	14.9 (CH_3)
5	1.30 (m)	50.1 (CH)	26	0.85 (3H, s)	28.2 (CH_3)
6	A) 1.48 (1H, m) B) 1.45 (1H, m)	18.2 (CH_2)	27	1.05 (3H, s)	25.9 (CH_3)
7	A) 1.96 (1H, d, 12.6) B) 1.35 (1H, m)	40.9 (CH_2)	28	A) 2.98 (1H, dd, 5.4, 10.4) B) 2.86 (1H, dd, 2.1, 10.4)	63.2 (CH_2)
8		38.7 (C)	29	0.92 (3H, s)	21.5 (CH_3)
9	1.50 (1H, m)	48.0 (CH)	30	0.91 (3H, s)	33.5 (CH_3)
10		36.9 (C)	1’		166.2 (C)
11	A) 1.60 (1H, m) B) 1.45 (1H, m)	16.9 (CH_2)	2’	6.29 (1H, d, 16.8)	114.7 (CH)
12	A) 1.20 (1H, m) B) 1.15 (1H, m)	32.4 (CH_2)	3’	7.42 (1H, d, 16.8)	144.8 (CH)
13		37.5 (C)	1”		125.6 (C)
14		157.7 (C)	2”	7.01 (1H, d, 2.0)	115.0 (CH)
15	5.42 (1H, dd, 2.9, 7.7)	115.9 (CH)	3”		145.5 (C)
16	A) 2.10 (1H, dd, 8.0, 14.6) B) 1.55 (1H, m)	30.2 (CH_2)	4”		148.2 (C)
17		38.7 (C)	5”	6.72 (1H, d, 8.0)	115.7 (CH)
18	0.45 (1H, dd, 3.2, 13.6)	44.1 (CH)	6”	6.99 (1H, dd, 2.0, 8.0)	121.2 (CH)
19	A) 1.38 (1H, t, 13.6) B) 1.00 (1H, dd, 3.2, 13.6)	35.5 (CH_2)	OH-28	4.30 (1H, t, 5.0)	
20		29.7 (C)	OH-6’	9.05 (1H, s)	
21	A) 1.60 (1H, m) B) 1.45 (1H, m)	33.1 (CH_2)	OH-7’	9.56 (1H, s)	

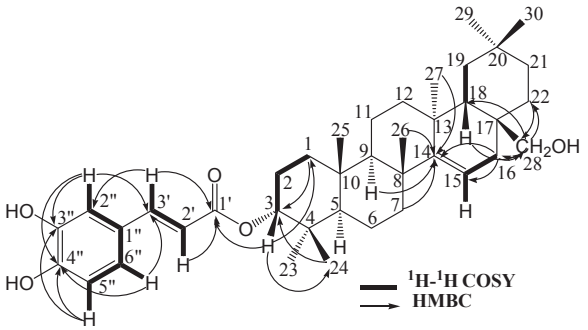

Fig. 1. Key ^{1}H-^{1}H COSY and HMBC correlations of **1**.

Table II. *In vitro* antioxidant activity of compounds **1**, **2**, and **5**–**10**.

Compound	DPPH scavenging activity IC_{50} [μM]
3-*O*-*trans*-Caffeoylisomyricadiol (**1**)	3.56
N-*trans*-Feruloyltyramine (**2**)	16.83
Isoferulic acid methyl ester (**5**)	167.80
Ellagic acid (**6**)	11.50
Ellagic acid-3-methyl ether (**7**)	13.27
Naringenin (**8**)	144.51
Kaempferol-7,4'-dimethyl ether (**9**)	228.31
Kaempferide (**10**)	63.37
Quercetin	5.72

have a taraxer-14-en skeleton (Corbett and Cumming, 1972).

The proton at position 3 (δ_H 4.60; δ_C 76.9) was deduced to be in β-position based on the coupling constant (J = 2.5 Hz) with H-2 and the upfield carbon resonance of C-3 when compared to myricadiol (Corbett and Cumming, 1972; Sakurai *et al*., 1987; Merfort *et al*., 1992). The chemical shift of H-3 furthermore indicated the presence of an acyl substituent that was esterifed to the triterpene core structure at C-3 (Sultanova *et al*., 2004). The remaining signals in the ^{1}H and ^{13}C NMR spectra of **1** that had not been assigned to the triterpene moiety indicated the presence of a caffeic acid substituent. The coupling pattern of three aromatic protons, appearing at δ_H 7.01 (1H, d, J = 2.0 Hz, H-2"; $\delta_{C\text{-}2"}$ 115.0), δ_H 6.72 (1H, d, J = 8.0 Hz, H-5"; $\delta_{C\text{-}5"}$ 115.7) and δ_H 6.99 ppm (1H, dd, J = 8.0 Hz, J = 2.0 Hz, H-6"; $\delta_{C\text{-}6"}$ 121.2), revealed an 1,3,4-trisubstituted aromatic ring system. This was further confirmed by analysis of the ^{1}H-^{1}H COSY spectrum. Two doublet proton resonances at δ_H 6.29 and 7.42 ppm (J = 16.8 Hz) revealed the *trans*-orientated olefinic protons H-2' and H-3' of the caffeic acid side chain. Attachment of the C_3 side chain to the 1,3,4-trisubstituted aromatic ring was proven by HMBC correlations (Fig. 1) of H-3' (δ_H 7.42 ppm) with C-2" (δ_C 115.0 ppm) and C-6" (δ_C 121.2 ppm). Attachment of the caffeoyl moiety at C-3 of the triterpene moiety was unequivocally confirmed by inspection of the HMBC spectrum which revealed a correlation of H-3 at δ_H 4.60 ppm with the ester carbonyl carbon atom C-1' at δ_C 166.2 ppm. On the basis of the spectral data, **1** was identified as the new 3α-(3",4"-dihydroxy-*trans*-cinnamoyloxy)-D-friedoolean-14-en-3α,28-diol for which we propose the trivial name 3-*O*-*trans*-caffeoylisomyricadiol (**1**) (Fig. 2).

Based on the spectral data and by comparison with those of the reported literature, the other isolated compounds were identified as *N*-*trans*-feruloyltyramine (**2**) (Wu *et al*., 1995), β-sitosterol (**3**) (Rubinstein *et al*., 1976; Wright *et al*., 1978), clematine (**4**) (Chen *et al*., 1993), isoferulic acid methyl ester (**5**) (Bowden *et al*., 1975), ellagic acid (**6**) (Nawwar *et al*., 1982), its 3-methyl ether **7** (Bai *et al*., 2008), naringenin (**8**) (Shen *et al*., 1993), kaempferol-7,4'-dimethyl ether (rhamnocitrin-4'-methyl ether) (**9**) (Rossi *et al*., 1997), kaempferide (**10**) (Barberan *et al*., 1990), and dillenetin (**11**) (Simonsen *et al*., 2003).

All phenolic compounds isolated were studied for their antioxidant activity *in vitro* by the DPPH assay using the flavonol quercetin as a standard antioxidant. The assay results (Table II) revealed that 3-*O*-*trans*-caffeoylisomyricadiol (**1**) has a potent antioxidant activity with an IC_{50} value of 3.56 μM compared to quercetin (5.72 μM), which indicated its effectiveness as a free radical scavenger. Moreover, *N*-*trans*-feruloyltyramine (**2**), ellagic acid (**6**), and its 3-methyl ether **7** showed significant radical scavenging activities with IC_{50} values of 11.50–16.83 μM. All isolated compounds were also screened for cytotoxicity against a mouse lymphoma (L5178Y) cell line using the MTT assay, but were found to be nontoxic to the cells, when tested at a concentration of 10 μg/mL (data not shown). The antioxidant activity of the major phenolic constituents of *T. nilotica* including the new compound **1** might possibly contribute to the reported anti-inflammatory properties of the plant (Ghazafar, 1994).

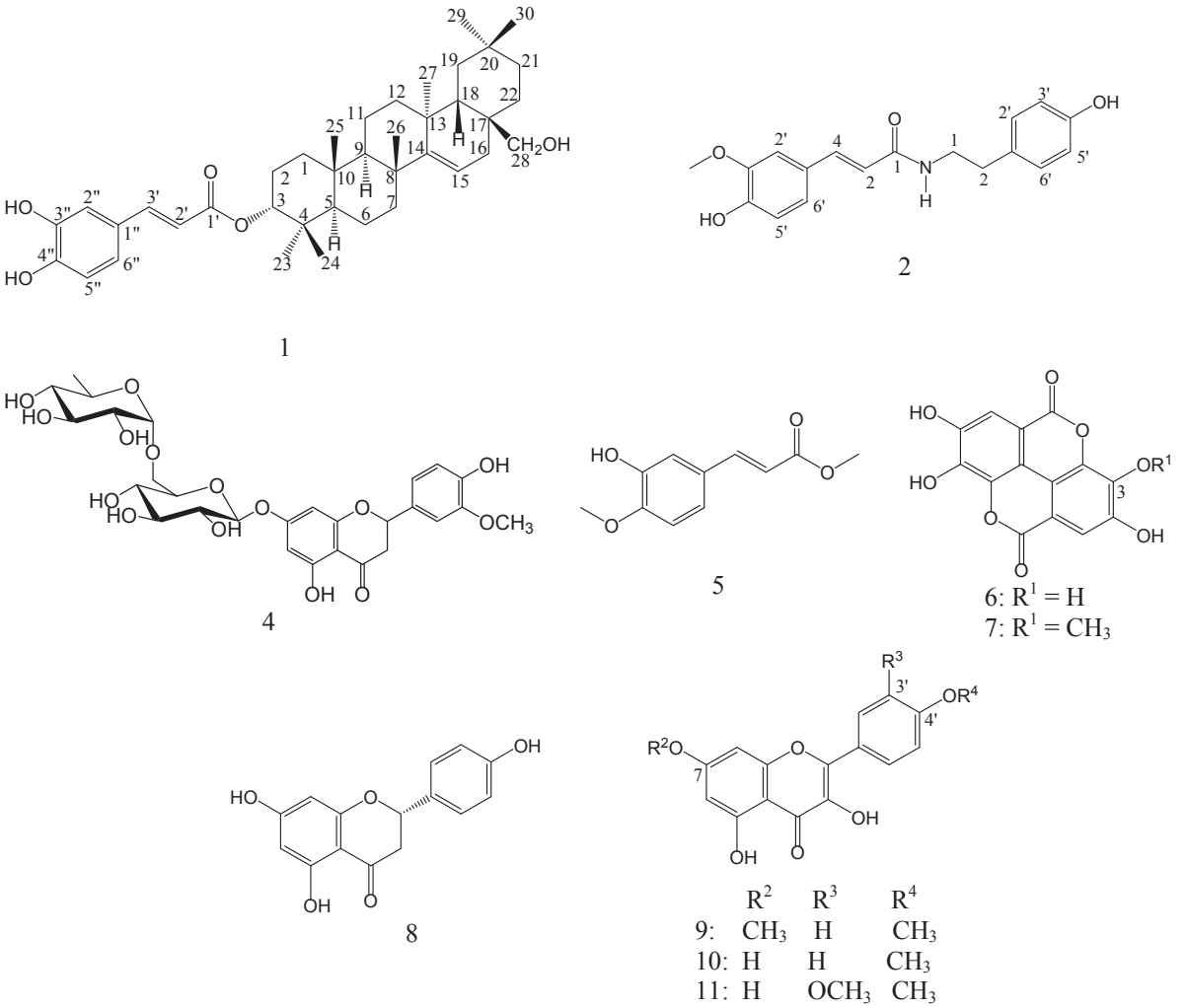

Fig. 2. Polyphenolic constituents from the aerial parts of *Tamarix nilotica*.

Material and Methods

Plant material

Aerial parts of *Tamarix nilotica* (Ehrenb.) Bunge were collected at the campus of King Saud University, Riyadh, Saudi Arabia in June 2007. The plant was kindly identified by Prof. Dr. Jaber Salim Al-Qahtani, Pharmacognosy Department, School of Pharmacy, King Saud University, and a voucher specimen of the authenticated plant was deposited in the same department.

General experimental procedures

Vacuum liquid chromatography (VLC) was performed on silica gel 60 (0.040–0.063 mm; Merck, Darmstadt, Germany). Column chromatography was carried out on silica gel 60 and Sephadex LH-20. For silica gel, varying ratios of DCM and MeOH were used as mobile phase. For Sephadex LH-20, the mobile phase was either 100% MeOH or a mixture of DCM/MeOH (1:1).

For analytical HPLC, samples were injected into a HPLC system equipped with a photodiode array detector (Dionex, Munich, Germany). Rou-

tine detection was at 235, 254, 280, and 340 nm. The separation column (125 × 4 mm ID) was prefilled with Eurosphere 100–5 C-18, 5 μm (Knauer, Berlin, Germany). Separation was achieved by applying a linear gradient from 90% H_2O (pH 2.0) to 100% MeOH over 40 min. TLC analysis was carried out using aluminium sheets precoated with silica gel 60 F_{254} (Merck). Preparative HPLC separations were performed on a Varian HPLC machine, equipped with a Prepstar 218 pump, Prostar 320 UV-detector and a C18 column (Varian Dynamax Column, 250 mm length, 21.4 mm ID, flow rate 20 mL/min, UV detection at 280 nm, prepacked with Microsorb 60–8 C-18, with an integrated pre-column). Semipreparative HPLC separations were done on a LaChrom-Merck Hitachi HPLC machine, equipped with a L-7100 pump, L-7400 UV detector and a C-18 column (Knauer, 300 × 8 mm ID, prefilled with Eurosphere 100–10 C-18, flow rate 5 mL/min, UV detection at 280 nm). For both preparative and semipreparative HPLC separations, the solvent system consisted of MeOH and nanopure H_2O that has been acidified with 0.1% formic acid. ESI mass spectra were obtained on a ThermoFinnigan LCQ DECA mass spectrometer coupled to an Agilent 1100 HPLC system equipped with a photodiode array detector. HRESI mass spectra were recorded on a LTQ FT-MS-Orbitrap instrument (ThermoFinnigan, Bremen, Germany). 1D and 2D NMR spectra were recorded at 300 K on either a Bruker ARX-500 or AVANCE DMX-600 NMR spectrometer. Samples were dissolved in different deuterated solvents, the choice of which was dependent on the solubility of each sample.

Extraction and isolation

The plant was extracted with EtOH and the solvent was removed under reduced pressure. The crude extract was partitioned between H_2O and petroleum ether, EtOAc, DCM, and *n*-butanol. The DCM-soluble fraction was then subjected to VLC and eluted using a stepwise gradient system from 100% *n*-hexane to 100% EtOAc, and from 100% DCM to 100% MeOH. VLC fractions II [*n*-hexane/EtOAc (7:3)] and IV (100% EtOAc) were further purified by CC using a silica gel stationary phase, eluted with *n*-hexane/EtOAc (8:2) and DCM/MeOH (9:1), respectively, yielding 40 mg of **1** and 35 mg of **2**. The petroleum ether-soluble fraction was subjected to CC using a silica gel stationary phase, eluted with *n*-hexane/EtOAc (8:2), to give 30 mg of **3**. The EtOAc-soluble fraction was fractionated by CC using Sephadex LH-20 as a stationary phase and MeOH as mobile phase. Further purification was achieved either by CC using silica gel or reversed phase stationary phases followed by semipreparative reversed phase HPLC (C-18 Eurosphere 100) when required using the appropriate gradient elution of MeOH/H_2O to afford 4 mg of **4**, 24 mg of **5**, 3 mg each of **6**, **7**, and **11**, 12 mg of **8** and **9**, and 6 mg of **10**.

Radical scavenging (DPPH) assay

Extracts, fractions and/or pure compounds were evaluated for their ability to function as free radical scavengers. The qualitative test was performed with a rapid TLC screening method using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Analytical TLC on silica gel 60 F_{254} plates was performed under appropriate conditions after application of 10 μL of each test compound solution (1 mg/mL); the plates were dried and sprayed with DPPH solution (0.2% w/v, MeOH); 5 min later active compounds appeared as yellow spots against a purple background. The purple stable free radical DPPH was reduced to the yellow coloured diphenylpicryl hydrazine. Quercetin was used as a standard antioxidant.

The quantitative assay was carried out at room temperature as described in 2007 by Tsevegsuren *et al.*: 10 μL of a methanolic solution of the test compound(s) were added to 490 μL of a 100 μM DPPH solution in MeOH. Serial concentrations, ranging from 1.56 to 200 μM, were prepared and analyzed in triplicate. 490 μL of 100 μM DPPH solution in MeOH plus 10 μL of 100 μM propyl gallate solution were used as positive control. 490 μL of 100 μM DPPH solution plus 10 μL of MeOH were used as blank. The absorbance at 517 nm was determined after 30 min of incubation, and the percentage of DPPH reduction was calculated. The difference between a DPPH blank solution and the positive control was taken as 100% antioxidative activity. The percent antioxidative activity was then calculated from the difference in absorption between the test sample and the DPPH blank as follows:

$$\alpha_A\ (\%) = [(A_B - A_P)/(A_B - A_{Pos})] \cdot 100,$$

where α_A is the percentage of antioxidative activity in comparison with the positive control, A_B is the absorption of the DPPH solution as blank,

A_P is the absorption of the test sample, and A_{Pos} is the absorption of the positive control (propyl gallate). IC_{50} values were calculated by linear regression (Tsevegsuren *et al*., 2007). Quercetin was taken as reference compound under the same experimental conditions.

Cell proliferation assay

Cytotoxicity was tested against L5178Y mouse lymphoma cells using the microculture tetrazolium (MTT) assay (Carmichael *et al*., 1987) as described earlier (Ashour *et al*., 2006). All experiments were carried out in triplicate and repeated three times. As controls, media with 0.1% EGMME/DMSO were included in the experiments.

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