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Note

A new kaempferol triglycoside from *Fagonia taeckholmiana*: cytotoxic activity of its extracts

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Abstract—In addition to apigenin, apigenin 7-*O*-glucoside, kaempferol 3-*O*-glucoside, kaempferol 3,7-di-*O*-rhamnoside, quercetin, and quercetin 3-*O*-glucoside, the methanolic extract of *Fagonia taeckholmiana* afforded a new compound identified as kaempferol 3-*O*-β-L-arabinopyranosyl-(1→4)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside. Identification of the isolated compounds was based on chemical and spectroscopic analyses including UV, FABMS, ^{1}H, ^{13}C and 2D NMR, and DEPT. The cytotoxic activities of the compounds against several cancer cell lines were determined.

Keywords: *Fagonia taeckholmiana*; Zygophyllaceae; Flavonoid triglycoside; Cytotoxic activity

The family Zygophyllaceae is common in the hyperarid and arid zones of the old and new worlds. Engler divided it into seven subfamilies.[1] The subfamily Zygophylloideae contains two of the most common genera in Egypt, *Zygophyllum* and *Fagonia*. The genus *Fagonia* L. includes 20 species that are grouped into eight complexes.[2] *Fagonia* species are reported to be medicinal plants in the scientific literature as well as in folklore, and their medicinal values are well documented.[3–6] Their properties are attributed to a variety of active phytochemical constituents. Most of the flavonoid compounds have been isolated from various Egyptian *Fagonia* species, and their phylogenetic affinities have also been investigated.[7–13] The present study deals with the isolation and structure elucidation of a new flavonoid triglycoside **1**, along with six known flavonoids from *Fagonia taeckholmiana*. The cytotoxic activities of different extracts of *F. taeckholmiana* are also reported.

The methanolic extract was subjected to polyamide 6S column chromatography using a water–methanol step gradient to give four fractions. Fraction I containing compound **1** was eluted from the column with 10:20 methanol–water. This fraction was further subjected to preparative paper chromatography, using H_2O, 15% AcOH, BAW (4:1:5 *n*-BuOH–AcOH–H_2O upper phase) to give compound **1**. Then it was purified using TLC (silica gel, 65:45:12 CH_2Cl_2–MeOH–H_2O), and final purification was carried out on a Sephadex LH-20 column to give compound **1**, which was recognized to be a flavonol glycoside from its positive reactions with the Molish and Shinoda reagents.[14] Acid hydrolysis (2 N HCl, 1 h, 100 °C) of compound **1** afforded kaempferol, rhamnose, and arabinose that were identified by co-chromatography with authentic samples. The UV spectral data of **1** indicated a flavonol with free –OH groups at positions C-5 and C-4′, while those at C-7 and C-3 are substituted.[15,16] Its IR spectrum exhibited absorptions at *ν*(KBr) 3510–3485 (OH), 2950 (CH), 2868 (C=C), 1690 (C=O) 1130–1015 (O-glycosidic linkage), and 825 cm^{-1}. The positive-ion FABMS spectrum of compound **1** showed a molecular ion peak $[M+H]^{+}$at *m*/*z*

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711, corresponding to $C_{32}H_{38}O_{18}$. The ^1H NMR spectrum of compound **1** showed two pairs of doublets one at δ 7.75 (2H, J 7.8 Hz) which was assigned to H-2′ and H-6′, and the other at δ 6.94 (2H, J 7.8 Hz) which was assigned to H-3′ and H-5′. The two meta coupled protons resonated at δ 6.70 and 6.44 (J 2.1 Hz), each with a one-proton integration, were assigned for C-8 and C-6, respectively. This downfield chemical shift confirmed that C-7 is substituted in ring A of the compound.[17]

The presence of three anomeric protons at δ 5.55 (d, J 1.4 Hz), 5.54 (d, J 1.4 Hz), and 5.26 (d, J 2.3 Hz), as well as two methyl groups at δ 0.8 and 1.2 indicated that the two rhamnose units are attached to the aglycon through an α-linkage, and the arabinose sugar unit is attached to the rhamnose through a β-linkage.[17–19] The ^{13}C NMR spectrum of compound **1** displayed 32 carbon resonances (Table 1), 15 of which were assigned to kaempferol as the aglycon moiety, 12 for two rhamnose units and five for the arabinose moiety.[20] The downfield chemical shift of the C-4 (δ 179.7) indicated the presence of a chelated hydroxyl function at C-5, which was also in accord with the chemical shift of C-5, (163.0).The DEPT experiment showed the presence of two methyl groups, one methylene, and 20 methine groups. The remaining carbon resonances were attributed to nine quaternary carbon atoms. In the HMBC spectrum the anomeric proton of one rhamnopyranosyl unit (H-1″, δ 5.55) showed a correlation with C-7 (δ 163.5), and the second rhamnopyranosyl unit (H-1‴, δ 5.45) showed a correlation with C-3 (δ 133.4). The terminal sugar unit was found to be arabinose in the pyranose form,[21] (H-1⁗, δ 5.26) showed a cross peak with C-4‴ (δ 78.3). The rhamnose and arabinose units at C-3 were found to be linked (1→4) as the signal at δ 78.3 is characteristic for C-4‴ in a 4-substituted rhamnose unit.[22]

Table 1. ^{13}C NMR data for compound **1**[a]

Carbon no.	δ_C ppm	DEPT
Aglycon		
2	159.9	C
3	133.4	C
4	179.7	C
5	163.0	C
6	100.5	CH
7	163.5	C
8	95.6	CH
9	158.1	C
10	107.6	C
1′	122.4	C
2′	132.0	CH
3′	116.6	CH
4′	161.7	C
5′	116.6	CH
6′	132.0	CH
Rha-1		
1″	99.9	CH
2″	71.3	CH
3″	71.4	CH
4″	72.1	CH
5″	69.2	CH
6″	18.1	CH_3
Rha-2		
1‴	102.8	CH
2‴	70.5	CH
3‴	71.0	CH
4‴	78.3	CH
5‴	69.0	CH
6‴	18.0	CH_3
Ara		
1⁗	111.0	CH
2⁗	79.4	CH
3⁗	65.6	CH
4⁗	80.5	CH
5⁗	64.2	CH_2

[a] DMSO-d_6 at 125 MHz.

The assignment of the various sugar protons were made by their spin-pattern analysis, COSY-45°, and HMQC experiments. The three sugar moieties were identified as two rhamnose units and one terminal arabinose in the pyranose form by comparing their chemical shift values in the ^{13}C NMR spectrum with the reference data.[23] Upon complete acid hydrolysis, compound **1** yielded rhamnose and arabinose in a ratio of 2:1, along with yellow needles of the kaempferol aglycone. Their configuration was proved by GLC analysis of their respective thiazolidine derivatives.[24] These results confirmed the new structure of compound **1** as kaempferol 3-*O*-β-L-arabinopyranosyl-(1→4)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (Fig. 1).

The cytotoxic activity (see Section 1.3) of the alcohol and water extracts against MCF7 human breast tumor cells in culture showed an IC_{50} of 8.72 and 9.80 μg/mL, respectively. No activity was found in HEPG2 liver carcinoma and U251 brain tumor systems. (for details, see Supplementary data).

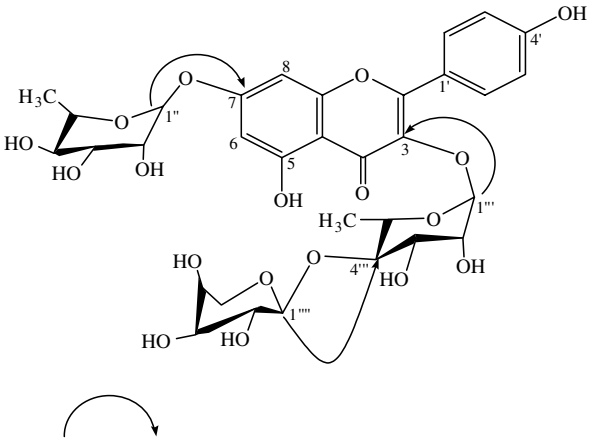

Figure 1. Most significant HMBC correlations.

1. Experimental

1.1. Plant material

Fresh plant material was collected from the National Research Centre (NRC) garden in March 2006 and authenticated by Dr. S. A. Kawashty. A voucher specimen was deposited in the Herbarium of NRC (CAIRC).

1.2. Investigation of the flavonoid constituents

1.2.1. Isolation of kaempferol 3-*O*-β-L-arabinopyranosyl-(1→4)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (1). Two kilograms of the dried powdered, aerial parts were extracted with 70:30 MeOH–water (3 × 4 L), and the methanolic extract was concentrated to dryness in vacuo at 70 °C. The residue (200 g) was dissolved in water (500 mL). The aqueous solution was successively extracted with *n*-hexane (2 × 500 mL) for defatting. Fractionation of the aqueous extract on polyamide 6s (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanover, Germany) column chromatography (125 cm × 5 cm), eluting with H_2O–MeOH mixtures of increasing ratio of MeOH gave four fractions. The fractions were monitored and isolated by paper chromatography using H_2O, 15% AcOH, BAW (4:1:5 *n*-BuOH–AcOH–H_2O upper phase) and thin-layer chromatography using (silica gel, 65:45:12 CH_2Cl_2–MeOH–H_2O). Final purification was carried out on a fine Sephadex LH-20 column (35 cm × 2 cm) two times. Fraction I gave 12 mg of compound **1**, yellow amorphous powder: mp 218–220 °C; R_f 0.65 (BAW), 0.39 (15:85 HOAc–H_2O); UV (λ_{max}-MeOH): 266, 340, and 344; +NaOMe 266 and 380; +NaOAc 266, 348, 352, and 455; +NaOAc/H_3BO_3 266, 290.293, 344, 426, 439, and 454; +$AlCl_3$ 272, 305, and 361; +$AlCl_3$/HCl 302, 325, and 360 nm; IR (KBr): 3510:3485 (OH), 2950 (CH), 2868 (C=C), 1690 (C=O), 1130:1015 (O-glycoside linkage), and 825 cm^{-1}; FABMS: *m*/*z* 711 $[M+H]^+$, 579 $[(M+H)-Ara]^+$, 565 $[(M+H)-Rha]^+$, and 287 $[(M+H)-(Ara+2Rha)]^+$; HRMS: *m*/*z* 711.6398 (calcd *m*/*z* 711.6414); ^1H NMR (DMSO-d_6, 500 MHz): δ 7.75 (d, 2H, *J* 7.8 Hz), 6.94 (d, 2H, *J* 8.9 Hz), 6.70 (d, 1H, *J* 2.1 Hz, H-8), 6.44 (d, 1H, *J* 2.1 Hz, H-6), 5.55 (d, 1H, *J* 1.4 Hz, H-1″), 5.45 (d, 1H, *J* 1.4 Hz, H-1‴), 5.26 (d, 1H, *J* 2.3 Hz, H-1⁗), 3.14:3.88 (m, 26 H, remaining sugar protons overlapped by OH protons); for ^{13}C NMR data see Table 1.

1.2.2. Isolation of additional compounds. In addition, six known flavonoids were isolated. Kaempferol 3,7-di-*O*-L-rhamnoside was obtained from Fraction II eluted by 40:60 MeOH–H_2O. Apigenin 7-*O*-β-D-glucoside, kaempferol 3-*O*-β-D-glucoside, and quercetin 3-*O*-β-D-glucoside were obtained from fraction III eluted by 60:40 MeOH–water. Fraction IV, eluted by 80:20 MeOH–H_2O, gave apigenin and quercetin. Their structures were determined according to standard methods.[15,16] These included complete, mild acid hydrolysis, co-chromatography, UV, and ^1H NMR spectrometry. Acid hydrolysis: An alcoholic solution (20 mg) was refluxed on a boiling water bath with 1 N HCl. The solution was monitored by paper chromatography, at intervals of 5 min for 1 h. At the end of this time the excess acid was precipitated with Ag_2O_3, the alcohol was evaporated, and the aglycon was extracted with EtOAc and recrystallized from MeOH. The sugars in the aqueous solution were identified by co-chromatography with authentic samples using the solvent system 1:5:3:3 benzene–*n*-butanol–pyridine–water. The chromatograms were sprayed with aniline phthalate reagent,[26] and their data were identical to those previously reported.[7–13]

1.3. Determination of cytotoxic activity

The *F. taeckholmiana* L. extracts were screened for cytotoxicity using the method of Skehan.[25] Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the extract to allow attachment of the cell to the wall of the plate. Different concentrations of the extracts (0, 1, 2.5, 5, and 10 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with compounds for 48 h at 37 °C and in an atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained with sulforhodamine B. stain. Excess stain was washed with acetic acid, and the attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between the surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line,[25] comparing with doxorubcin as anticancer drug.

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Supplementary data

Supplementary data (Tables S1 and S2) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.10.011.

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