New Erythroxane-Type Diterpenoids from *Fagonia boveana* (Hadidi) Hadidi & Graf

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The aerial parts of *Fagonia boveana* afforded two new erythroxane-type diterpenes, 3β, 15, 16-trihydroxy-erythrox-4(18)-ene (**2**) and 15, 16-dihydroxy-*cis*-ent-erythrox-3-ene (fagonene) (**3**) together with two known ones; 16-O-acetylfagonone (**1**) and 7β-hydroxy fagonene (**8**). Also a new guaiane sesquiterpene alcohol, 6,10-epoxy-4α-hydroxy guaiane type sesquiterpene (**4**) has been isolated In addition three 8-methoxy flavonols, 8-methoxy-quercetin-3, 7, 3′-trimethyl ether (ternatin) (**5**), gossypetin, 3, 8, 3′, 4′ tetramethyl ether (**6**) and herbacetin-3, 8-dimethyl ether (**7**) were also isolated. The structures of the isolated compounds have been determined on the basis of spectroscopic evidences as well as physical and chemical correlation with known compounds. On performing different assays for biological activities, **6** displayed significant cytotoxic activity against KA3IT and NIH3T3 cell lines, **8** was the most active antiviral against *Herpes simplex* type 1 while **7** was the most active cancer-preventive agent using protein-tyrosine kinase inhibitory method.

Key words: Fagonia boveana, Diterpenes, Cytotoxic Assays

Introduction

Fagonia boveana is an erect hispid shrublet wildly distributed in Sinai (Tackholm, 1974). The plants belonging to the genus *Fagonia* (Zygophyllaceae) are characterized by their triterpenoid saponins, alkaloids, coumarins and flavonoids (Rizk, 1986). A series of novel diterpenoids of the ent-erythroxane type was recently separated from two related species of *Fagonia*, *Fagonia bruguieri* D. C., and *Fagonia glutinosa* Del. (Abdel-Kader *et al.*, 1993; Abdel-Kader, 1994; Abdel-Kader *et al.*, 1994; Abdel-Kader *et al.*, 1997) and denote for the first time the occurrence of diterpenoids in this family. In continuation of our studies on diterpenoid-containing plants (Gedara, 1994; Khalil *et al.,* 1996; 1997; Gedara *et al.*, 2002), the present communication deals with the diterpenoid content of the aerial parts of *Fagonia boveana.* Four diterpenes, an epoxy guaiane sesquiterpene alcohol and three 8-methoxy flavonols were characterized. Different assays for biological activities, viz. cytotoxic and antiviral activities in addition to the inhibitory effect against protein-tyrosine kinase were performed on the isolated pure compounds.

Results and Discussion

Fractionation of the petroleum ether and chloroformic fractions of the alcoholic extract of the dried powdered aerial parts of *Fagonia boveana* on repeated silica gel and alumina column chromatography and preparative TLC, afforded compounds **1–4** and **5–8** from the two fractions respectively.

Compounds **1** and **8** were identified as 16-O-acetylfagonone **1** and 7β-hydroxy fagonene **8** respectively, on the basis of physical and spectral data (UV, IR, MS, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC, HMBC and 2D-NOESY). Both compounds are first reported in the title plant. It is worth to note that extensive spectral analysis of **1** and **8** led to the full assignment of all proton and carbon atoms and further correcting some of the previously reported data (Abdel-Kader *et al.*, 1993; Abdel-Kader *et al.*, 1994) as shown in Tables I and II. For example in compound **1**, the previously reported values for C-11 and C-2 should be exchanged since the HMQC experiment revealed the carbon at δ 34.75 is correlated with the two protons at δ 1.42 and δ 1.81, while, HMBC analysis revealed many long range correlations

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Table I. ^{13}C-NMR data of compounds 1,2–4,8 and the reported data of 16-O-acetylfagonone and 7β-hydroxy fagonene.

Proton no.	**1** ($CDCl_3$)	Reported for 16-O-acetyl fagonone ($CDCl_3$)	**2** (CD_3OD)	**3** (DMSO d_6)	**8** (DMSO-d_6)	**8** (CD_3OD)	Reported for **7**β-hydroxy Fagonene (CD_3OD	**4** (DMSO-d_6)
1	1.9 (1H,m) 2.02 (1H, m)	1.86 (2H, m)	1.55 (1H, m) 1.8 (1H, m)		1.80 1.84	1.92 (1H, m) 1.99 (1H, m)	1.85–2.15^{c}	1.7 (1H,m)
2	2.04 (2H, m)	1.37, 1.75 (2H, m)	1.15 (1H, m)2.35 (1H, m)	1.91 (1H, m) ^{f}1.97 (1H, m)	1.92 1.96	2.03 (1H, m) 2.08 (1H, m)	1.1–1.3^{c} 1.57–1.78^{c}	0.98 (1H, m)
3	5.32 (1H, s)	5.28 (1H, brs)	4.5 (1H, dd, *J* = 5, 1.2	5.2 (1H, s)	5.2	5.28 (1H, br s)	5.25 (1H, br s)	0.84 (1H, m) 1.46 (1H, m)
4	–	–	–	–	–	–	–	
5	–	–	–	–	–	–	1.55 (1H, m)	
6	2.21 (1H, d, *J* = 14.6) 2.74 (1H, d, *J* = 14.6)	2.16, 2.7 (2H, d, *J* = 14.8)	1.62 (1H, m) 1.69 (1H, m)		0.96 2.18	1.08 (1H, m) 2.35 (1H, dd, *J* = 4, 13)	1.1–1.3^{c} 2.32 (1H, dd, *J* = 3.5, 9.5)	3.87 (1H, br s)
7	–	–	1.25 (1H, m) 1.5 (1H, m)		2.97	3.18 (1H, ddd, *J* = 4, 10, 11.5) (1H, m)	3.15 (1H, m)	0.95 (1H, m)
8	2.42 (1H, dd, *J* = 3.5, 12.5)	2.41 (1H, dd, *J* = 3.9, 12.0)	1.25 (1H, m)		1.01	1.20 (1H, m)	1.1–1.3^{c}	1.15 (1H, m), 1.32 (1H, m
9	–	–	–		–	–	–	1.25 (1H, m) 1.72 (1H, m)
10	1.75 (1H, br t, *J*= 3.5	1.75 (1H, m)	0.92 (1H, m)		1.05	1.12 (1H, m)	1.1–1.3^{c}	–
11	1.42 (1H, m) 1.81 (1H, ddd, *J* = 3.5, 3.5, 13.5)	1.99 (2H, m)	1.07 (1H, m) 1.56 (1H, m)		1.0 1.58	1.14 (1H, m) 1.71 (1H, m)	1.82–2.15^{c}	1.22 (1H, m)
12	1.17 (1H, br. d, *J* = 11.5) 1.61 (1H, ddd, *J* = 4.5, 13.5, 13.5)	1.13, 1.55 (2H, dd, *J* = 3.6, 12.9)	1.08 (1H, m) 1.7 (1H, m)		1.06 1.54	1.22 (1H, m) 1.62 (1H, m)	1.1–1.3^{c} 1.57–1.78^{c}	0.92 (3H, d, *J* = 6.2)
13	–	–	–			–	–	0.85 (3H, d, *J* = 6.2)
14	1.45 (1H, m) 1.55 (1H, t, *J* = 12.5)	1.41, 1.42 (2H, d, *J* = 3.2, 19.4	1.34 (1H, m) 1.39 (1H, m)		0.9 1.54	1.05 (1H, m) 1.70 (1H, m)	1.1–1.3^{c}	1.08 (3H, s)
15	3.46 (1H, d, *J* = 9.2)	3.43 (1H, m)	3.17 (1H, dd, J = 2.5, 9.0)	2.98 (1H, dd, *J* = 2.5, 8.0)	3.0	3.21 (1H, dd, J = 2.5, 9.0)	3.2 (1H, dd, *J* = 2.1, 5.0)	1.02 (3H, s)
16	4.03 (1H, dd, *J* = 9.2, 11.5), 4.33 (1H, dd, *J* = 1.9, 11.5)	3.98 (1H, dd, *J* = 2.3, 11.4), 4.28 (1H, dd, *J* = 2.3, 11.4)	3.41 (1H, dd, *J* = 9, 11), 3.7 (1H, dd, *J* = 2.5, 11)	3.19 (1H, dd, *J* = 8, 10.7), 3.5^{b} (1H)	3.2 3.48	3.45 (1H, dd, *J* = 9, 11.5), 3.73 (1H, dd, *J* = 2.5, 11)	3.45 (1H, dd, *J* = 5, 13.5), 3.7 (1H, dd, *J* = 2.1, 6.9)	
17	0.93 (3H, s)	0.89 (3H, s)	0.93 (3H, s)	0.78 (3H, s)	0.76	0.9 (3H, s)	0.87 (3H, s)	
18	1.7 (3H, d, *J* = 1.5)	1.65 (3H, d, *J* = 1.4)	4.69 (1H, s) 4.82 (1H, s)	1.6 (3H, brs)	1.65	1.73 (3H, br d, *J* = 1.5)	1.71 (3H, d, *J* = 1.0)	
19	1.26 (3H, s)	1.21 (3H, s)	1.1 (3H, s)	0.9 (3H, s)	1.0	1.11 (3H, s)	1.08 (3H, s)	
20	0.78 (3H, s)	0.74 (3H, s)	0.78 (3H, s)	0.78 (3H, s)	0.76	0.93 (3H, s)	0.87 (3H, s)	
CH_3CO	2.11 (3H, s)	2.06 (3H, s)	–	–		–		

[a] *J* in Hz. [b]: This peak is completely hidden under the solvent peak. [c] unresolved multiplet. [d] Abdel-Kader *et al.* (1993); Abdel-Kader (1994); Abdel-Kader *et al.* (1994). [e] Abdel-Kader (1994); Abdel-Kader *et al.* (1994).

Table II. ^{13}C-NMR data of compounds 1,2–4,8 and the reported data of 16-O-acetylfagonone* and 7β-hydroxy fagonene**.

Carbon no.	**1** ($CDCl_3$)	Reported for 16-O-acetyl fagonone ($CDCl_3$)	**2** (CD_3OD)	**3** (DMSO)	**8** (DMSO)	**8** (CD_3OD)	Reported for 7β-hydroxy fagonene (CD3OD	**4** (DMSO)
1	18.45	18.50	19.38	17.29	17.18	17.86	17.8	45.82
2	23.57	34.80	32.76	23.91	23.89	24.61	36.5	23.08
3	123.07	123.1	82.37	123.30	122.90	124.12	124.0	40.63
4	139.00	138.9	157.98	139.90	139.67	140.16	140.1	79.89
5	41.79	35.8	40.56	37.17	37.56	37.12	37.0	55.33
6	50.46	50.4	37.47	38.17	47.96	47.87	47.7	69.83
7	212.19	212.8	25.65	35.84	66.37	68.41	68.5	51.30
8	52.56	52.6	41.95	41.57	48.10	48.72	48.7	20.56
9	41.93	41.9	36.97	38.02	38.73	39.67	39.6	47.68
10	52.98	53.0	56.25	51.90	51.54	52.92	52.7	73.40
11	34.75	23.5	34.86	35.69	35.85	36.66	24.6	29.71
12	28.38	28.4	29.03	29.33	29.31	29.93	30.0	21.51
13	36.11	41.7	36.51	56.71	36.43	38.58	38.5	21.14
14	27.18	27.3	35.57	27.67	29.10	29.70	29.6	22.98
15	78.40	78.4	81.25	80.70	80.74	82.44	82.3	21.76
16	65.86	65.9	62.09	62.20	62.36	63.39	63.2	
17	18.18	18.2	18.49	19.86	19.98	19.65	19.6	
18	20.33	20.3	99.10	20.34	20.45	20.14	20.4	
19	33.53	33.5	20.84	33.27	33.46	33.56	33.7	
20	14.02	14.0	11.33	12.13	13.27	13.28	13.4	
CH_3**CO**	171.40	171.4	–	–	–			
CH_3CO	21.00	20.9	–	–	–			

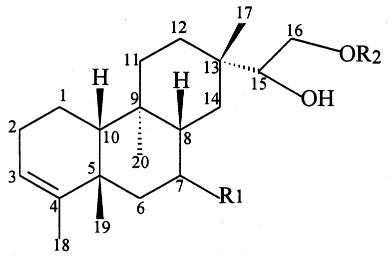
* Abdel-Kader *et al.* (1993); Abdel-Kader (1994); Abdel-Kader *et al.* (1994)

** Abdel-Kader (1994); Abdel-Kader *et al.* (1994).

(Fig. 1), which readily proved that these δ values should be assigned to the CH_2 moiety at position 11. On the other hand, the value of H-2 was deduced from the COSY spectrum, where H-3 at δ 5.32 (1H, br. s.) showed cross peak with adjacent protons at δ 2.04 (2H, m, H-2a, H-2b) and another cross peak with methyl protons at δ 1.70 (3H, d, *J* = 1.5, H-18) as a result of long range allylic coupling. The C-2 assignment was also deduced from HMBC correlations (Fig. 1). Also the previously reported values for the two quaternary carbons C-5 and C-13 in **1** were exchanged based on HMBC experiment (Fig. 1).

In compound **8**, C-2 and C-11 were exchanged based on COSY, HMQC and HMBC measurements. COSY experiment recorded in DMSO revealed that H-3 (δ 5.2) crossed with adjacent protons H-2a (δ 1.92) and H-2b (δ 1.96) as well as long ranged methyl protons H-18 (δ 1.65). In HMQC, the value of H-2a, H-2b has cross peaks with the corresponding carbon at δ 23.89 in DMSO (= δ 24.61 in CD_3OD) which should be reassigned to C-2 instead of C-11. The signal at δ 35.85 in DMSO (= δ 36.66 in CD_3OD) was reassigned to C-11 (Table II) on the basis of HMBC experiment (Fig. 1).

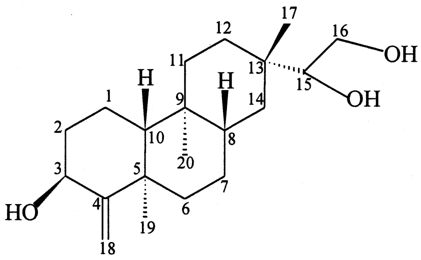
Compound **2** was isolated as a white amorphous powder, in a yield of 0.00057% of the dry weight. Comparing the collective spectral data of **2** with those of **8**, the most significant difference resides in the absence of double bond signals at δ 124.12 and 140.16 in compound **8** in CD_3OD, and in the meantime, the DEPT spectrum of **2** showed the presence of three methyl groups instead of four. This was supported from ^1H and ^{13}C NMR data as the resonance signals of the methyl group at δ 20.14 (C-18) and δ 1.73 (H-18) in compound **8** were replaced by an exomethylene resonance signal at δ 99.1 (C-18), δ 4.69 (1H, s, H-18a) and δ 4.82 (1H, s, H-18b). The other difference resides in the downfield shift of the carbinol carbon from δ 68.41 (H-7) in compound **8** to δ 82.37 in com-



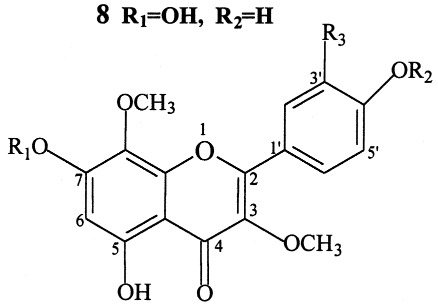
1 R_1=O, R_2=Ac

3 R_1=H, R_2=H

8 R_1=OH, R_2=H



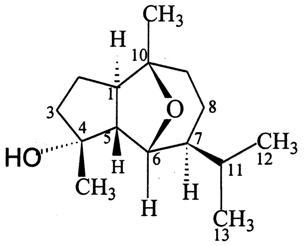
2



5 R_1=CH_3, R_2=H, R_3=OCH_3

6 R_1=H, R_2=CH_3, R_3=OCH_3

7 R_1= R_2= R_3= H



4

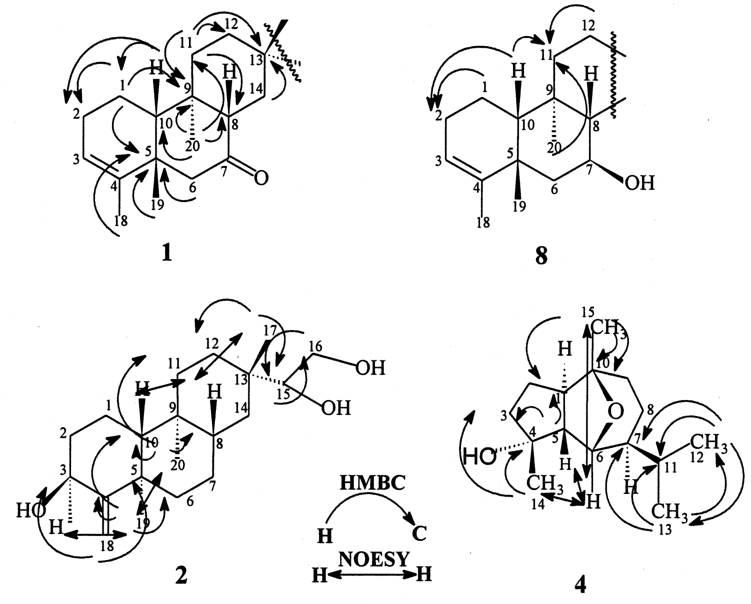


Fig. 1. Important correlations in HMBC for 1,2,4 and 8 and in 2D-NOESY for 2 and 4.

pound **2**. The position of the hydroxyl group relative to the exomethylene group was confirmed by the HMBC correlations (Fig. 1), as the signals at δ 4.69 (H-18a) and 4.82 (H-18b) showed three bond correlation ($^3J_{CH}$) with the carbons at δ 82.37 (C-3) and 40.65 (C-5). Complete assignments of the rest of proton and carbon signals were possible through the interpreted information obtained from the different 2-dimensional spectra. The location of methyl groups were confirmed by the HMBC experiment as shown in Fig. 1.

The relative stereochemistry of compound **2** was determined on the basis of chemical shifts of the carbon signals as well as the results of 2D-NOESY experiment. The large shift of C-19 from 33.56 ppm in 8 to 20.8 in **2** is very close to the compound with a-configuration at C-5 in an A/B transfused structure (Gough *et al.*, 1972; Bohlmann *et al.*, 1985).

The a-configuration of methyl group at δ 1.1 (H-19) was also confirmed by NOESY experiment as H-19 has cross peak with H-20 (δ 0.78). Furthermore, a cross peak between H-19 and H-3 (δ 4.5) proved the β-configuration of the hydroxyl group at C-3. The NOESY experiment further confirmed the β-configuration of the protons at C-8 (δ 1.25), C-10 (δ 0.92) and C-17 (δ 0.93) based on the cross peak between H-8 and both of H-10 and H-17. The deduced relative stereochemistry completely matched the computer-generated model of compound **2** after energy minimization. Based on the above data, **2** is deduced to be 3β, 15, 16-trihydroxy-erythrox-4(18)-ene. This is the first report of isolating this compound from a natural source.

Compound **3** was isolated as a colorless gummy solid, in a yield of 0.00038% of the dry weight. Comparative spectral data of compound **3** and **8**, revealed the close chemical similarity. The only significant difference was the loss of one hydroxyl group as indicated from the molecular ion peak at *m/z* 306, being 16 mass unit less than that of compound **8**. The presence of an extramethylene group in the DEPT experiment and the absence of a methine carbon at δ 66.37 (in DMSO) indicated that the lost hydroxyl was the one attached to C-7. This was substantiated by the up-field shift of C-6 (– 9.79 ppm) and C-8 (– 6.53 ppm) (Table II). On the other hand, the presence of a glycol moiety attached to C-13 was confirmed from the presence of a fragment at *m/z* 245 indicated the direct loss of this moiety $[M^+-CH_2(OH)-CH(OH)]$. Also the NMR data revealed the only two oxygenated carbons at δ 80.7 (C-15) and δ 62.2 (C-16) correlated with the proton signals at δ 2.98 (1H, dd, J = 2.4, 8.0, H-15) and 3.19 (1H, dd, J= 8.0, 10.7, H-16a) and 3.5 (1H, H-16b). Further confirmation was achieved from the COSY experiment, as the proton at δ 2.98 (H-15) crossed with the protons δ 3.19 (H-16a) and 3.5 (H-16b). Based on the above data, compound **3** was thus, 15, 16-dihydroxy-*cis*-ent-erythrox-3-ene which was trivially given the name fagonene by Abdel-Kader *et al.* (1994) as a basic nucleus for all the separated erythroxane diterpenes from *Fagonia* species. To the best of the authors' knowledge and after thorough literature review, this compound constitutes a new addition to the natural products.

Compound **4** was isolated as a colorless gummy solid, in a yield of 0.00057% of the dry weight. **4** was found by mass spectrometry and ^{13}C NMR spectroscopy to have the molecular formula $C_{15}H_{26}O_2$, showing three degrees of unsaturation. The structure of **4** was thus tricyclic due to the absence of any type of double bond. The presence of three oxygenated carbons and only two oxygen atoms in the molecular formula suggested the presence of an epoxide ring. The IR spectrum showed absorption for a hydroxyl group at 3412 cm^{-1}. The EIMS displayed a molecular ion peak at *m/z* 238 and other peaks at *m/z* 220 $[M-H_2O]^+$, 205$[M-(H_2O+CH_3)]^+$. DEPT spectrum as well as HMQC experiment confirmed the presence of 4 methyl groups (δ 21.14, 21.51, 21.78 and 22.98), 4 methylene (δ 20.56, 23.08, 40.63 and 47.68), 5 methine one of them is oxygenated (δ 29.71, 45.82, 51.30, 55.33 and 69.83) and 2 oxygenated quaternary carbons (δ 73.4 and 79.89). The presence of an isopropyl group was confirmed by the presence of an ion fragment at *m/z* 195 [M-isopropyl]$^+$, two methyl signals at δ 0.85 (3H, d, J = 6.2, H-13) and δ 0.92 (3H, d, J = 6.2, H-12) both have the same J value, most possibly split by the same proton. The ^1H NMR spectrum of **4** also displayed the presence of two tertiary methyls at δ 1.08 (Me-14), δ 1.02 (Me-15) and one methine proton attached to oxygen-bearing carbon at δ 3.87 (1H, br. s., H-6). The detailed ^1H NMR, ^{13}C NMR data were compatible with a guaiane sesquiterpene type skeleton (Bohlmann and Jakupovic, 1979; Bruno *et al.*, 1993). HMBC (Fig. 1) associated with ^1H-^1H COSY permitted the complete assignment of the structure of **4**. The result of this analysis revealed the connectivity of carbons as well as the connectivity of the substituents to the skeleton of **4**. For example, the location of a methyl group at C-15 was confirmed through two and three bond correlation from the methyl protons at δ 1.02 to C-10, C-1, C-9, from H-14 at δ 1.08 (3H, s) to C-3 and C-5, from H-12 at δ 0.92 (3H,d, $J = 6.2$) to C-11, C-7 and C-12 and from H-5 at δ 1.55 (1H, m) to C-1, C-4, C-6, C-10 and C-14.

The relative stereochemistry of **4** was determined on the basis of a 2D-NOESY experiment, as the proton at δ 3.87 (1H, br. s., H-6) showed a cross peak with H-14 and H-15. The proton at δ 1.55 (1H, m, H-5) showed cross peak with H-15. Thus providing that H-5, H-6, H-14 and H-15 were on the same face of the molecule (β-face). The β-configuration of the oxygen bridge was deduced from the computer generated model of **4** after minimization of its energy. The β-configuration of the oxygen bridge in a closely related sesquiterpene cyclo-epoxy puliglene, was deduced through x-ray crystallographic analysis (Mossa *et al.*, 1992).

Based on the previously presented data, compound **4** was thus, 6,10-epoxy-4α-hydroxy guaiane type sesquiterpene. To the best of the authors knowledge, this is the first report of isolation of this compound from any natural source.

Compounds **5–7** were identified as 8-methoxyquercetin-3, 7, 3′-trimethyl ether (ternatin) **5** (Ensemeyer and Langhammer, 1982), gossypetin, 3, 8, 3′, 4′ tetramethyl ether **6** and herbacetin-3, 8-dimethyl ether **7**, on the basis of physical and spectral data (UV, IR, MS, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC and HMBC). This is the first report of isolation of these compounds from *Fagonia boveana* and also the first time to carry out 2D-NMR experiments for these compounds which were helpful in the determination of the exact positions of the methoxyl groups as well as complete proton and carbon assignments (See experimental). These compounds were reported before in *Fagonia bruguieri* D. C. (Abdel-Kader *et al.*, 1993)

Different biological assays have been performed including cytotoxicity, antiviral and protein-tyrosine kinase inhibitory assay. Cytotoxicity assays (Shier, 1983; Abbas *et al.*, 1992) for the individual compounds after purification were carried out *in vitro* using two proliferating mouse cell lines, a normal fibroblast cell line, NIH3T3 and a virally transformed form of cells, KA3IT (Table III).

As shown in Table III, compound **2** displayed significant cytotoxic activity on the cancer cell lines, KA3IT and NIH3T3 with IC50 2 μg/ml (6.2 nM) and 1 μg/ml (3.1 nM), respectively. Among the tested compounds, **6** displayed the most significant cytotoxic activity on the cancer cell lines, KA3IT and NIH3T3 with $IC_{50} < 1$ μg/ml (2.6 nM) and 2 μg/ml (5.2 nM), respectively, while **3** and **5** exhibited substantial cytotoxic activity and **7** showed moderate activity.

Table III. Cytotoxicity [IC_{50} μg/ml & nM]* of the isolated compounds from *Fagonia boveana* in cultured mammalian cell lines.

Sample	Cell Lines			
	KA3IT		NIH3T3	
	[μg/ml]	[nM]	[μg/ml]	[nM]
Compound **1**	5	13.8	10	27.6
Compound **2**	2	6.2	1	3.1
Compound **3**	10	32.6	20	65.2
Compound **4**	–	–	–	–
Compound **5**	5	13.3	2	5.2
Compound **6**	<1	<2.6	2	5.2
Compound **7**	5	15.1	5	15.1
Compound **8**	20	62.1	–	–

* IC_{50} is defined as the concentration of the sample which gives approximately 50% inhibition of cell proliferation.

– indicates that the maximum concentration used in these assays 200 μg/ml did not kill 50% of the cells.

On testing all isolated compounds for their antiviral activity against *Herpes simplex* type 1 (Abou-Karum and Shier, 1990), only compound **8** showed a significant antiviral activity with IC_{50} 10 μg/ml (31.05 nM). Its little cytotxicity on the normal cells, NIH3T3 ($IC_{50} > 200$ μg/ml) is considered as a further advantage as a potential antiviral agent.

On performing the protein-tyrosine kinase (PTK) inhibitory assay for cancer preventive agents (Gazit *et. al.*, 1989), 8-methoxy flavonols, isolated from *Fagonia boveana* **5–7**, were selected for this study because of their phenolic nature and the potential competition for the tyrosine binding site of EGFRK (epidermal growth factor receptor kinase) and thus inhibiting the phosphorylation of the tyrosine residue of the protein involved in the signaling pathway. Each compound is tested in two concentrations (10 and 100 μg/ml). Compound **7**, herbacetin-3, 8-dimethyl ether was found to be the most active among the tested compounds as it showed 57% inhibition at 10 μg/ml concentration This is probably due to the presence of the only free *p*-hydroxyl group in ring B of the structure which is similar to the phenolic moiety of tyrosine suggesting its fitting into the receptors. The low % inhibition exhibited by the other two compounds **5** (0.74%) and **6** (31.6%) at the same concentration (10 μg/ml) explains the steric hindrance as a probable cause. Using high concentration (100 μg/ml) of compound **7** did not increase the inhibition significantly and thus indicating the saturation of the receptors at low concentration. These results are consistent with the reported data (Chang and Geahlen, 1992) which showed that the free 4′-OH group, planarity of ring B and the free 5-OH are essential for the inhibiting effect of flavonoids on PTK. Thus, compound **7** may be a potential cancer-preventive agent at a small concentration, in addition to its moderate cytotoxic activity as shown in Table III.

Experimental

General

Mps. uncorr., and were determined on melting point apparatus, Fisher-John Scientific Co., U. S. A.; IR spectra were recorded in KBr using a Nicolet MX-IFT-IR, USA; UV on ultra-violet spectrophotometer (UV-1601 PC. Shimadzu, Japan); ^{1}H NMR spectra were recorded at 400 or 500 MHz and ^{13}C NMR at 100 or 125 MHz, on a Bruker DRX-400 (Germany) or VI-500 (USA) spectrometer. Chemical shifts are given in δ (ppm) relative to TMS as internal standard in $CDCl_3$, CD_3OD and DMSO-δ_6; EIMS: 70 ev. on a Kratos MS-25 spectrometer, FABMS: using xenon as a bombardment gas and 3-nitrobenzyl alcohol or glycerol as a viscous matrix; TLC: Silica gel (kieselgel 60, F254) of 0.25 mm layer thickness, the spots were visualized by spraying with anisaldhyde and vanillin-sulfuric acid; preparative TLC was performed on silica gel plates (20 cm × 20 cm) of 0.5 mm thickness.

Plant material

The aerial parts of *Fagonia boveana* (Hadidi) Hadidi & Garf. were collected in April 1996 during the flowering season from Sinai, Egypt. A voucher specimen was identified by Professor Dr. N. El-Hadidi, Faculty of Science, Cairo University and deposited in Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Extraction and isolation

The air-dried powdered aerial parts (5.5 kg) were extracted to exhaustion with ethanol (90%) in a percolator at room temperature. After filtration, the alcoholic extract was evaporated under reduced pressure to solvent-free residue. The residue was suspended in water and successively extracted with petroleum ether (8 × 500 ml), chloroform (5 × 500 ml) and ethyl acetate (4 × 500 ml). The obtained extracts were separately dehydrated with anhydrous sodium sulfate, evaporated under vacuum to afford a dark residues, 211.0 g (3.84%), 50.0 g (4.4%) and 3.9 g (0.071%) respectively. The petroleum ether extract (200.0 g) was chromatographed on a silica-packed column (Merck, type 60, mesh 70–230). The column was gradiently eluted with n. hexane, chloroform/n- hexane (5–60%), chloroform and MeOH/chloroform (5–30%) mixtures. The collected fractions (500 ml, each) were concentrated and monitored by silica gel TLC plates using EtOAc in hexane (5–50%) as solvent systems. Similar fractions were pooled, concentrated to get three combined fractions (Fr. 13–18, Fr. 21–24 and Fr. 37–43). Fr. 13–18, eluted with 40% $CHCl_3$/hexane were further fractionated on a neutral alumina packed column (aluminium oxide, Brockmann grade 1), eluted with 50% $CHCl_3$/hexane to afford **1**.

Fr. 21–24, eluted with 60% $CHCl_3$/hexane were further fractionated with silica gel C. C. using 20% $CHCl_3$/hexane and PTLC using 40% EtOAc/hexane to afford **2** (R_f 0.17) and **3** (R_f 0.33).

Fr. 37–43, eluted with 20% MeOH/$CHCl_3$ were further purified by silica gel C. C. using 25% EtOAc/hexane and PTLC using 30% EtOAc/hexane to afford **4**.

The chloroform extract (45.0 g) was chromatographed on a silica-packed column and was gradiently eluted with mixtures of MeOH/$CHCl_3$ (0–30%). The effluent was collected in 100 ml fractions, concentrated and monitored by silica gel TLC plates using MeOH/$CHCl_3$ (1–15%) as solvent systems. Similar fractions were pooled, concentrated to get three combined fractions (Fr. 30–41, Fr. 46–50 and Fr. 58–64). Fr. 30–41, eluted with 3% MeOH/$CHCl_3$ were further purified by silica gel C. C. using 2% MeOH/$CHCl_3$ to afford **5** as yellow amorphous powder. Fr. 46–50, eluted with 6% MeOH/$CHCl_3$, on concentration, afford a yellow ppt. which on crystallization from methanol gave yellow needle crystals of **6**.

Fr. 58–64, eluted with 8% MeOH/$CHCl_3$, were further fractionated on silica gel C. C. using MeOH/$CHCl_3$, gradiently eluted, to give two subfractions, the first afforded on concentration and crystallization from MeOH **7** as yellow amorphous powder, the second was further purified by PTLC (12% MeOH/$CHCl_3$) to afford **8**.

The ethyl acetate extract was subjected to a similar processing but failed to afford any diterpenoid isolates.

16-O-acetylfagonone **1**: colorless needle crystals from methanol (50 mg, 0.00095% dry wt.); mp. 120–122° ($CHCl_3$); UV λ^{MeOH}_{max} nm: 208; IRν^{KBr}_{max} cm^{-1}: 3472(OH), 1745 (-OAc), 1696 (C = O); FABMS *m/z*: 363 $(M+1)^+$; ^1H NMR ($CDCl_3$, 400 MHz): Table I; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table II.

3β, 15, 16-trihydroxy-erythrox-4(18)-ene **2**: colorless gummy solid (30 mg, 0.00057% dry wt.); UV λ^{MeOH}_{max} nm: 222; IRν^{KBr}_{max} cm^{-1}: 3397 (OH), 1650 (C = C); ^1H NMR (CD_3OD, 400 MHz): Table I; ^{13}C-NMR (CD_3OD, 100 MHz): Table II.

15, 16-Dihydroxy-cis-ent-erythrox-3-ene (fagonene) **3**: colorless gummy solid (20 mg, 0.00038% dry wt.); UV λ^{MeOH}_{max} nm: 214; IRν^{KBr}_{max} cm^{-1}: 3450 (OH), 1640 (C = C); EIMS *m/z* (rel. int.): 306 (48) $[M]^+$, 291 (17) $[M\text{-}CH_3]^+$, 273 (13) $[M\text{-}(CH_3+H_2O)]^+$, 245 (60) $[M\text{-glycol}]^+$, 95 (100); ^1H NMR (DMSO-δ_6, 400 MHz): Table I; ^{13}C-NMR (DMSO-δ_6, 100 MHz): Table II.

6,10-Epoxy-4α-hydroxy guaiane type sesquiterpene **4**: white amorphous powder (30 mg, 0.00057% dry wt.); UV λ^{MeOH}_{max} nm: 210; IRν^{KBr}_{max} cm^{-1}: 3413 (OH); EIMS *m/z* (rel. int.): 238 (10) $[M]^+[C_{15}H_{26}O_2]$, 220 (22) $[M\text{-}H_2O]^+$, 205 (12) $[M\text{-}(H_2O+CH_3)]^+$, 195 (9) $[M\text{-}C_3H_7]^+$, 164 (48), 139 (90), 94 (100), 81 (82); ^1H NMR (DMSO-δ_6, 400 MHz): Table I; ^{13}C-NMR (DMSO-δ_6, 100 MHz): Table II.

8-Methoxy-quercetin-3,7,3′-trimethyl ether (ternatin) **5**: yellow amorphous powder (30 mg, 0.00060% dry wt.); UV λ^{MeOH}_{max} nm: 364, 272, 266; +MaOMe: 412, 264; +$AlCl_3$: 422,355, 280, 272; +$AlCl_3$+HCl: 420, 368, 280, 242; +NaOAc: 413, 264; IRν^{KBr}_{max} cm^{-1}: 3342 (OH), 1650 (C = O), 1615 (C = C); EIMS *m/z* (rel. int.): 374 (92) $[M]^+$, 359 (100) $[M\text{-}CH_3]^+$, 331 (15), 278 (12), 173 (8), 151 (18); ^1H NMR ($CDCl_3$, 500 MHz): δ 6.42 (1H, s, H-6), 7.8 (1H, s, H-2′), 7.07 (1H, d, *J* = 9, H-5′), 7.8 (1H, d, *J* = 9, H-6′), 3.89 (3H, s, 3-OCH_3), 3.95 (3H, s, 7-OCH_3), 3.92 (3H, s, 8-OCH_3), 3.98 (3H, s, 3′-OCH_3), 6.01 (1H, s, 4′-OH), 12.48 (1H, s, 5-OH); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 146.3 (C-2), 138.5 (C-3), 178.4 (C-4), 157.3 (C-5), 95.3 (C-6), 158.2 (C-7), 128.2 (C-8), 157.3 (C-9), 105.0 (C-10), 122.7 (C-1′), 110.7 (C-2′), 148.4 (C-3′), 134.0 (C-4′), 114.7 (C-5′), 122.8 (C-6′), 60.1 (3-OCH_3), 55.9 (7-OCH_3), 61.5 (8-OCH_3), 56.3 (3′-OCH_3).

Gossypetin, 3, 8, 3′, 4′ tetramethyl ether **6**: yellow needle crystals (50 mg, 0.001% dry wt.); mp. 235–236° (MeOH); UV λ^{MeOH}_{max} nm: 340, 274, 256; +MaOMe: 390, 316, 282; +$AlCl_3$: 416, 362, 282; +$AlCl_3$+HCl: 414, 356, 282; +NaOAc: 388, 318, 282; +NaOAc/H_3BO_3: 332, 274; IRν^{KBr}_{max} cm^{-1}: 3422 (OH), 1660 (C = O), 1612 (C = C); FABMS *m/z*: 375 $(M+H)^+$; EIMS *m/z* (rel. int.): 374 (68) $[M]^+$, 359 (100) $[M\text{-}CH_3]^+$, 331 (8), 187 (9), 165 (10); ^1H NMR (DMSO-δ_6, 500 MHz): δ 6.30 (1H, s, H-6), 7.66 (1H, s, H-2′), 7.17 (1H, d, *J* = 8.5, H-5′), 7.73 (1H, d, *J* = 8.5, H-6′), 3.85 (3H, s, 3-OCH_3), 3.85 (3H, s, 8-OCH_3), 3.85 (3H, s, 3′-OCH_3), 3.85 (3H, s, 4′-OCH_3), 12.3 (1H, s, 5-OH); ^{13}C-NMR (DMSO-δ_6, 125 MHz): δ 155.6 (C-2), 138.1 (C-3), 178.03 (C-4), 154.8 (C-5), 98.6 (C-6), 155.9 (C-7), 127.5 (C-8), 156.9 (C-9), 104.1 (C-10), 122.3 (C-1′), 110.7 (C-2′), 148.4 (C-3′), 151.2 (C-4′), 111.7 (C-5′), 121.8 (C-6′), 61.9 (3-OCH_3), 55.8 (8-OCH_3), 55.3 (3′-OCH_3), 59.7 (4′-OCH_3).

Herbacetin-3, 8-dimethyl ether **7**: yellow amorphous powder (15 mg, 0.0003% dry wt.); UV λ^{MeOH}_{max} nm: 356, 324, 272; +MaOMe: 404, 330, 280; +$AlCl_3$: 414, 354, 310, 280; +$AlCl_3$+HCl: 414, 348, 310, 282; +NaOAc: 400, 316, 280; +NaOAc/H_3BO_3: 360, 316, 272; IRν^{KBr}_{max} cm^{-1}: 3418 (OH), 1660 (C = O), 1615 (C = C); FABMS *m/z*: 331 $(M+H)^+$; ^1H NMR (DMSO-δ_6, 500 MHz) : δ 6.29 (1H, s, H-6), 7.95 (1H, d, *J* = 8.8, H-2′), 7.0 (1H, d, *J* = 8.8, H-3′), 7.0 (1H, d, *J* = 8.8, H-5′), 7.95

(1H, d, J = 8.8, H-6′), 3.79 (3H, s, 3-OCH_3), 3.81 (3H, s, 8-OCH_3), 12.37 (1H, s, 5-OH); ^{13}C-NMR (DMSO-δ_6, 125 MHz): δ 157.3 (C-2), 138.0 (C-3), 178.5 (C-4), 155.9 (C-5), 99.2 (C-6), 156.3 (C-7), 127.9 (C-8), 149.0 (C-9), 104.5 (C-10), 121.1 (C-1′), 130.4 (C-2′), 116.1 (C-3′), 160.6 (C-4′), 116.2 (C-5′), 130.4 (C-6′), 60.1 (3-OCH_3), 61.4 (8-OCH_3).

7β,15, 16-Trihydroxy-cis-ent-erythrox-3-ene (*7β-hydroxy fagonene*) **8**: white amorphous powder (250 mg, 0.005% dry wt.); mp. 193–195°; UV λ^{MeOH}_{max} nm: 213; IRν^{KBr}_{max} cm^{-1}: 3392(OH); EIMS *m/z* (rel. int.): 322 (48) $[M]^+$, 261 (25) $[M\text{-glycol}]^+$, 243 (84) $[M\text{-}(glycol+H_2O)]^+$, 123 (51), 107 (78), 95 (84), 43 (100); ^{1}H NMR (DMSO-δ_6, CD_3OD, 400 MHz): Table I; ^{13}C-NMR (DMSO-δ_6, CD_3OD, 100 MHz): Table II.

Cytotoxicity

Cytotoxic assay was determined using two proliferating mouse cell lines, a normal fibroblast line NIH3T3 (ATCC: CRL1658) which was established from NIH Swiss mouse embryos and a virally transformed form, KA3IT (3T3 mouse fibroblast cell line transformed by the K ras oncogene-containing Kirsten strain of Malony sarcoma virus) in accordance with the method of Shier (1983) as modified by Abbas *et al.*, (1992). Sample of each of the isolated compounds (5 mg) was dissolved in 62.2 μl of DMSO, and working solutions made by diluting 20 μl of the DMSO solution into 2 ml of sterile medium (Dulbecco's modified Eagle's medium, Sigma Chemical Co. St. Louis, MO, USA). Two-fold or 2.5-fold dilutions from 200 μg/ml to 0.5 μg/ml were prepared in triplicate in the wells of 96-well culture trays (Falcon Micro Test III, # 3072, Becton Dickinson Labware, Lincoln Park, NJ, USA) in 200 μl of medium containing 5% (v/v) calf serum (Hyclone Laboratories, Logon, Utah, USA). An inoculum of 2×10^3 cells was added to each well in a 100 μl aliquot of 10% calf serum in medium. The 96-well trays of cells were cultured under standard conditions until uninhibited cultures (control) became confluent. The contents of the wells were decanted, and each cell layer washed with a small amount of the medium. The wells were filled with formal saline (3.7% w/v formaldehyde in 0.15 M NaCl), and allowed to stand at room temperature for at least 30 min. The trays were washed with tap water, and attached cells stained by adding two drops of 0.5% (w/v) crystal violet solution in 20% (v/v) aqueous methanol added to each well. The trays were washed with tap water, and the IC_{50} estimated visually as the approximate concentration that causes 50% reduction in the number of stained cells adhering to the bottom of the wells.

Antiviral assay (Abou-Karum and Shier, 1990):
Sample preparation: as in cytotoxicity assay.

Herpes simplex type 1 (HS-1) was the gift of R. G. Hughes, Roswell Park Memorial Institute, Buffalo, NY. Working stocks of HS-1 that could conveniently be diluted to give about 30 plaques per culture well were prepared by titering virus by serial dilution in culture medium and assaying in triplicate on vero monolayers in the wells of microtiter trays. Working stocks were stored at 4° until used. *Vero* African green monkey kidney cells (ATCC: CCL81) were grown in Dulbecco's modified Eagle's (DME) medium supplemented with 10% (v/v) calf serum, 60 10 μg/ml penicillin G, and 100 10 μg/ml streptomycin sulfate. Cells and viruses were cultured at 37° in humidified atmosphere containing about 15% (v/v) CO_2 in air.

Vero cultures of virus titration and antiviral assays were grown to confluence in the wells of microtiter trays (Falcon Microtest III 96-wells trays) inoculated with *Vero* cells suspended at 2×10^4 cells per 200 μl culture in medium containing 10% (v/v) calf serum.

Microtiter trays with confluent monolayer cultures of *vero* cells were inverted and the medium was shaken out and replaced with serial dilution of serial samples in triplicate in 100 μl of medium. Tray wells were inoculated with 30 plaque forming units of HS-1 virus in 100 μl medium containing 10% (v/v) calf serum. In each tray the first and the last rows of wells were reversed for controls. The trays were inoculated under normal culture conditions for 66 h. After incubation, the trays were inverted onto the paper towels, and the remaining cells were rinsed carefully with medium and fixed with 3.7% formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water, stained with 0.5% crystal violet in 20% aqueous EtOH for 30 min, rinsed with water and examined visually. Antiviral activity was identified as confluent, relatively unaltered monolayers of stained *Vero* cells at dilutions of test compounds that are not cytotoxic in assay trays with plaques in control wells.

The antiviral activity was estimated by the IC50 value (defined as the concentration of the substance which gives approx. 50% protection from the cytopathic effect of the virus).

Tyrosine-specific protein kinase inhibitor assay (Gazit *et al.*, 1989)

Partially purified EGFRK (epidermal growth factor receptor kinase) was prepared by the method of Panayotou *et al.*, 1990 from A431 human epidermoid carcinoma cells (American Type Culture Collection, Rockville, Maryland) in Dulbecco's modified Eagle's medium supplemented with 4% (v/v) fetal calf serum, harvested with 1 mᴍ EDTA in saline and stored at −80 °C until used. Tyrosine-specific protein kinase inhibitory assay was carried out in triplicate with titered aliquots of this enzyme preparation according to the method of Gazit *et. al.* (1989), except that counting of radioactive precipitates in paper cutting acquired more extensive washing and use of a liquid scintillation cocktail. This method measures the effect of inhibitors on the transfer of radioactivity from [γ^{-32} P]ATP to a synthetic polypeptide substrate, poly (glutamate, alanine, tyrosine).

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