FAGONILIN: A NEW TRITERPENE FROM *FAGONIA INDICA* BURM. F. VAR. *INDICA*

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Abstract

Faganilin (**1**), a new lupene type triterpene has been isolated from the aerial parts of *Fagonia indica* Burm. f. var. *indica* along with *β*-amyrin (**2**), lupeol (**3**) and *β*-sitosterol (**4**). The structure of the new isolated compound was elucidated as 3*β,* 19, 21-trihydroxy-20(29)-lupene with the help of extensive spectroscopic studies depending on 1D and 2D-NMR.

Introduction

Fagonia indica Burm. f. var. *indica* belongs to the Family Zygophyllaceae and a medicinally important taxon (Ghafoor, 1974; Pullaiah, 2006) commonly known as Sachchi booti in Pakistan. All species of *Fagonia* are shrub, shurblets or herbs, usually higher than (60 to 100) cm, and near about 100 cm wide. The genus *Fagonia* is confined to arid and dry areas of all continents other than Australia (Beier, 2005). It is found mostly in Indo-Pakistan subcontinent westwards to North and East tropical Africa in arid and semi arid region. The plant parts have been used by local people for the cure of fever, asthma, vomiting, dysentery, urinary discharge, leucoderma, biliousness and typhoid and as blood purifier. The twigs are commonly applied as tooth brushes and bark in the scabies (Shinwari and Shah, 2003). Aerial parts of *Fagonia indica* Burm. f. are used as a remedy for tumors and leaf and twigs are used for treating cancer (Graham, 2000).

The genus is a rich source of triterpenes and saponins (Rahman, *et al*., 1982, 1984; Ansari *et al*., 1987, 1988; Perrone *et al*., 2007). In this article, we present the isolation and identification of one new triterpene lupene (1**)** and three known compounds (2-4) from the methanolic extract Burns *et al* (2000). Compounds 2 and 3 are reported first time from this source.

Experimental
Materials and Methods

General: Column chromatography was carried out on silica gel 60 (70-230 mesh size, *E. Merck*,). Aluminum cards precoated with silica gel PF_{254} (0.5 mm thickness, *E. Merck*) were used for the purpose of TLC. For infrared spectra VECTOR 22 spectrophotometer was used. NMR spectra were measured on Advance AMX-400 spectrometer operating at 400 MHz and 100 MHz for ^{1}H NMR and ^{13}C NMR respectively. EI and HR-EIMS were recorded on MS-Route mass spectrometer and Jeol-JMS-HX-110 instrument respectively.

Plant Material: The aerial parts of *Fagonia indica* var. *indica* were collected from Hyderabad, Sind region during the month of August to September 2009. The plant was authenticated by Afshen Ather, Curator of the Centre for Plant Conservation, Botany Dept. University of Karachi and a voucher No. (86583) was submitted in the Herbarium of the same department.

Extraction and Isolation: The aerial parts of *Fagonia indica* (5 kg) were many times extracted within methanol (3x15L) at room temperature (28°C). This was concentrated under vacuum (rotary) and devided between EtOAc and water layers. The upper layer (EtOAc layer) was washed with water, dried (anhydrous Na_2SO_4), charcoaled and concentrated on rotary. The residue (60 g) was separated into two parts, petroleum ether soluble and insoluble. Petroleum ether soluble fraction was hydrolyzed with 10 % ethanolic KOH for 4 h, reduced to half of its volume and shaken out with EtOAc. The EtOAc layer was washed (water), dried (anhydrous Na_2SO_4) and reduced under vacuum to give a gummy residue (4.15 g). It was subjected to column chromatography eluting with pet ether, pet ether- EtOAc, $CHCl_3$, MeOH, $CHCl_3$-MeOH, in sequence of increasing polarity to give 40 fractions from which four pure compounds were obtained and identified as fagonilin (**1**) (10.8mg), *β*-amyrin (**2**) (32.3 mg), lupeol (**3**) (25.6mg) and *β*-sitosterol (**4**) (15.0 mg).

Characterization of fagonilin **(1):** Gummy white solid, HREIMS =458.7282 ($C_{30}H_{50}O_3$; M^+calcd. for $C_{30}H_{50}O_3$ 458.7232); IR (KBr) ν_{max} cm^{-1}: 3416, 3093, 2945, 1730, and 1628; $[\alpha]_D^{24}$ -11.0° (*c*= 0.3, MeOH). ^1H and ^{13}C NMR data are given in **Table1**.

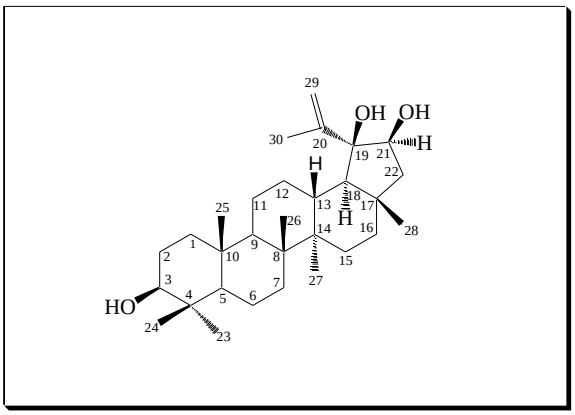
Characterization of β-amyrin **(2):** White powder, HREIMS=426.2975 ($C_{30}H_{50}O$); IR (KBr) ν_{max} cm^{-1}: 3360, 3093, 2850, and 1650. ^1H-NMR (400 MHz) δ = 3.15 (dd, *J*=4.4 Hz; 10.8 Hz, H-3), 1.55, 1.35, (m, H-6), 5.12 (t, *J*=3.2 Hz, H-12), 1.89 (dd, *J*=4.0 Hz; 14.0 Hz, H-15), 1.70 (dd, *J*=4.3 Hz, 13.5, H-16), 1.83, 1.30 (m, H-19), 0.77 (s, H-23) 0.87 (s, H-29), 0.80 (s, H-30).

Characterization of lupeol **(3):** White powder, HREIMS =426.2092 ($C_{30}H_{50}O$); IR (KBr) ν_{max} cm^{-1}: 3370, 2936 and 2865, 1139, 1456 and 1379. ^1H-NMR Data: (400 MHz) δ= 3.18 (dd, 10.8 Hz, 5.0 Hz, H-3), 1.38 (m, H-6), 1.61, 1.10 (m, H-12), 1.35 (m, H-18), 2.36 (m, H-19), 4.68, 4.56 (m, H-29), 1.67 (s, H-30).

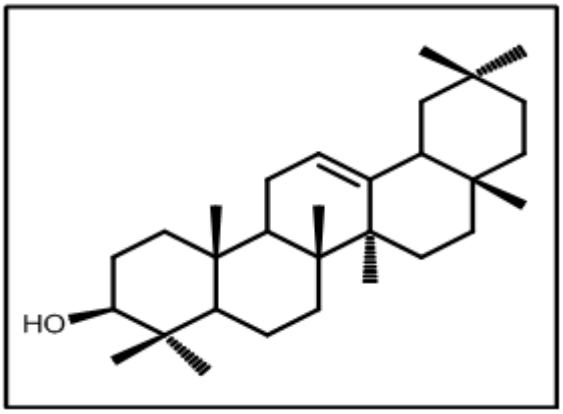
Characterization of β-sitosterol **(4):** White powder, HREIMS =414.3562 ($C_{29}H_{50}O$, calcd. for 414.3861 $C_{29}H_{48}O$) (100), IR (KBr) ν_{max} cm^{-1}: 3600, 2900 and 1640. ^1H-NMR (400 MHz) 5.35 (m, H-6) δ= 3.52 (m, H-3), 0.72 (s, H-18), 1.01(s, H-19), 0.92 (d, 6.7 Hz, H-21), 2.38 (m, H-22), 0.84 (br. s, H-24), 0.87 (d, *J*= 6.7 Hz, H-26), 0.85 (d, *J* = 6.7 Hz, H-28), 0.89 (t, *J* = 7.4 Hz, H-29).

Results and Discussion

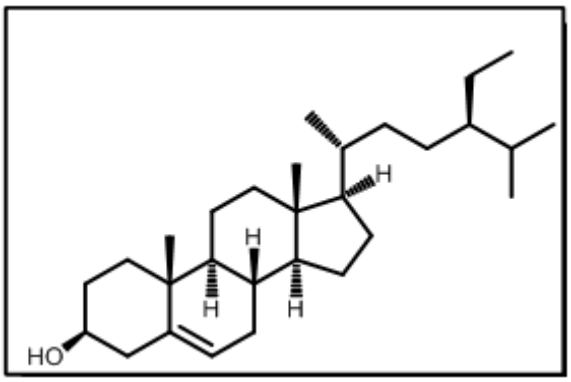
Aerials parts of *Fagonia indica* were extracted with MeOH and it was divided in to petroleum ether, ethyl acetate, *n*-butanol and (polar) water soluble fractions. The compounds **1-4** were extracted from the petroleum ether extract through column chromatography (Fig.1). The known compounds **2**-**4** were identified as *β* – amyrin, lupeol and *β*- sitosterol respectively by the relative comparison of its data (spectral and physical) with those published in literature.



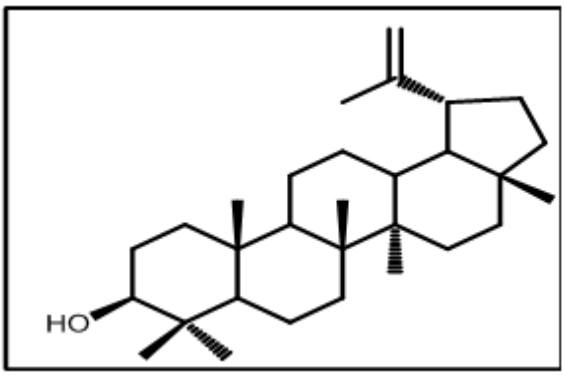
Compound 1



Compound 2



Compound 3



Compound 4

Fig.1. Structure of the isolated compounds from *Fagonia indica*.

Faganilin (**1**) was isolated as a colorless gummy (sticky) solid. Its IR spectrum indicated characteristic absorptions for hydroxyl (3416 cm^{-1}) and olefinic (1628 cm^{-1}) functionalities. Its molecular formula $C_{30}H_{50}O_3$

was deduced with the help of HREIMS which exhibited molecular ion peak at *m/z* 458.7282 (calc.458.7232). The ^{1}H NMR spectrum indicated six methyl singlets at δ 0.75, 0.82, 0.94, 0.95, 1.10 and 1.30 and one vinylic methyl at δ 1.71 and their respective connectivity to carbons at δ 16.3, 16.4, 14.7, 27.9, 16.1, 15.3 and 16.42 was established with the help of HSQC spectrum. The ^{13}C NMR spectra (specially BB and DEPT) showed 30 signals (**Table 1**) comprising seven quaternary carbons, seven methyl, ten methylene and six methine. A pair of downfield protons at δ 4.81 (d, *J*=1.6 Hz, H_a) and 4.93 (d, *J*=1.6 Hz, H_b) showing HSQC correlation with C-29 (δ 110.8) and a vinylic methyl singlet at δ 1.71 were characteristic of an isopropylene group of the lupane skeleton Burns *et al* (2000). The molecular formula depicted six double bond equivalents; five of these were accounted by the rings of carbocyclic skeleton and one by the double bond at C-20 (29). The existance of three hydroxyl groups in this compound was verified by ^{13}C NMR signals at 78.9, 75.0 and 76.2. One of the hydroxyl groups was placed at C-3 (δ 78.9) on biogenetic grounds in *β* - orientation (δ 3.16, dd, *J*= 11.2, 5.2 Hz H-3α) while the second hydroxyl group was placed at C-21 (δ 76.2) based on the HMBC interactions of H-21 (δ 4.01, dd, *J*=4.8, 4.4 Hz) with C-18 (δ 49.8), C-19 (δ 75.0) and C-20 (δ 142.0). The third hydroxyl group was located at C-19 (δ 75.0) on the basis of the HMBC correlations of H-30 with C-19, C-20 and C-29. The stereochemistry was determined from the NOE interactions of various chiral centers, of H-3 with H-5 and H-23; H-18 with H-21 and H-27; H-13 with H-26 and H-28; H-24 with H-25 and H-21 with H-30. In the light of above evidences the structure of fagonilin (**1**) has been elucidated as 3*β,* 19, 21-trihydroxy-20(29)-lupene.

Table 1. ^{1}H-(400 MHz) and ^{13}C- (100 MHz) NMR data of fagonilin (1) in $CDCl_3$ δ in ppm, *J* in Hz.

Carbon	δ_H, J_{Hz}	δ_C	Type C	Important HMBC correlations
1	0.94 (m)	39.0	C H_2	_
	1.66 (m)			
2	1.41 (m)	27.5	C H_2	_
	1.10 (m)			
3	3.16 (dd, 11.2, 5.2)	78.9	C H	C-1, C-2, C-5, C-23
4	_	37.9	C	_
5	0.70 (m)	55.8	C H	C-1, C-3, C-23, C-24
6	1.40 (m)	18.2	C H_2	_
	1.50 (m)			
7	1.25 (m)	33.9	C H_2	_
8	_	40.3	C	_
9	1.28 (m)	50.6	C H	C-1, C-5, C-7,
10	_	37.1	C	_
11	0.83 (m)	21.4	C H_2	_
	1.19 (m)			
12	1.65 (m)	25.4	C H_2	_
	1.10 (m)			
13	1.67 (m)	37.8	C H	C-14, C-19
14	_	42.3	C	_
15	0.95 (m)	27.5	C H_2	_
	1.60 (m)			
16	1.23 (m)	35.1	C H_2	_
	1.50 (m)			
17	_	50.0	C	_
18	1.49 (d 2.9)	49.8	C H	_
19	_	75.0	C	_
20	_	142.0	C	_
21	4.01 (dd, 4.8, 4.4)	76.2	C H	C-18, C-19, C-20
22	2.17 (d 3.2)	43.3	C H_2	C-19, C-21
23	0.95 (s)	27.9	C H_3	C-3, C-5
24	1.30 (s)	15.3	C H_3	C-3, C-5
25	0.75 (s)	16.3	C H_3	C-8, C-10
26	1.10 (s)	16.1	C H_3	_
27	0.94 (s)	14.7	C H_3	_
28	0.82 (s)	16.4	C H_3	_
29	H_a 4.1 (d 1.6)	110.8	C H_2	C-19, C-20
	H_b 4.93 (d 1.6)			
30	1.71 (s)	16.42	C H_3	C-19, C-20, C-29

Conclusion

One new pentacyclic trtiterpene (fagonilin) and three known compounds (*β*-amyrin, lupeol and *β*-sitosterol) are isolated from the pet. ether soluble fraction of methanolic extract of the aerial parts of *Fagonia indica* var. *indica* (Zygophyllaceae).

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