

## Characterization and Isolation of a New Flavonoid from *Viburnum grandiflorum*

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(Received May 10, 2014; Revised August 28, 2014; Accepted September 20, 2014)

**Abstract:** In the present study a new flavonoid **1** along with Betulin **2**, Betulinic acid **3**, Oleanolic acid **4** and Ursolic acid **5** were isolated from the aerial part of *Viburnum grandiflorum* Wall. ex DC as white amorphous powder from the chloroform fraction. On the bases of different modern spectroscopic techniques such as EIMS, UV, IR, NMR, HSQC, HMBC, COSY and NOESY the structure of compound **1** was elucidated as 6,7-bis(propenyloxy)-2-ethoxy-5-hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one (Grandinoid).

**Keywords:** *Viburnum grandiflorum*; grandinoid. © 2014 ACG Publications. All rights reserved.

### 1. Plant Source

The various classes of secondary metabolites such as triterpenoids, iridoid glycosids, flavon glucoside neovibsanin, triterpene saponin, furcadin, norisoprenoids, phenolic compounds, new vibsanes, diterpenes and lupane triterpenes etc has been reported from *Viburnum* genus [1-4].

*Viburnum grandiflorum*., roots were collected from Tandyani district Hazara, Khyber Pakhtunkhwa, Pakistan in the month of July 2009. Plant was identified by an eminent taxonomist Abdul Majid lecturer in Botany, Department of Botany University of Hazara, and specimen voucher No 02 was deposited in the University Herbarium.

### 2. Previous Studies

We first time subjected this valuable plant for phytochemical studies.

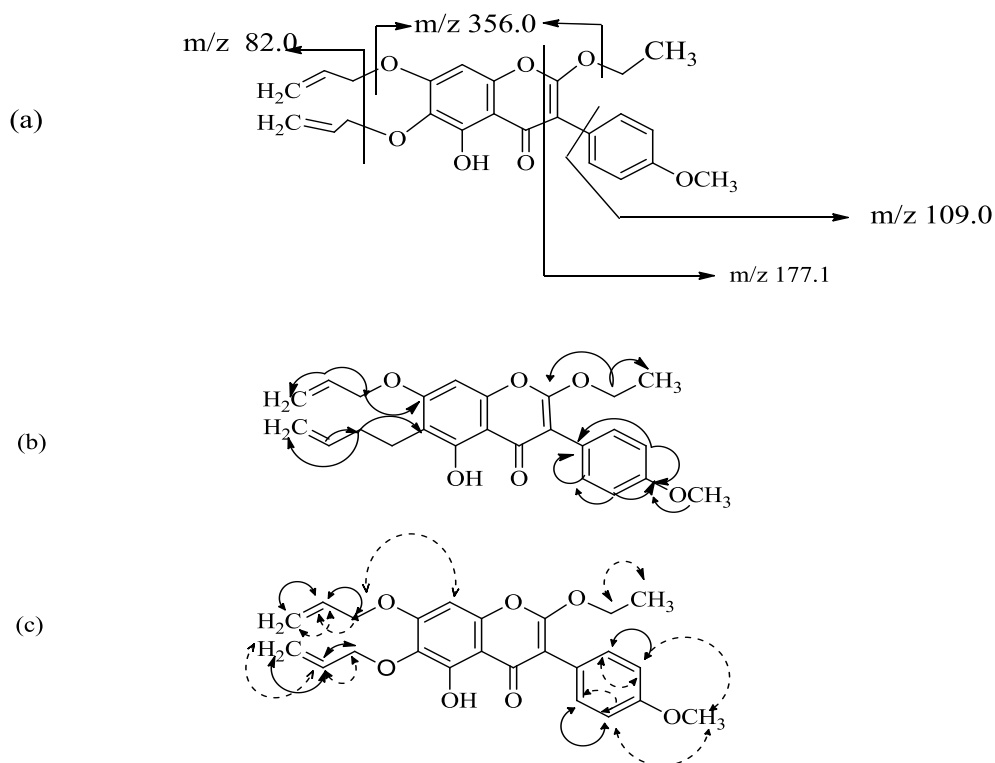
### 3. Present Study

The shade dried aerial parts (14.5 kg) of *V. grandiflorum* were pulverized to powder and (×3) repeatedly extracted with ethanol at room temperature. The combined ethanolic crude extract was concentrated under reduce pressure in rotavapor. The brownish gummy residue 1.5 kg was obtained

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and partitioned between *n*-hexane and water to remove *n*-hexane soluble fraction (365 g). The *n*-hexane insoluble fraction was further partitioned with chloroform, ethyl acetate and butanol to obtained chloroform soluble fraction (225 g), ethyl acetate soluble fraction (245 g) and butanolic fraction (285 g) which were concentrated under reduce pressure. The chloroform fraction (225 g) was again shaken with *n*-hexane to remove as possible *n*-hexane soluble portion. A portion of defatted chloroform fraction (90 g) was subjected to column chromatography (silica gel, 90 mm) and eluted with *n*-hexane-EtOAc with increasing polarity obtained 150 fractions of 20 ml each were collected and compiled to 12 fractions (F<sub>1</sub>-F<sub>12</sub>) based on TLC profile. The fraction F<sub>10</sub> was subjected again to CC followed by preparative TLC precoated with silica gel 60 GF<sub>254</sub> (0.5 mm thickness, Merck) and purified a flavonoid (**1**). Fraction F<sub>8</sub> yield Betulin **2** and Betulinic acid **3** when subjected to CC using ethyl EtOAc and *n*-hexane as a solvent system with increasing polarity. In the similar fashion fraction F<sub>9</sub> yielded Oleanolic acid **4** and Ursolic acid **5** when subjected to CC over silica gel eluted with increasing polarity of EtOAc:*n*-hexane.

**Grandinoid (1):** Compound **1** isolated as white amorphous powder from the chloroform fraction of *V. grandiflorum* aerial parts. The EIMS molecular ion peak observed at *m/z* 424 a.m.u while base peak appeared at *m/z* 356 a.m.u. The molecular formula of the compound **1** deduced as C<sub>24</sub>H<sub>24</sub>O<sub>7</sub>. The U.V spectrum indicates absorption band at 282 nm and characteristic IR absorption bands appeared at 2915 (C-H stretching aliphatic), 1730 (ester carbonyl), 1610 (aromatic double bonds). The <sup>13</sup>C NMR spectra (BB and DEPT) (Table 1) of **1** exhibited two methyl's, five methylenes, seven methines and eleven quaternary carbons. The protons chemical shift values for methyl's displayed at δ<sub>H</sub> 1.01 (t, J=7.2 Hz, H-2'') and δ<sub>H</sub> 3.81 (s, H-4') while these protons were linked with carbon values at δ<sub>C</sub> 13.5 (C-2'') and δ<sub>C</sub> 56.4 (C-4') respectively, in the HMQC spectrum. The proton NMR values for methylene appeared at δ<sub>H</sub> 4.14 (q, J= 7.2 Hz, H-1'''), 4.54 (d, J= 5.6 Hz, H-1'''), 5.43 (ddd, (J= 17.2, 3.2, 1.6 Hz), (10.8, 3.2, 1.5 Hz), H-3'''), 4.69 (d, J= 4.8 Hz, H-1''') and δ<sub>H</sub> 5.54 (ddd, J= 17.2, 3.2, 1.2 Hz, H-3'''). Their carbon NMR absorption peaks displayed at δ<sub>C</sub> 62.3 (C-1''), 75.3 (C-1'''), 118.4 (C-3'''), 70.2 (C-1''') and δ<sub>C</sub> 118.6 (C-3''') respectively. The proton NMR resonated for methines at δ<sub>H</sub> 6.67 (s, H-8), 7.18 (dd, J= 7.2, 2.0 Hz, H-2'/6'), 6.93 (dd, J= 7.2, 2.0 Hz, H-3'/5'), 6.23 (ddt, J= 16.4, 10.0, 6.0 Hz, H-2''') and δ<sub>H</sub> 6.05 (ddt, J= 15.6, 10.0, 4.8 Hz, H-2''') while their respective carbon NMR values appeared at δ<sub>C</sub> 97 (C-8), 131 (C-2'/6'), 113.7 (C-3'/5'), 131.1 (C-2''') and δ<sub>C</sub> 118.6 (C-2'''). Analysis of the <sup>1</sup>H, <sup>13</sup>C NMR, HSQC, and HMBC data (Table 1) revealed the flavonoid structure; containing substituted aromatic rings, a 5, 6, 7 trisubstituted ring A (δ<sub>H</sub> 6.67 (s, H-8), δ<sub>C</sub> 97 (C-8)) and a monosubstituted ring B (( δ<sub>H</sub> 7.18 (dd, J=7.2, 2.0 Hz), δ<sub>C</sub> 131 (C-2'/6') and δ<sub>H</sub> (6.93 (dd, J=7.2, 2.0 Hz), δ<sub>C</sub> 113.7 (C-3'/5')). The spectral data and <sup>13</sup>C NMR chemical shifts for C-4 (δ<sub>C</sub> 174.7), C-5 (δ<sub>C</sub> 113.5), C-6 (δ<sub>C</sub> 161.4), C-7 (δ<sub>C</sub> 158.8) showed chrysin nucleus (Chen, et al., 2003) The <sup>1</sup>H NMR data further revealed two propenyloxy groups attached, at C-6 (( δ<sub>H</sub> 5.54 (d, 5.6 Hz), δ<sub>C</sub> 75.3, C-1''', δ<sub>H</sub> 6.23 (ddt, 16.4, 10.0, 6.0 Hz), δ<sub>C</sub> 131.1, CH-2''', δ<sub>H</sub> 5.43 (ddd, 17.2, 3.2, 1.6 Hz), δ<sub>C</sub> 118.4, C-3''') and at C-7 (δ<sub>H</sub> 4.69 (d, J=4.8 Hz), δ<sub>C</sub> 70.2, C-1'''), δ<sub>H</sub> 6.05 (1H, ddt, J=15.6, 10.0, 4.8 Hz), δ<sub>C</sub> 133.6, CH-2''') and (δ<sub>H</sub> 5.54, ddd, J=17.2, 3.2, 1.2 Hz), δ<sub>C</sub> 118.6, C-3''') of the ring A. Analysis of the <sup>1</sup>H-<sup>1</sup>H COSY correlation Figure (c) provided the sequential connectivity of all protons resonances of the propenyloxy chains. The HMBC spectrum exhibited correlations between H-1'''' and C-6 and also between H-1''' and C-7 suggested that one propenyloxy group attached with C-6 and other propenyloxy group attached with C-7. The NOESY correlation Figure (c) between H-1'''' and H-8 further confirmed the position of propenyloxy at C-7. These spectral data and mass fragmentation profile Figure (a) of **1** and related compound data in literature led to elucidate the structure of **1** as 6,7-bis (propenyloxy)-2-ethoxy-5-hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one (Grandinoid) (Figure 1).

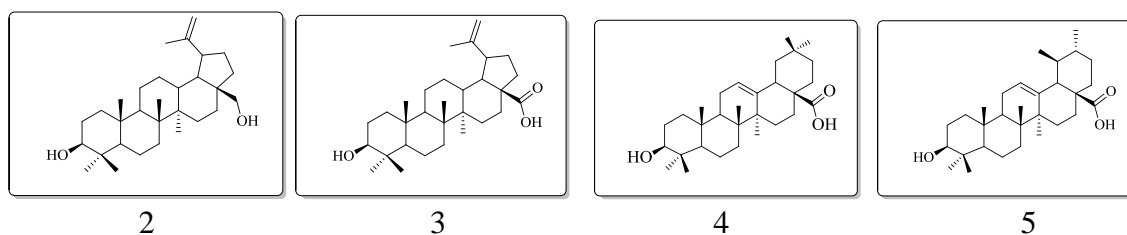


**Figure 1.** (a) Mass fragmentation pattern, (b) HMBC correlations (c) Important  $^1\text{H}$ - $^1\text{H}$  COSY correlations (bold arrows), NOESY correlation (dotted arrow) observed for Grandinoid (**1**).

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR (1D and 2D NMR in  $\text{CDCl}_3$ ) of compound **1**

Number	$\delta_{\text{H}}$ , Multiplicity, $J$ (Hz)	$\delta_{\text{C}}$	TYPE	HMBC
2	-	148.7	C	-
3	-	126.7	C	-
4	-	174.7	C	-
4a	-	84.8	C	-
5	-	161.4	C	-
6	-	113.5	C	-
7	-	158.8	C	-
8	6.67, s	97.0	CH	C-8a, C-7, C-6, C-4a
8a	-	159.3	C	-
1'	-	123.3	C	-
2'/6'	7.18, dd, 7.2, 2.0	131.0	CH	C-1', C-3', C-5', C-4'
3'/5'	6.93, dd, 7.2, 2.0	113.7	CH	C-1', C-2', C-4', C-6'
4'	-	159.7	C	-
1''	4.14, q, 7.2	62.3	$\text{CH}_2$	C-2''
2''	1.01, t, 7.2	13.5	$\text{CH}_3$	C-1''
1'''	4.54, d, 5.6	75.3	$\text{CH}_2$	C-2''', C-3''', C-7
2'''	6.23, ddt, 16.4, 10.0, 6.0	131.1	CH	C-1'''
3'''	5.43, ddd, 17.2, 3.2, 1.6	118.4	$\text{CH}_2$	C-2'''
-	10.8, 3.2, 1.6	-	-	-
1''''	4.69, d, 4.8	70.2	$\text{CH}_2$	C-2'''', C-3''''
2''''	6.05, ddt, 15.6, 10.0, 4.8	133.6	CH	C-1''''
3''''	5.54, ddd, 17.2, 3.2, 1.2	118.6	$\text{CH}_2$	C-1'''', C-2''''
-	10.8, 3.2, 1.5	-	-	-
4'- $\text{OCH}_3$	3.81, s	55.2	$\text{CH}_3$	C-4'

The structures of compound **2**, **3**, **4**, **5** were elucidated by comparing their spectral data with the known compounds from literature [5 – 8]. The previous studies on this plant reflect the plant is a valuable source of various phytochemicals [9] such as benzofuran [10] and triterpene [11].



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