

Guaianolide and *seco*-Guaianolide from *Salvia nubicola* (Lamiaceae)

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A sesquiterpene-lactone of the guaianolide class (nubenone) and a *seco*-guaianolide (nubenoic acid) have been isolated from *Salvia nubicola* collected in Quetta, Pakistan. Structures of both sesquiterpenes were elucidated with the aid of spectroscopic techniques including two dimensional NMR spectroscopy.

Key words: Guaianolide, Nubenone, *seco*-Guaianolide, Nubenoic Acid, *Salvia nubicola*

Introduction

Salvia is one of the famous genera of the family Lamiaceae previously called Labiatae comprising about 800 species throughout the world [1]. Most of the members of this genus are known due to their biologically active chemical constituents specifically reported for *anti*-tumor activity [2]. A number of *Salvia* species are used in folk medicines. For example, *S. cavaleriei* is used for the treatment of dysentery, boils and fall injuries [3], *S. desoleria* for the treatment of menstrual, digestive and CNS diseases [4] and *S. bucharica* as traditional medicine for the treatment of hepatic problems. Most of the *Salvia* plants are rich in essential oils and among their constituents guaiane-sesquiterpenes are the most common [5 – 9].

In continuation of our research on the chemical constituents of *Salvia* [10 – 19], we have recently published two articles on *S. hypoleuca* [18] and *S. nubicola* [19]. Now we wish to report the isolation and spectroscopic characterization of further new sesquiterpenes: guaianolide (**1**, nubenone) and a *seco*-guaianolide (**2**, nubenoic acid).

Results and Discussion

The methanolic extract of *Salvia nubicola* collected in Quetta (Pakistan) yielded a guaianolide (**1**, nubenone) and a *seco*-analog of the same class (**2**, nubenoic acid).

Nubenone (1): Compound **1** was obtained as a colorless powder melting at 96 °C. The IR spectrum of **1** displayed strong absorption bands at 3423, 1750, 1690 and 1615 cm⁻¹ due to the presence of hydroxyl, α,β -

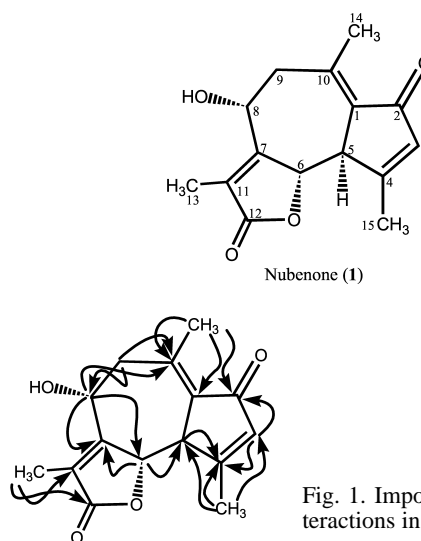


Fig. 1. Important HMBC interactions in **1**.

unsaturated lactone carbonyl, α,β -unsaturated ketone carbonyl and olefinic functionalities, respectively. The molecular mass and corresponding formula were determined based on a field desorption mass spectrum (FDMS) and HREIMS with a molecular ion peak at m/z 260 as C₁₅H₁₆O₄. The fragments and their formulae resulted from HREIMS give the loss of methyl group and removal of a water molecule from the molecular ion observed at m/z 245 and 242, respectively, in the mass spectra (EIMS and HREIMS).

The NMR spectra were scanned at low temperature (15 °C) in order to avoid the possible resonance due to the two conjugated systems in the molecule. The proton NMR spectrum of **1** displayed three methyl singlets at δ = 1.85, 2.40 and 2.10. Their positions

Table 1. ^1H NMR spectral data of nubenone (**1**) and nubenoic acid (**2**).

H. No.	Nubenone (CDCl_3 , 500 MHz) (J in Hz)	Nubenoic acid (CD_3OD , 300 MHz) (J in Hz)
2	—	4.32 (br.s)
3	6.13 (s)	5.78 (br.s)
5	3.51 (d, 10.50)	3.19 (d, 10.47)
6	4.22 (d, 10.61)	4.81 (d, 10.11)
8	4.93 (dd, 10.51, 3.18)	4.73 (dd, 12.19, 4.43)
9a	2.73 (dd, 13.43, 3.18)	2.70 (dd, 14.32, 4.40)
9b	2.37 (dd, 13.50, 10.62)	2.00 (dd, 14.30, 12.22)
13	1.85 (s)	2.06 (s)
14	2.40 (s)	1.57 (s)
15	2.10 (s)	2.09 (s)

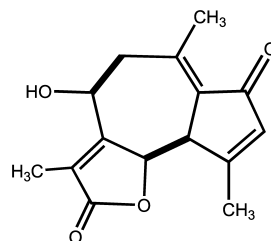
Table 2. ^{13}C NMR spectral data of nubenone (**1**) and nubenoic acid (**2**).

C-No.	Nubenone	Multiplicity	Nubenoic acid	Multiplicity
1	132.5	C	131.1	C
2	196.8	C	80.0	CH
3	135.6	CH	129.5	CH
4	167.2	C	144.3	C
5	52.0	CH	50.3	CH
6	76.3	CH	71.5	CH
7	162.4	C	163.4	C
8	63.0	CH	77.3	CH
9	42.4	CH_2	39.1	CH_2
10	142.6	C	148.9	C
11	123.3	C	123.2	C
12	173.9	C	174.1	C
13	8.1	CH_3	10.2	CH_3
14	20.6	CH_3	19.9	CH_3
15	16.1	CH_3	23.0	CH_3

were assigned as Me-13 ($\delta = 1.85$), Me-14 ($\delta = 2.40$) and Me-15 ($\delta = 2.10$) with the aid of HMQC and HMBC experiments (Fig. 1). The carbon-signals of the methyls group were located at $\delta = 8.1$ (Me-13), 20.6 (Me-14) and 16.1 (Me-15).

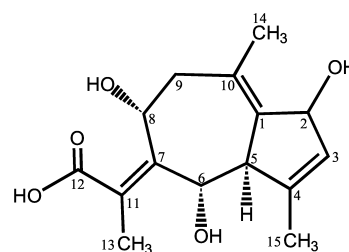
The information gathered from the NMR spectra were cross-checked by HMBC (Fig. 1) and COSY (Fig. 2) experiments. Finally, the structure was deduced **1** and named nubenone possessing a “guaiane” skeleton. Such olides have already been reported in the literature [20]. Guaianolides are very common natural sesquiterpene-lactones found in various terrestrial plants [5–7]. Nubenone has not been reported so far from any natural source.

A similar metabolite named desangeloyl shairidin with the reversed stereochemistry at C-8 was reported from *Guillonea scabra* by Pinar *et al.* [21]. The possibility of the hydroxyl group at C-8 being in the β -position in **1** is clearly ruled out as proton-proton *trans*-diaxial coupling ($J = 10.51$ Hz) was observed for the

Fig. 2. COSY interactions (—) in **1**.

signals of H-8 as well as H-9 ($J = 10.62$) (see Table 1). These couplings are not possible in case of a β -oriented hydroxyl at C-8. Furthermore, the relative stereochemistry of the stereogenic centers (C-5, C-6 and C-8) is assigned on comparison with recently reported guaianolide named nubenolide, the structure of which was solved by single crystal X-ray crystallography [19].

Nubenoic acid (2): Compound **2** was obtained as an off white powder, which melted at 164 °C. The IR spectrum of **2** exhibited absorptions at 3425, 1720 and 1625 cm^{-1} due to the hydroxyl, conjugated acid carbonyl and olefinic functionalities in the molecule. The FAB mass spectrum in positive mode displayed a pseudo-molecular ion peak ($M+1$) at m/z 281 and the formula of this peak was determined as $\text{C}_{15}\text{H}_{21}\text{O}_5$ through HRMS. Compound **2** contains six degrees of unsaturation, at least two degrees of which can be assigned on the grounds of IR spectral information which exhibited the presence of a conjugated acid moiety in **2**. The other mass spectral details are given in the Experimental Section.

Nubenoic acid (**2**)

The NMR spectra of **2** were quite similar when compared with **1** (see Table 1 and 2). However, disappearance of the ketone signal and appearance of an additional oxy-methine at $\delta = 80.0$ (C-2) and $\delta = 4.32$ (br.s, H-2) was noted in the NMR spectra. A down-field shift of C-8 methine signal ($\delta = 77.3$) was also observed in the carbon NMR spectrum of **2** when compared with **1** which showed the same methine at $\delta = 63.0$. This change may be due to the *seco*-lactone

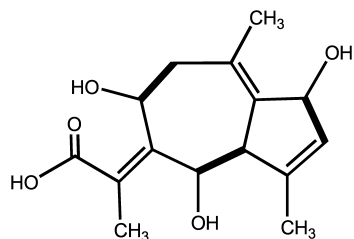


Fig. 3. Important COSY (–) interactions in **2**.

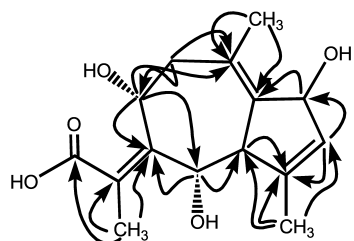


Fig. 4. Important HMBC interactions in **2**.

presence of a moiety as well as to the γ -effect of an acid carbonyl group. Similarly, change in the chemical shifts of C-3 and C-4 (towards the upfield as compared to **1**) at $\delta = 129.5$ and 144.3 , respectively, also helped to decide the position of the additional hydroxyl function in the cyclo-pentene ring (see Table 2).

When the proton NMR spectra of **1** and **2** were compared (Table 1), a drastic change in the chemical shifts of Me-14 ($\delta = 1.57$ in **2** and 2.40 in **1**) was noticed. This change may be due to the reduction of the keto (C-2 in **1**) to the hydroxyl function (C-2 in **2**). All proton and carbon assignments were finally made with the aid of COSY (Fig. 3) and HMBC (Fig. 4) experiments and structure was elucidated as **2** named nubenoic acid. However, the n.O.e. experiments could not help to deduce the stereochemistry of C-2. This *seco*-guaianolide is a new addition in the list of natural products. *seco*-Guaianolides [22] are not as common in nature as guaianolides.

Experimental Section

General

^1H NMR and ^{13}C NMR spectra were recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$ at 500/300 MHz and at 100/125 MHz, respectively. All the 1D and 2D NMR spectra were scanned at 15°C .

Collection and identification

Plant material was collected from Urhat-Juniper forest, near Zearat (Quetta), Pakistan, in the month of July (1999) and identified by Dr. Rasool Baksh Tareen, Department of Botany, Baluchistan University, Quetta, where the

voucher specimen (No. 613) of the plant is deposited in the herbarium.

Extraction and isolation

The fresh plant material (all parts, 13 kg) was dried under shade (6.5 kg) for a period of two weeks then soaked in hexane ($12\text{ l} \times 2$) and methanol ($12\text{ l} \times 2$) for ten days in each solvent. Solvents were evaporated through vacuum distillation. The concentrated and crude methanolic extract (217 g) was then partitioned between water and ethyl acetate. The ethyl acetate layer was again concentrated (167 g) and subjected to column chromatography using hexane; hexane-ethyl acetate; ethyl acetate and ethyl acetate-methanol as mobile phase.

Fractions eluted with 10% ethyl acetate in hexane yielded **1** as a colorless powder (9.13 mg).

Nubenone (**1**)

M. p. 96°C . – $[\alpha]_{\text{D}}^{28} = +72.3^\circ$ (chloroform, c 0.778). – IR (film): $\nu = 3423$ (OH), 1751 (α,β -unsaturated lactone $\text{C}=\text{O}$), 1690 (α,β -unsaturated ketone $\text{C}=\text{O}$), 1615 ($\text{C}=\text{C}$) cm^{-1} . – MS (EI, 70 eV): m/z (%) = 260 (7) [M^+], 245 ($\text{M}-\text{CH}_3$) $^+$, 242 [$\text{M}-\text{H}_2\text{O}$] $^+$, 227, 214, 199, 186, 171, 135, 134 and 107. – MS (FD): m/z 260. – MS (HREI): m/z 260.10390 ($\text{C}_{15}\text{H}_{16}\text{O}_4$ requires m/z 260.10484), 245.15246 ($\text{C}_{14}\text{H}_{13}\text{O}_4$ requires m/z 245.08137), 242.13654 ($\text{C}_{15}\text{H}_{14}\text{O}_3$ requires m/z 242.09428), 227.06919 ($\text{C}_{14}\text{H}_{11}\text{O}_3$ requires m/z 227.07081), 214.09232 ($\text{C}_{14}\text{H}_{14}\text{O}_2$ requires m/z 214.09937), 199.07035 ($\text{C}_{13}\text{H}_{11}\text{O}_2$ requires m/z 199.07590), 186.10424 ($\text{C}_{13}\text{H}_{14}\text{O}$ requires m/z 186.10446), 171.08152 ($\text{C}_{12}\text{H}_{11}\text{O}$ requires m/z 171.08098), 135.07379 ($\text{C}_9\text{H}_{11}\text{O}$ requires m/z 135.08098), 134.06947 ($\text{C}_9\text{H}_{10}\text{O}$ requires m/z 134.07316), 107.09073 (C_8H_{11} requires m/z 107.08607). – ^1H NMR: Table 1. – ^{13}C NMR: Table 2. – COSY-45: Fig. 1. – HMBC: Fig. 2.

Fractions eluted with 20% ethyl acetate in hexane yielded as an off white solid, which on washing with diethyl ether yielded **2** as a white solid (7.88 mg).

Nubenoic acid (**2**)

M. p. 164°C . – $[\alpha]_{\text{D}}^{28} = +36.3^\circ$ (py, c 0.613). – IR (KBr): $\nu = 3425$ (OH), 1720 (α,β -conjugated acid $\text{C}=\text{O}$), 1625 ($\text{C}=\text{C}$) cm^{-1} . – MS (FAB + ve): m/z 281 [$\text{M}+1$] $^+$, 266 [$\text{M}+1-\text{CH}_3$] $^+$, 263 [$\text{M}+1-\text{H}_2\text{O}$] $^+$, 245 [$\text{M}+1-2\text{H}_2\text{O}$] $^+$, 237 [$\text{M}+1-\text{CO}_2$] $^+$. – (HRFAB + ve): m/z 281.136992 ($\text{C}_{15}\text{H}_{21}\text{O}_5$ requires m/z 281.138887), 263.130098 ($\text{C}_{15}\text{H}_{19}\text{O}_4$ requires m/z 263.1283238), 245.119320 ($\text{C}_{15}\text{H}_{17}\text{O}_3$ requires m/z 245.1177605), 237.151117 ($\text{C}_{14}\text{H}_{21}\text{O}_3$ requires m/z 237.1490589). – ^1H NMR: Table 1. – ^{13}C NMR: Table 2. – COSY-45: Fig. 3. – HMBC: Fig. 4.

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