# Guaianolides from *Salvia nubicola* (Lamiaceae)

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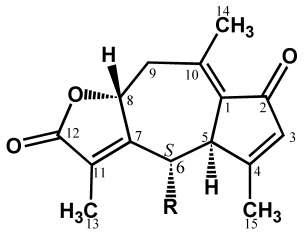
**A new sesquiterpene-lactone (nubenolide) belonging to the guaianolide class along with its acetate (nubenolide acetate) and a dimer (bisnubenolide) have been isolated from *Salvia nubicola* collected from Quetta, Pakistan. Structures of all three new metabolites were elucidated with the aid of spectroscopic techniques including 2D-NMR. However, the structure of nubenolide was finally confirmed *via* single-crystal X-ray diffraction method.**

**Key words** *Salvia nubicola*; Lamiaceae; guaianolide; 2D-NMR; X-ray

*Salvia* is the largest genus of the family Lamiaceae having about 800 species throughout the world.[1)] Most of the plants of this genus are well known for their biologically active constituents, specifically those with anti-tumor activity.[2)] Several species of *Salvia* are used in folk medicines, for instance, *Salvia cavaleriei* is used for the treatment of dysentery, haemoptysis, boils, and fall injuries,[3)] *Salvia desoleria* for the treatment of menstrual, digestive, and central nervous system diseases,[4)] and *Salvia bucharica* as a traditional medicine for the treatment of liver disorders. The plants of this genus are rich in essential oils and among their constituents, 1,8-cineol and guaiane-type mono- and sesquiterpenes are very common.[5,6)]

In continuation of our research on the chemical constituents of *Salvia*,[7—14)] herein wish to report the isolation and spectroscopic characterization of three new guaianolides: nubenolide (**1**), bisnubenolide (**2**), and nubenolide acetate (**3**) from *Salvia nubicola* collected from Quetta, Pakistan.

## Results and Discussion



**(1) R = OH (nubenolide)**
**(1a) R = (*R*) - MTPA**
**(1b) R = (*S*) - MTPA**
**(3) R = OAc (nubenolide acetate)**

The ethyl acetate-soluble part of the methanolic extract of *S. nubicola* collected from Quetta (Pakistan) yielded a guaianolide (**1**, nubenolide), its dimer (**2**, bisnubenolide), and aceteate (**3**, nubenolide acetate).

**Nubenolide (1)** Compound **1** was obtained as colorless needles. This was melted at 110—112 °C. The IR spectrum exhibited presence of hydroxyl (3440 $cm^{-1}$), $\alpha,\beta$-unsaturated ketone and/or lactone (1750 $cm^{-1}$) and olefinic (1608 $cm^{-1}$) functionalities in the molecule. The molecular ion peak was observed at *m*/*z* 260 in field desorption mass spectrum (FD-MS) and the formula of this peak was depicted as $C_{15}H_{16}O_4$ *via* high resolution (HR)-EI-MS. The other fragments are mentioned in the experimental section.

Most of the signals in $^1$H-NMR spectrum showed confusing multiplicities with minor coupling constants inferring the existence of long-range couplings in the molecule. Therefore the spectrum was simplified by recording at low temperature (10 °C). A singlet at $\delta$ 6.30 was assigned to H-3. A pair of doublets having the coupling constants 10.1 Hz appeared at $\delta$ 3.45 and 4.55 due to H-5 and H-6. An ABX system was found in the molecule due to H-8, H-9$\alpha$, and 9$\beta$ at $\delta$ 4.76 (dd , 11.8, 3.4), 2.50 (dd, 12.9, 11.8), and 2.68 (dd, 12.9, 3.4), respectively. The three quaternary methyls appeared at $\delta$ 2.37 (H-13), 2.45 (H-14), and 2.44 (H-15). A complete picture of the proton NMR spectrum is given in Table 1.

The $^{13}$C-NMR spectrum showed 15 carbon signals altogether which were resolved into three methyls, a methylene, four methines, and seven other quaternary carbons with the aid of Distortionless Enhancement by Polarization Transfer (DEPT) experiments. The signals due to the ketone and lactone carbonyls were located in the spectrum at $\delta$ 195.8 and 174.2, respectively. The carbinylic carbon appeared at $\delta$ 75.1. The olefinic methine was found at $\delta$ 135.3. The olefinic-quaternary carbons, which are also the part of ring junctions, resonated at $\delta$ 135.4 (C-1) and 164.6 (C-7). The signals of the three remaining olefinic-quaternary carbons to which methyls are attached appeared at $\delta$ 173.1(C-4), 141.7 (C-10), and 122.7 (C-11). Their corresponding methyls appeared at $\delta$ 20.1, 21.0 and 10.5, respectively. An oxy-methine and the only methylene in the molecule resonated at $\delta$ 77.6 (C-8) and 41.7 (C-9). Detailed $^{13}$C-NMR data are given in Table 2.

The obtained information through NMR spectra were further correlated *via* $^1$H–$^1$H correlation spectroscopy (COSY) and heteronuclear multiple-bond correlations (HMBC) (Fig. 1) experiments. The HMBC connectivity of H-6 ($\delta$ 4.55) with C-7 ($\delta$ 164.6) clearly confirmed the position of hydroxyl function at C-6. The structure of **1** was elucidated when the information gathered from COSY and HMBC experiments was coupled with obtained NMR spectral data. Finally, the relative stereochemistry of chiral centers (C-5, C-8) and complete structure of **1** were cross-checked by means of single-crystal X-ray crystallography (Fig. 2).

However, the (*S*) absolute configuration at C-6 was established by modified Mosher's method.[15)] Esterification of **1**

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Table 1. $^1$H-NMR Spectral Data of Nubenolide (**1**), (*R*) MTPA-Ester (**1a**), (*S*) MTPA-Ester (**1b**), Bisnubenolide (**2**) and Nubenolide Acetate (**3**)

| H. No. | Nubenolide (*J* in Hz) | (*R*) MTPA-ester (*J* in Hz) | (*S*) MTPA-ester (*J* in Hz) | Bisnubenolide (*J* in Hz) | Nubenolide acetate (*J* in Hz) |
|---|---|---|---|---|---|
| 3 | 6.30 (s) | 6.29 (s) | 6.29 (s) | 5.99 (s) | 6.21 (s) |
| 5 | 3.45 (d, 10.1) | 3.39 (d, 10.1) | 3.45 (d, 10.1) | 3.19 (d, 10.8) | 3.47 (d, 10.6) |
| 6 | 4.55 (d, 10.1) | 5.23 (d, 10.1) | 5.26 (d, 10.1) | 4.10 (d, 10.8) | 5.35 (d, 10.6) |
| 8 | 4.76 (dd, 11.8, 3.4) | 4.76 (dd, 11.8, 3.4) | 4.76 (dd, 11.8, 3.4) | 4.45 (d, 11.8) | 4.69 (dd, 12.1, 3.3) |
| 9$\alpha$ | 2.50 (dd, 12.9, 11.8) | 2.52 (dd, 12.9, 11.8) | 2.50 (dd, 12.9, 11.8) | 2.54 (d, 11.8) | 2.43 (dd, 13.3, 12.1) |
| 9$\beta$ | 2.68 (dd, 12.9, 3.4) | 2.68 (dd, 12.9, 3.4) | 2.69 (dd, 12.9, 3.4) | — | 2.78 (dd, 13.3, 3.3) |
| 13 | 2.37 (s) | 2.37 (s) | 2.34 (s) | 1.95 (s) | 1.97 (s) |
| 14 | 2.45 (s) | 2.44 (s) | 2.44 (s) | 2.24 (s) | 2.45 (s) |
| 15 | 2.44 (s) | 2.42 (s) | 2.44 (s) | 2.20 (s) | 2.23 (s) |
| OAc | — | — | — | — | 2.20 (s) |
| Ph | — | 7.22 (m) | 7.23 (m) | — | — |
| OMe | — | 3.58 (s) | 3.56 (s) | — | — |

At 300 MHz in $CDCl_3$.

Table 2. $^{13}$C-NMR Spectral Data of Nubenolide (**1**), Bisnubenolide (**2**) and Nubenolide Acetate (**3**)

| C. No. | Nubenolide (125 MHz) | Nubenolide acetate (75 MHz) | Bisnubenolide (100 MHz) |
|---|---|---|---|
| 1 | 135.4 | 133.9 | 134.6 |
| 2 | 195.8 | 194.8 | 196.6 |
| 3 | 135.3 | 137 | 134.7 |
| 4 | 173.1 | 167.5 | 173.7 |
| 5 | 53.3 | 50.5 | 52.7 |
| 6 | 75.1 | 75.4 | 74.5 |
| 7 | 164.6 | 158.5 | 163.8 |
| 8 | 77.6 | 77 | 77.3 |
| 9 | 41.7 | 42 | 41.3 |
| 10 | 141.7 | 143.3 | 142.5 |
| 11 | 122.7 | 122.5 | 122.8 |
| 12 | 174.2 | 172.9 | 174.6 |
| 13 | 10.5 | 9.6 | 9.4 |
| 14 | 21 | 20.7 | 20.4 |
| 15 | 20.1 | 19.9 | 20.1 |
| $CH_3\underline{C}O$ | — | 168.7 | — |
| $\underline{C}H_3CO$ | — | 20.9 | — |

In $CDCl_3$.

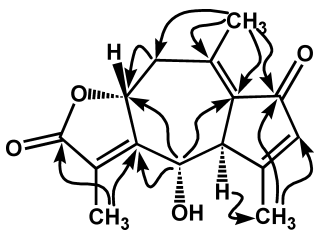



Fig. 1. Important HMBC Interactions in **1**

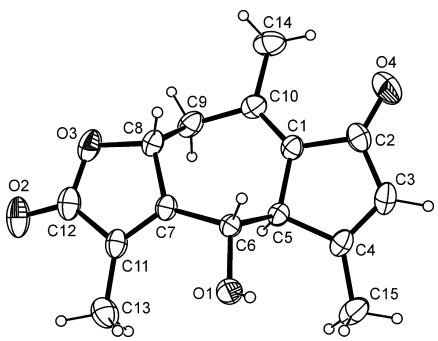
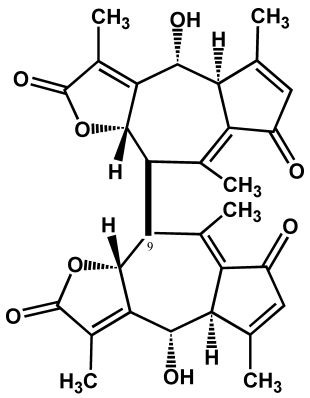



Fig. 2. X-Ray Structure of **1**




**Bisnubenolide (2)**

with *R*-(−)MTPA-Cl and *S*-(+)MTPA-Cl afforded the corresponding esters **1a** and **b**, respectively. The positive value of $\Delta(\delta_S-\delta_R)$ for H-5 indicated (*S*)-configuration at C-6 in **1**.

This new guaianolide has not been reported so far from any natural source and is named nubenolide. Guaianolides are very common natural sesquiterpene-lactones found in various terrestrial plants.[16—18)]

**Bisnubenolide (2)** Compound **2** was obtained as a white powder. Spectral analysis of **2** suggested the structure as a dimer of **1**. Brief mass and IR spectral data are given in the experimental section.

Compound **2** was melted at 131 °C. The molecular mass and corresponding formula were confirmed by FD and HR-EI mass spectra at *m/z* 518 as $C_{30}H_{30}O_8$. As compared with the NMR spectra of **1**, only disappearance of methylene and appearance of an additional methine were observed in the NMR spectra of **2** (see Tables 1, 2). Instead of ABX system, the $^1$H-NMR spectrum showed a mutually coupled pair of doublets at $\delta$ 4.45 (H-8) and 2.45 (H-9) with the coupling constant 11.8 Hz. Similarly, $^{13}$C-NMR spectrum displayed an extra methine at $\delta$ 41.3 with the loss of methylene signal. Thus the structure of **2** was concluded as a dimer of **1** with the carbon-carbon bond across C-9 of both monomeric units.

With the aid of X-ray data of nubenolide (**1**) (CCDC 1768121), the dihedral angles of 9-H$\alpha$ and 9-H$\beta$ with H-8$\beta$ could be calculated and were found to be 184.3° and 76.5°, respectively. Since 9-H$\beta$ is missing in bisnubenolide (**2**) depicted by the disappearance of 3.4 Hz coupling in the H-8

NMR signal (see Table 1), so the linkage of the other monomeric unit in **2** should be from $\beta$-face. The molecular model of **2** also suggested the $\beta$-orientation of attachment to avoid the steric factor from the $\alpha$-face. This new dimer is named bisnubenolide.

**Nubenolide Acetate (3)** Compound **3** was obtained as an off-white powder melted at 128 °C. Most of the spectral data were matched with the data of **1** except for a few signals/peaks described here. The FD-MS of **3** displayed the molecular weight 302 atomic mass unit and the formula was observed as $C_{17}H_{18}O_5$ in the HR-EI-MS. The status of an additional two carbons, an oxygen, and two hydrogen atoms in the molecule was inferred by the presence of an acetate moiety that was cross-checked *via* NMR spectra. The carbon spectrum of **3** showed two extra signals at $\delta$ 20.9 and 168.7 due to the methyl and carbonyl carbons of acetoxyl function. The methyl corresponds to the acetoxyl function appearing in the proton NMR spectrum at $\delta$ 2.20. Detailed NMR spectral data of **3** are given in Tables 1 and 2. The slight shift of carbinylic signal towards downfield in the $^1$H-NMR spectrum suggested the position of the acetate moiety at C-6. In the $^{13}$C-NMR spectrum, the upfield shift of both signals adjacent to C-6 carbons at $\delta$ 50.5 (C-5) and 158.5 (C-7) additionally supports the position of the acetate moiety at C-6. Furthermore, the position of this additional moiety was reconfirmed with the aid of HMBC and Heteronuclear Multiple-Quantum Coherence (HMQC) experiments. This acetate-guaianolide named nubenolide acetate is another new addition in the constituents of *S. nubicola*.

The NMR spectra of chemically acetylated product of **1** exactly matched with **3**, confirming the *S*-configuration at C-6 in **3**.

## Experimental

**General Procedures** IR spectra were recorded by Jasco-320-A spectrometer. $^1$H-NMR spectra were recorded in $CDCl_3$ by a Bruker AM-300 spectrometer while the $^{13}$C-NMR spectra were recorded by Bruker AM-300, AM-400 and AM-500 spectrophotometers at 75, 100, and 125 MHz in the same solvent. Chemical shifts are given relative to TMS ($\delta$: 0.00) as internal standard ($^1$H) and $\delta$: 77.0 (ppm) from $CDCl_3$ as standard ($^{13}$C). Optical rotations were measured by a JASCO DIP-360 digital polarimeter in $CHCl_3$ using a 10 cm cell tube. Mass spectra (EI, FD, and HR-EI-MS) were measured in electron impact mode by Finnigan MAT 12 or MAT 312 spectrometers and ions are given in *m/z* (%). TLC was performed with precoated silica gel G-25-UV$_{254}$ plates and detection was done by spraying with ceric sulphate in 10% $H_2SO_4$. Silica gel (E. Merck, 230—400 mesh) was used for column chromatography. Melting points were determined by Gallenkemp apparatus and are uncorrected.

**Plant Material** *S. nubicola* was collected from Urhat-Juniper forest, near Zearat (Quetta), Pakistan, in the month of July (1999) and identified by Dr. Rasool Baksh Tareen, Department 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 2 weeks then soaked in hexane (12 l×2) and methanol (12 l×2) for 10 d in each solvent. Solvents were evaporated through vacuum distillation. The condensed and crude methanolic extract (217 g) was then partitioned between water and ethyl acetate. The ethyl acetate layer was again condensed (167 g) and subjected to column chromatography using hexane; hexane–ethyl acetate; and ethyl acetate and ethyl acetate–methanol as mobile phase.

Fractions eluted with hexane–ethyl acetate, 19 : 1 yielded off-white powder **3** (8.30 mg).

Nubenolide Acetate (**3**): mp 128 °C. $[\alpha]_D^{28}$ +21.7 ° (*c*=0.733, $CHCl_3$). IR (KBr) cm$^{-1}$: 1756 (broad, ester, $\alpha,\beta$-unsaturated ketone and ester C=O), and 1618 (C=C). EI-MS *m/z*: 302 $[M]^+$, 260 $[M-\text{ketene}]^+$, 242 $[M-AcOH]^+$ and 227 $[M-AcOH+CH_3]^+$. FD-MS *m/z*: 302. HR-EI-MS *m/z*: 302.1159 (Calcd for $C_{17}H_{18}O_5$: 302.1154). $^1$H-NMR: see Table 1. $^{13}$C-NMR: see Table 2.

Fractions eluted with hexane–ethyl acetate, 17 : 3 yielded colorless needles, which on washing with methanol yielded **1** (9.13 mg).

Nubenolide (**1**): mp 110—112 °C. $[\alpha]_D^{28}$ +16.1° (*c*=0.613, $CHCl_3$). IR (KBr) cm$^{-1}$: 3440 (OH), 1750 (broad, $\alpha,\beta$-unsaturated ketone and ester C=O) and 1608 (C=C). EI-MS *m/z*: 260 $[M]^+$, 245 $[M-CH_3]$, 242 $[M-H_2O]$, and 227 $[M-H_2O+CH_3]$. FD-MS *m/z*: 260. HR-EI-MS *m/z*: 260.1044 (Calcd for $C_{15}H_{16}O_4$: 260.1048), 245.0808 (Calcd for $C_{14}H_{13}O_4$: 245.0813), 242.0938 (Calcd for $C_{15}H_{14}O_3$: 242.0942), and 227.0704 (Calcd for $C_{14}H_{11}O_3$: 227.0708). $^1$H-NMR: see Table 1. $^{13}$C-NMR: see Table 2. HMBC: see Fig. 1. X-ray: a colorless crystal with dimensions 0.16×0.15×0.13 mm was selected for the crystallographic measurements. $C_{15}H_{16}O_4$: *Mr* 260.28; monoclinic, *a*=6.468(3), *b*=16.952(9), *c*=12.070(7) Å, $\beta$=101.42(3)°, *V*=1297.2 (12) Å$^3$, space group=$P2_1$, *Z*=4, $D_x$=1.333 mg/m$^3$, *F*(000)=552, Mo$K\alpha$=0.71073 Å. Unit cell dimensions were determined by least-square fit of 352 reflections measured at 293(2) K using Mo$K\alpha$ radiations on Nanius Kappa CCD diffractometer. The intensity data within ($\theta$) range of 4.0 to 27.5° were collected at 293(2) K. A total of 5057 reflections were collected, of which 3052 reflections were monitored on the basis of $I>2\sigma(I)$. The structure was solved by the direct method and expanded using Fourier transformation technique. The structure was refined by full-matrix least-square calculation on $F^2$ with the aid of program SHELXL 97. The final *R* and *Rw* factors were measured as 0.040 and 0.079, respectively. The figure was plotted with the aid of ORTEP program. See Fig. 2. [The X-ray data are deposited with the X-ray Crystallographic Centre, Cambridge, U.K. (CCDC 176812).]

**Preparation and Purification of Mosher's Esters** *R*-(−)MTPA-Cl/*S*-(+)MTPA-Cl (10 $\mu$l) was treated with a stirred solution of **1** (0.01 mmol) in pyridine (200 $\mu$l) for 8 h. After removal of the solvent, the products (**1a**, **b**) were purified by preparative TLC ($CHCl_3$ : hexane 9.5 : 0.5) as gums. See $^1$H-NMR data in Table 1.

**Acetylation of 1** Compound **1** (0.01 mmol) was dissolved in pyridine (0.2 ml) and treated with acetic anhydride (0.2 ml) overnight. Then the reaction mixture was quenched with ice-cold water and the unreacted pyridine removed by adding saturated $CuSO_4$ solution. The organic mass was recovered in ethyl acetate and the organic layer was washed with water, condensed under reduced pressure, and chromatographed.

Fractions eluted with hexane–ethyl acetate, 4 : 1 yielded a white powder, which on washing with methanol yielded **2** (8.43 mg).

Bisnubenolide (**2**): mp 131 °C. $[\alpha]_D^{28}$ *ca.* 0° (*c*=0.666, $CHCl_3$). IR (KBr) cm$^{-1}$: 3435 (OH), 1735 (broad, $\alpha,\beta$-unsaturated ketone and ester C=O) and 1615 (C=C). FD-MS *m/z*: 518. HR-EI-MS *m/z*: 518.1945 (Calcd for $C_{30}H_{30}O_8$: 518.1940). $^1$H-NMR: Table 1 and $^{13}$C-NMR: Table 2.

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