

Structure and Absolute Stereochemistry of Salvimirzacolide, a New Sesterterpene from *Salvia mirzayanii*

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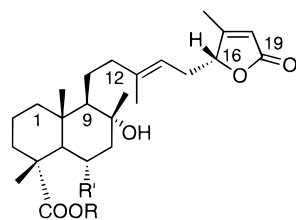
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A new sesterterpene, salvimirzacolide (**1**), was isolated from the aerial parts of *Salvia mirzayanii* and its structure established by X-ray diffraction analysis. Full assignments of ¹H- and ¹³C-NMR spectral data of salvimirzacolide are presented.

Plants belonging to genus *Salvia* (Lamiaceae) are pharmacologically active and are used in folk medicine all around the world. Several species of *Salvia* have been reported to exhibit antibacterial, estrogenic, anti-oxidant, and antitumor activities^{1–4} and are used in the treatment of eczema, psoriasis, and tuberculosis.^{5,6} As part of our continuing chemical and pharmacological investigations of the Iranian *Salvia* species, we have studied *S. mirzayanii* for its terpenic constituents. Only flavonoid constituents of this species have been reported in the literature.⁷ In this report, we describe the isolation, characterization, and the X-ray diffraction analysis of a new sesterterpenoid (**1**), along with eupatorin, a previously known flavone, which has not hitherto been reported from this plant.

Extraction of the aerial parts of *Salvia mirzayanii* followed by column chromatography yielded, from the medium polar fractions, a crystalline compound [**1** (0.024%)]. A HRFAB of **1** exhibited a peak at *m/z* 441.2615 [(M + Na)⁺], indicating the molecular ion at *m/z* 418 corresponding to the molecular formula C₂₅H₃₈O₅, which is supported by ¹³C-NMR (APT and DEPT displayed five methyl, eight methylene, five methine, and seven quaternary carbons) data. The IR spectrum displayed a hydroxyl group absorbance between 3500 and 2500 cm⁻¹, indicating the possible presence of a carboxylic group, along with two carbonyl absorptions at 1760 cm⁻¹ (γ -lactone) and 1715 cm⁻¹ (carboxylic acid).



1. R = R' = H
2. R = Me, R' = OH

Table 1. Positional and Equivalent Isotropic Thermal Parameters^a

atom	x	y	z	U _{eq}
O1	0.7797(1)	0.9422(1)	0.7435(3)	0.0431(7)
O2	0.8291(2)	0.8785(1)	0.9517(3)	0.068(1)
O3	0.6844(1)	0.7970(1)	1.0928(3)	0.0335(6)
O4	1.0178(1)	0.6736(1)	0.7951(3)	0.0407(7)
O5	1.0518(1)	0.5625(1)	0.8369(3)	0.0522(8)
C1	0.7327(2)	0.6536(2)	0.6014(4)	0.0313(9)
C2	0.7898(2)	0.5983(2)	0.5181(4)	0.038(1)
C3	0.8891(2)	0.6007(2)	0.5748(4)	0.0356(9)
C4	0.8971(2)	0.5906(1)	0.7586(3)	0.0264(8)
C5	0.8347(2)	0.6459(1)	0.8425(3)	0.0231(7)
C6	0.8432(2)	0.6448(2)	1.0268(3)	0.0311(9)
C7	0.8057(2)	0.7123(2)	1.0958(4)	0.0323(9)
C8	0.7061(2)	0.7257(1)	1.0477(3)	0.0270(8)
C9	0.6928(2)	0.7181(1)	0.8600(3)	0.0239(7)
C10	0.7331(2)	0.6488(1)	0.7879(3)	0.0247(7)
C11	0.5919(2)	0.7341(1)	0.8158(4)	0.0292(8)
C12	0.5729(2)	0.7717(1)	0.6547(4)	0.0326(9)
C13	0.6108(2)	0.8443(1)	0.6374(3)	0.0305(8)
C14	0.6701(2)	0.8588(1)	0.5203(4)	0.0311(8)
C15	0.7085(2)	0.9296(2)	0.4818(4)	0.038(1)
C16	0.7966(2)	0.9461(1)	0.5706(4)	0.038(1)
C17	0.8721(2)	0.8956(2)	0.5434(4)	0.045(1)
C18	0.8915(2)	0.8649(2)	0.6817(5)	0.054(1)
C19	0.8341(2)	0.8929(2)	0.8084(4)	0.048(1)
C20	0.9123(4)	0.8856(3)	0.3784(7)	0.081(2)
C21	0.5744(2)	0.8971(2)	0.7528(5)	0.044(1)
C22	0.6412(2)	0.6823(2)	1.1501(4)	0.038(1)
C23	0.9967(2)	0.6062(1)	0.8026(3)	0.0310(8)
C24	0.8789(2)	0.5149(1)	0.8069(4)	0.037(1)
C25	0.6770(2)	0.5839(1)	0.8372(4)	0.0343(9)

$$^a U_{eq} = (1/3)\epsilon_{ij}U_{ij}a^*i a^*j a^*k$$

The ¹H NMR showed five methyl singlets resonating at δ 0.8, 1.10, 1.13, 1.65, 2.03; two vinylic protons at δ 5.07 dd ($J = 7.5, 7$ Hz) and 5.85 dq ($J = 4.5, 6.5$ Hz); and a proton on an oxygen-bearing carbon (δ 4.90 dd, $J = 7.2, 6.5$ Hz). Comparison of the ¹H NMR of **1** with that of **2**, which was previously isolated from *Salvia sahendica*,^{8,9} indicated a great similarity between these two compounds. Only two signals (resonating at δ 3.61 and 3.64) in the ¹H NMR and one carbon [C-6] signal in the ¹³C NMR were displaced in compound **1**.⁹

The structure of **1** was established by single-crystal X-ray diffraction. The final atomic parameters of the nonhydrogen atoms are listed in Table 1. A perspective ORTEP drawing of a single molecule of **1** is given in Figure 1, which shows the atom numbering scheme. The absolute configuration is based on the X-ray structure

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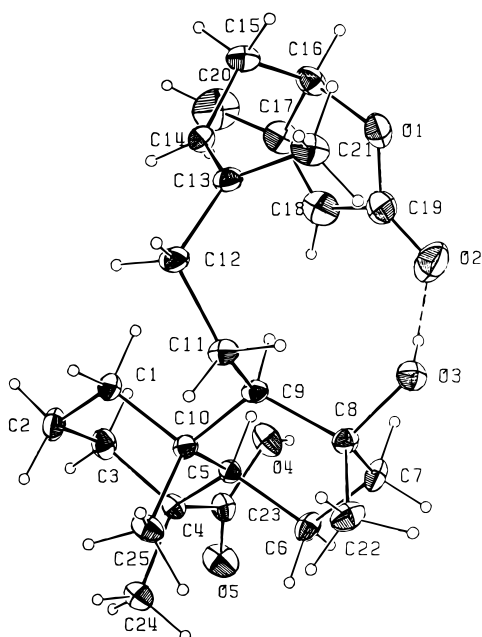


Figure 1. Perspective ORTEP drawing of **1** showing the atom numbering. Thermal ellipsoids are at the 35% level. The dashed line indicates a hydrogen bond.

of salvileucolide methyl ester reported earlier.¹⁰ The molecule possesses an intramolecular O–H~O hydrogen bond (O3–H~O2 = 2.882 Å), which links the hydroxyl group on C-8 with the carbonyl oxygen of the lactone ring, giving the overall molecule a globular geometry. In contrast, **2** is relatively flat and elongated. The major conformational difference between the two molecules occurs in the linear chain, which is folded in **1** and extended in **2**. The torsion angle about the bond, C9–C11–C12–C13, is -63.3° in structure **1** and 171.0° in compound **2**. The two six-membered rings are in a chair conformation, and the lactone ring is planar. In the crystal, molecules are linked through a strong intermolecular hydrogen bond, O4–H~O3 ($0.5 + x, 1.5 - y, 2 - z$) = 2.672 Å, to form unending chains.

This molecule (**1**) belongs to the normal cyclic sesterterpenoid series (10*R*), the same as salvileucolide, and has *R*-configuration at C-16. Extensive 2D NMR experiments (¹H–¹H COSY, ¹H–¹³C COSY, HMBC, HMQC) allowed the assignments of all carbons and protons. Table 2 shows ¹H- and ¹³C-NMR data of compound **1** along with the ¹³C NMR of **2**.

Experimental Section

General Experimental Procedures. ¹H-, ¹³C-, and 2D NMR experiments were performed on Bruker AMX-400 (CDCl₃), GE-300, and GE-600 (CDCl₃) instruments. HRMS was recorded at the Nebraska Mass Spectrometry Center, Department of Chemistry, University of Nebraska, Lincoln, Nebraska.

Plant Material. The aerial parts of *Salvia mirzayanii* Rech. and Esfandieri were collected from Malek and Adori villages near Kerman (central Iran) in April 1994, and identified by Dr. G. R. Amin, Faculty of Pharmacy, University of Tehran. A voucher specimen is deposited at the herbarium of the Faculty of Pharmacy, Tehran University.

Extraction and Isolation. The air-dried aerial parts (800 g) of *S. mirzayanii* were extracted with Me₂-

Table 2. ¹H- and ¹³C-NMR Spectral Data of **1** and **2** (δ value, CDCl₃)

position	1		2
	¹ H ^a	¹³ C	¹³ C
1	1.06 ddd, 1.68 m	38.3	39.0
2	1.55 m	17.3	15.5
3	1.62 m, 1.78 m	36.7	35.8
4		47.1	44.3
5	1.78 dd	50.0	55.8
6	1.34 m, 1.55 m	23.5	67.0
7	1.42 ddd, 1.82 m	43.7	53.7
8		74.2	73.3
9	1.10 m	60.9	60.1
10		38.3	38.1
11	1.28 m, 1.42 dq	23.1	23.1
12	2.05 m	42.9	42.6
13		140.9	140.9
14	5.07 dd	117.3	116.1
15	2.27 ddd, 2.64 ddd	30.2	30.2
16	4.90 dd	84.3	84.2
17		168.3	168.2
18	5.85 dq	116.0	117.3
19		173.3	173.3
20	2.03 s	13.9	13.9
21	1.65s	16.4	16.3
22	1.10 s	23.7	24.9
23		184.1	181.2
24	1.13 s	15.75	16.2
25	0.80 s	16.05	16.3

^a The *J* values are the same as reported by Rustaiyan et al.⁹ for salvileucolide.

CO at room temperature for 3 days. The extract was evaporated to dryness and the residue triturated with MeOH. The MeOH-soluble portion was fractionated by column chromatography (Kieselgel 60, 70–230 mesh) eluted with increasing polarity of petroleum ether–EtOAc. Further column chromatography (gel, CHCl₃–MeOH, 10:1) of the medium polar fractions afforded eupatorin (5 mg) and compound **1** (200 mg, 0.024%).

Salvimirzacolide (1): colorless needles (CH₂Cl₂–petroleum ether), *R_f* = 0.57 (EtOAc–petroleum ether 1:2), mp 155 °C; [α]_D +0.15 (CHCl₃, *c* 1.4); IR ν_{\max} 3500–2500 (OH), 2964, 2910, 1760 (γ -lactone), 1715 (C=O carboxylic acid), 1650 (C=C), 1465, 1375, 1303, 1259, 1187, 1089, 982, 928, 821, 750 cm⁻¹; ¹H and ¹³C NMR, see Table 2; EIMS *m/z* (%rel int) 400.7 [M⁺ – H₂O] (8.5), 355.8 (36.8), 339.5 (5.6), 303.8 (39.6), 257.3 (11.3), 234.6 (16.0), 189.1 (19.8), 179.2 (100%), 161.2 (24.5), 121.3 (16.9), 109.3 (33.9), 81.3, (26.4), 69.3 (7.5); HRFABMS *m/z* 441.26162, calcd for C₂₅H₃₈O₅Na 441.26167.

Eupatorin (3,5-dihydroxy-4,6,7-trimethoxy flavone): yellow-greenish crystals, mp 192.3 °C (195–196 °C [lit.¹⁰]), *R_f* UV/vis, IR, ¹H- and ¹³C-NMR and EIMS data are in full agreement with reported values.¹¹

X-Ray Diffraction. Compound **1** crystallized from a mixture of CH₂Cl₂–petroleum ether as colorless rods. A crystal of size 0.60 × 0.25 × 0.12 mm was used for all X-ray measurements. Cell dimensions were determined by a least-squares fit to $\pm 2\theta$ of 25 reflections ($11 < \theta < 25^\circ$) measured at -50°C using Cu K α_1 radiation.

Crystal Data: C₂₅H₃₈O₅, FW = 418.6, orthorhombic, *P*2₁2₁2₁, *a* = 14.659 (1), *b* = 19.164 (2), *c* = 8.267 (1) Å, *V* = 2322.4 (3) Å³, *Z* = 4, *D_x* = 1.197 g/cm³, μ (Cu K α) = 5.8 cm⁻¹, λ = 1.54178 Å.

Intensities of 2716 unique reflections within $0 < 2\theta < 150^\circ$ were collected on an Enraf–Nonius CAD-4 diffractometer fitted with a N₂ low-temperature device using Cu K α radiation. The ω – 2θ scan technique was

employed with a variable scan angle, $(0.90 + 0.20 \tan \theta)^\circ$ and a variable horizontal aperture $(3.0 + 0.86 \tan \theta)$ mm. Three intensity control monitors were measured every 7200 s of X-ray exposure time, and they showed a maximum variation of <2%. Intensities were corrected for Lorentz and polarization factors, but no absorption correction was made; 2201 reflections with intensities greater than $2\sigma(I)$ were considered observed, and these were used in the structure determination and refinement.

The structure was solved by direct methods using the program MITHRIL.¹² The structure was refined by a full-matrix least-squares routine using the program SHELX76.¹³ The quantity $\sum w(F_o - F_c)^2$ was minimized, where w is the weighting function, $1/\sigma^2(F_o)$. All the hydrogen atoms were located from difference Fourier maps, and hydrogen atom positions were refined isotropically. The refinement converged to $R = 0.039$, $R_w = 0.040$ for 2201 observations and 423 variables, $S = 1.2$, $(\Delta/\sigma)_{\max} = 0.01$, $\rho_{\max} = 0.20 \text{ e}/\text{\AA}^3$.

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