New adduct of abietane-type diterpene from *Salvia leriifolia* Benth.

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**ABSTRACT**
A new adduct of abietane-type diterpene, salvialeriicone (1), was isolated from *Salvia leriifolia* Benth., along with a new chemical entity nor-abietane diterpene, 2-isopropyl-8,8-dimethyl-7,8-dihydrophenanthrene-1,4,5(6\(\text{H}\))-trione (2). Their structures were determined using mass spectrometry, and 1D- and 2D-NMR spectroscopy.

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1. Introduction
The genus *Salvia* (Lamiaceae), distributed in the temperate and warmer regions of the world, comprises over 900 species (Clebsch 1997). Extracts, fractions and pure compounds of the genus *Salvia* display a variety of biological activities, such as antioxidant, antiplasmodial, antimicrobial, anti-inflammatory, cytotoxicity, hypoxia-inducible factor-1 inhibition activities, etc. (Lu & Yeap 2002; Dat et al. 2007; Topçu & Goren 2007; Kabouche & Kabouche 2008;
Salvia leriifolia Benth. (S. leriaefolia) is a tall annual plant found in the north-west of Iran (Hosseinzadeh & Lari 2000). The plant has been reported for diverse pharmacological properties, such as cytotoxicity, analgesic, antioxidant, hypoglycemic and antibacterial activities (Hosseinzadeh et al. 1998; Habibi et al. 2000; Hosseinzadeh et al. 2007). We have previously reported cytotoxic diterpenoids and a diterpene–norditerpene dimer from this plant (Choudhary et al. 2012, 2013). In the course of our search for new secondary metabolites, a new compound salvialeriicone (1), and a new chemical entity 2, have been reported.

2. Results and discussion

Compound 1 (Figure 1) was obtained as a yellow amorphous solid. The UV spectrum exhibited absorption at 340 nm, while the IR spectrum displayed absorptions for the hydroxyl functionality at 3462 cm⁻¹ and for carbonyl groups at 1698, 1651 and 1617 cm⁻¹. The molecular formula, C₄₀H₅₀O₆, was deduced from HRESI-MS, which showed a quasi-molecular ion at m/z 627.3629 [M + H]+ (Calcd for C₄₀H₅₀O₆+H = 627.3635). The ¹H-NMR spectrum of 1 showed four doublets for secondary methyls at δ 1.06 (J₁₆,₁₅ = 7.0 Hz, H₃-16), 1.04 (J₁₇,₁₅ = 7.0 Hz, H₃-17), 1.07 (J₁₆',₁₅' = 7.0 Hz, H₃-16') and 1.05 (J₁₇',₁₅ = 7.0 Hz, H₃-17'). The ¹H- and ¹³C-NMR chemical shift values (Table S1) of partial structure (Rings A–C) of this compound were found to be distinctly similar to structures of compounds 1 and 2.
those of taxodone, reported in the literature (Zaghloul et al. 2008). COSY cross-peak between signals at δ$_1$ 1.82 (H-5) and 5.02 (H-6), indicated that the C-6 is an oxygenated carbon.

The HMBC correlations between H$_3$-20 and C-5, C-9; H-6 and C-7, C-8, C-10; H$_3$-19 and C-3, C-4, C-5; H-14 and C-7, C-8, C-9, C-12 and C-15; H-15 and C-13, C-12, and H-11 (OH) and C-9, C-11, C-12 helped in the assignment of the NMR chemical shifts of one of the units (rings A, B and C) of compound 1. Similarly, the chemical shifts in rings A’, B’ and C’ were assigned on the basis of HMBC correlations between H$_3$-18’ and C-3’, C-4’, C-5’, H-5’ and C-4’, C-6’, C-10’, C-9’ and C-20’; H$_3$-20’ and C-1’, C-9’; H-15’ and C-12’ and C-14’; H$_3$-16’ and C-13’. The presence of isopropyl groups at C-13 and C-13’ were also inferred using the HMBC correlations of H-15/H-15’ with the carbonyl carbons, C-12 and C-12’. An ether linkage between C-6 and C-7’ was proposed to satisfy the remaining degree of unsaturation. The downfield chemical shift of C-6 (δ 76.9) also supported the presence of an ether linkage between C-6 and C-7’ of two units.

Stereochemistry of compound 1 was deduced on the basis of biogenesis and the NOESY correlations, biogenetically in Salvia diterpenes, H-5 is α-oriented and CH$_3$-20 is β-oriented. The NOESY correlation between axially oriented CH$_3$-19 with CH$_3$-20 indicated the β-orientation of CH$_3$-20. The NOESY correlation of the α-oriented H-5 (assigned on biogenetic ground) with H-6 revealed the β-orientation of ether linkage between C-6 and C-7’. The NOESY correlations of H-14 with H-15’ and H-17’ and α-oriented H$_3$-20’ with H-18’ were also observed.

Compound 2 (Figure 1) was obtained as a yellowish gum from the chloroform soluble fraction. The absorption band at 343 nm in the UV spectrum indicated the presence of extended conjugation in the compound. The IR spectrum showed the absorptions for conjugated carbonyl groups at 1671 and 1617 cm$^{-1}$. The chemical formula, C$_{19}$H$_{20}$O$_3$, was deduced from the HREI-MS spectrum, which showed the molecular ion [M+] at m/z 296.1381 (Calcd for C$_{19}$H$_{20}$O$_3$ = 296.1407).

The $^1$H-NMR spectrum of 2 showed two resonances for secondary methyl groups at δ 1.15 (d, $^3$J$_{16,15}$ = 6.5 Hz, H$_3$-16), and 1.16 (d, $^3$J$_{17,15}$ = 6.5 Hz, H$_3$-17), coupled with a methine proton at δ 3.16 (sep, $^3$J$_{15,16/15,17}$ = 6.5 Hz, H-15). This indicated an isopropyl moiety in the molecule. A sharp singlet of six-proton integration was observed for the two tertiary methyl groups at δ 1.33 (s, H-18 and H$_3$-19). The two ortho-coupled aromatic protons at δ 7.63 (d, $^3$J$_{6,7}$ = 8.0 Hz, H-6) and 8.12 (d, $^3$J$_{7,6}$ = 8.0 Hz, H-7) were also observed. The $^{13}$C-NMR spectrum showed resonances for 19 carbon atoms, comprising 9 quaternary, 4 methine, 2 methylene and 4 methyl carbons. Presence of 1, 4-quinone moiety was inferred from the carbon resonances at δ 183.9 (C-11) and 185.3 (C-14) and three quaternary carbons in conjugation with the carbonyl carbons at δ 133.3 (C-8), 132.4 (C-9) and 155.2 (C-13) (Gao et al. 2004). The two aromatic quaternary carbons were observed at δ 157.6 (C-5) and 134.9 (C-10), showing HMBC correlations with two aromatic methine carbons both resonated at δ 129.1 which indicated the presence of a tetra-substituted phenyl group (Ulubelen et al. 1997).

Two AB doublets for the aromatic methines at δ 7.63 (H-6) and 8.11 (H-7) ($^3$J$_{6,7}$ = 8.0 Hz) were found to be ortho-coupled with each other in the $^1$H-$^1$H COSY spectrum. The aromatic signal at δ 7.63 was assigned to H-6 on the basis of its HMBC correlations with C-4 (δ 35.4) and C-10 (δ 134.9). The HMBC correlations of the C-15 methine proton (δ 3.16) of the isopropyl group with C-14 (δ 185.3) on one side and with the C-12 (δ 133.8) on the other side, suggesting the position of the isopropyl group at C-13 of ring C. Based on the $^1$H-$^1$H COSY, HSQC, HMBC, $^1$H- and $^{13}$C-NMR spectra, the structure of the new compound was deduced as 2-isopropyl-8,8-dimethyl-7,8-dihydrophenanthrene-1,4,5(6H)-trione (2).
3. Experimental

3.1. General experimental conditions

JASCO DIP-360 digital polarimeter was used for the measurement of optical rotations. The infrared (IR) spectra were recorded on JASCO A-302 infrared spectrophotometer. For ultraviolet (UV) spectra, Thermo Evolution-300 spectrophotometer was used. 1D- and 2D-NMR spectra were recorded on a 500 MHz on Bruker Avance-500 nuclear magnetic resonance spectrometer. Electrospray ionisation mass spectra were recorded on LC-MS/MS Q STAR XL mass spectrometer (Applied Biosystems). Low-resolution EI was performed on Finnigan MAT 311 mass spectrometer. HREI-MS was recorded on the Finnigan MAT 95 XP mass spectrometer. Column chromatography was performed on silica gel (230–400 mesh, E. Merck), while TLC was carried out on pre-coated preparative silica gel plates, GF-254 (20 × 20 cm, 0.5 mm thick, E-Merck).

3.2. Plant material

The whole plant of *S. leriifolia* Benth. was collected from Sabzewar, Khorasan province at the north-west of Iran, and was identified by Professor Jamzadeh in the Botanical Garden of Tehran, Tehran, Iran. The voucher specimen (A.R. No. 112) was deposited to the Herbarium of the Department of Botany, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

3.3. Extraction and isolation

The completely air-dried material of the plant *S. leriifolia* Benth., weighing 8.8 kg, was ground into a fine powder and extracted with a mixture of 95% EtOH/H₂O at room temperature (3 × 4 days × 20 L). The reduced pressure was applied to the soluble part of the extract for complete evaporation of the solvent. This process resulted in a brownish material, weighing 469 g. The major water insoluble part of this gummy extract separated from the soluble part. The water soluble part of the extract (229 g) was fractionated with water and organic solvents, including hexanes, chloroform, ethyl acetate and *n*-butanol, to get corresponding 80 g of hexanes, 23.6 g of CHCl₃, 4.2 g of ethyl acetate, 12.3 g of *n*-butanol and 102.1 g of water fractions.

The CHCl₃-soluble fraction, weighing 23.6 g, was subjected to cc [silica gel, 1.0 kg, column (5 × 60 cm)] with CHCl₃/hexanes (19:1) to afford 6.1 g of fraction CA. The fraction CA was again subjected to cc [silica gel (150 g), column (2 × 40 cm), CHCl₃/hexanes (1:4)] to get fractions 20.3 mg of fraction CA1, 2.8 g of fraction CA2, and 3.0 g of fraction CA3.

The CA2 fraction was subdivided into three fractions, CA2A (400.5 mg), CA2B (1.0 g), and CA2C (900.7 mg) by cc [silica gel (150 g), column (3 × 40 cm), EtOAc/hexanes (1:19)]. The CA2A fraction was subjected to normal-phase cc [silica gel (50 g), column (2 × 30 cm), EtOAc/hexanes (1:19) and (1.7:18.3)] to purify compound 1 (5.5 mg), while fraction CA2C was further subjected to cc [silica gel (95 g), column (3 × 38 cm), CHCl₃/hexanes (1:4)] to afford compound 2 (6.3 mg).
3.3.1. *Salvialeriicone* (1)

Yellow amorphous solid. m.p.: 237–239 °C. $[\alpha]_D^{26} + 114$ (c 0.1, CHCl$_3$). UV (MeOH) $\lambda_{\text{max}}$ nm (log $\varepsilon$): 340 (3.7). IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3462 (OH), 1698 (C=O), 1651 (C=O), 1617 (C=O).

HRESI-MS $m/z$: 627.3629 [M + H]$^+$ (Calcd for C$_{40}$H$_{49}$O$_6$+H, 627.3635). ESI-MS $m/z$ (%): [M + H]$^+$ 627 (20), 586 (25), 440 (4), 415 (5), 338 (7), 282 (10), 210 (15), 149 (20), 137 (100).

$^1$H-NMR (CDCl$_3$, 500 MHz): 5.02 (d, $J = 2.7$ Hz, H-6), 7.0 (s, H-14), 2.53 (sep, $J = 7.0$ Hz, H-15), 1.06 (d, $J = 7.0$ Hz, H-16), 1.04 (d, $J = 7.0$ Hz, H-17), 1.23 (s, H-18), 1.27 (s, H-19), 1.43 (s, H-20), 2.70 (s, H-5'), 2.53 (sep, $J = 7.0$ Hz, H-15'), 1.07 (d, $J = 7.0$ Hz, H-16'), 1.05 (d, $J = 7.0$ Hz, H-17'), 1.06 (s, H-18'), 1.03 (s, H-19'), 1.32 (s, H-20').

$^{13}$C-NMR (CDCl$_3$, 125 MHz): 36.4 (C-1), 20.1 (C-2), 43.1 (C-3), 34.2 (C-4), 50.8 (C-5), 76.9 (C-6), 138.4 (C-7), 128.3 (C-8), 125.8 (C-9), 39.1 (C-10), 145.8 (C-11), 181.3 (C-12), 143.5 (C-13), 131.8 (C-14), 29.7 (C-15), 21.5 (C-16), 21.2 (C-17), 22.3 (C-18), 24.8 (C-19), 20.1 (C-20), 37.6 (C-1'), 19.6 (C-2'), 42.3 (C-3'), 32.8 (C-4'), 62.2 (C-5'), 193.4 (C-6'), 151.8 (C-7'), 116.8 (C-8'), 126.6 (C-9'), 41.1 (C-10'), 144.7 (C-11), 178.8 (C-12'), 136.1 (C-13'), 139.2 (C-14'), 29.8 (C-15'), 21.8 (C-16'), 21.9 (C-17'), 28.7 (C-18'), 33.3 (C-19'), 20.3 (C-20').

3.3.2. 2-Isopropyl-8,8-dimethyl-7,8-dihydrophenanthrene-1,4,5(6H)-trione (2)

Yellow amorphous solid. m.p.: 167–168 °C. $[\alpha]_D^{26} + 35$ (c 0.1, CHCl$_3$). UV (CHCl$_3$) $\lambda_{\text{max}}$ nm (log $\varepsilon$): 343 (3.1). IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 1671 (C=O), 1617 (C=O). HRESI-MS $m/z$: 296.1381 [M]$^+$ (Calcd for C$_{19}$H$_{20}$O$_3$, $\text{M}^+$ = 296.1407). EI-MS: $m/z$ (%): [M]$^+$ 296 (37), 281 (8), 268 (78), 240 (100).

$^1$H-NMR (CDCl$_3$, 500 MHz): 2.92 (t, $J = 7.0$ Hz, H-2), 2.06 (t, $J = 7.0$ Hz, H-3), 7.63 (d, $J = 8.0$ Hz, H-6), 8.12 (d, $J = 8.0$ Hz, H-7), 6.75 (s, H-12), 3.16 (sep, $J = 6.5$ Hz, H-15), 1.16 (d, $J = 6.5$ Hz, H-16), 1.16 (d, $J = 6.5$ Hz, H-17), 1.33 (s, H-18), 1.33 (s, H-19).

$^{13}$C-NMR (CDCl$_3$, 125 MHz): 199.7 (C-1), 36.6 (C-2), 36.4 (C-3), 35.4 (C-4), 157.6 (C-5), 129.1 (C-6), 129.1 (C-7), 133.3 (C-8), 132.4 (C-9), 134.9 (C-10), 183.9 (C-11), 133.8 (C-12), 155.2 (C-13), 185.3 (C-14), 26.8 (C-15), 21.4 (C-16), 21.4 (C-17), 28.7 (C-18), 28.7 (C-19).

4. Conclusion

The phytochemical investigation of *S. leriifolia* resulted in the isolation and structural elucidation of one new class of compound and one new natural product.

Supplementary material

All the mass and NMR spectra (with table) of compounds 1 and 2 are available online.

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Disclosure statement

The authors declare that there are no conflict of interest.
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