SUPPLEMENTARY MATERIAL

The roots of *Salvia rhytidea*: a rich source of biologically active diterpenoids

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Abstract

Sahandinone (**1**), 12-deoxysalvipisone (**2**), miltirone (**3**), 7 α -acetoxyrooleanone (**4**), and labda-7,14-dien-13-ol (**5**) were isolated from the roots of *Salvia rhytidea* Benth. (Lamiaceae). Their structures were elucidated by a combination of spectroscopic analyses including EIMS and NMR. The ^{13}C NMR spectroscopic data were revised for the quaternary carbons of both **1** and **3** with the help of HMBC spectra in respect to the spectral data previously reported in the literature. Compounds **1** and **3**, two very potent anticancer agents, were isolated in high yields from the roots of the plant. The biological activities of the plants' constituents were reported in the literature as antimicrobial, cytotoxic and antimalarial, are discussed in this article.

Keywords: *Salvia rhytidea*, Abietane diterpenoids, Labdane diterpenoid

1. Experimental

1.1. General experimental procedures

The NMR spectra were recorded on Bruker Avance spectrometers (Bruker Biospin, Karlsruhe, Germany), operating at resonance frequencies of 500.13 and 400.13 MHz for ^1H and 125.75 and 100.62 MHz for ^{13}C , respectively. Samples were measured at 25°C in CDCl_3 using TMS as an internal standard. Standard tubes (5mm i.d.) and capillary tubes (2 mm i.d) were used for all NMR measurements. Data acquisition as well as processing was accomplished using Bruker Topspin 2.1. Unless otherwise specified, chemical shifts (δ) are expressed with reference to the solvent signals. MS: *MicromassMasSpec* spectrometer. Open column- and flash column chromatography (CC) were performed using silica gel (Merck Chemical Company, Darmstadt) 70–230 and 230–400 mesh, respectively. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F₂₅₄ aluminum sheets and preparative handmade TLC was performed using Merck silica gel 60 G on glass sheets.

1.2. Plant material

The roots of *S. rhytidea* were collected from Taftan Mountain in the altitude of 2497 m in Baluchestan Province of Iran in autumn 2012. The plant material was taxonomically identified by Jafar Valizadeh, plant taxonomist at the University of Sistan and Baluchestan, Department of Biology. A voucher specimen (No. 4938) has been deposited in the Herbarium of the School of Biology (Dr. Akhani Herbarium), University of Tehran.

1.3. Extraction of plant material, isolation of the phytochemicals

The roots of *S. rhytidea* (2100 g) were shade-dried and extracted at room temperature with petroleum ether (bp. 40-60°C) for five days to yield a brownish crude extract (12.6 g) after removal of its solvent under reduced pressure. The extract was subjected to column chromatography over silica gel (70-230 mesh) using petroleum ether as eluting solvent,

increasing the polarity of the mobile phase with a mixture of dichloromethane: acetone (90:10) yielded nine different fractions. The fraction number 2 was subjected to the repeated CC experiment using petroleum ether: ethyl acetate (99:1). The polarity of the solvent system was increased by adding increasing ratios of ethyl acetate ratio (50:50) to the solvent mixture to yield eleven subfractions. Compounds **4** (100 mg) and **1** (1.0 g) were crystallized as yellow and red-orange crystals from seventh and second subfractions, respectively. Compound **2** (20 mg) was isolated as a mixture with **1** by subjecting the third subfraction to CC and gradually changing the eluent mixture from pure petroleum ether to pure dichloromethane. Compound **3** (750 mg) was crystallized from the third and fourth subfractions of the second open CC as red crystals. Compound **5** (10 mg) was obtained from the eighth subfraction of second open column fraction using repeated flash column chromatography eluting with petroleum ether: dichloromethane system (50:50) and subjecting the resulting fractions to preparative TLC eluting petroleum ether: acetone (95:5).

1.4. Spectroscopic data of the purified compounds

1.4.1. *Sahandinone (1)*: Red crystals; C₂₀H₂₄O₂, EIMS (*rel. int. %*): *m/z* 296 [M]⁺(60), 253 (10), 227 (100), 211 (13), 200 (44), 185 (31), 141 (20), 69 (35). For NMR data, see Table S1.

1.4.2. *12-Deoxysalvipisone (2)*: red gummy material as a mixture with **1** (1: 4, based on ¹H NMR integral ratio of peaks) ; C₂₀H₂₄O₂, EIMS (*rel. int. %*): *m/z* 296 [M]⁺ (64), 253 (11), 227 (100), 211 (13), 200 (48), 185 (33), 141 (18), 69 (32). ¹H NMR (500 MHz, CDCl₃) δ 7.36 (d, *J* = 7.6 Hz, H-6), 7.09 (s, H-12), 7.05 (d, *J* = 7.6 Hz, H-7), 4.73 (br s, H-18), 3.04 (m, H-1, H-15), 2.38 (s, Me-20), 2.26 (t, *J* = 7.5 Hz, H-3), 2.19 (m, H-2), 1.79 (s, Me-19), 1.18 (d, *J* = 6.9 Hz, Me-16, Me-17). ¹³C NMR (125 MHz, CDCl₃) δ 182.30 (C-11), 181.45 (C-14), 148.72 (C-10), 145.72 (C-4), 144.64 (C-13), 140.11 (C-5), 136.72 (C-6), 134.92 (C-9), 128.41 (C-8), 128.23 (C-7), 110.11 (C-18), 38.44 (C-3), 30.07 (C-1), 26.86 (C-15), 26.69 (C-2), 22.45 (C-19), 21.52 (C-15, C-16), 19.85 (C-20).

1.4.3. Miltirone (3): red crystals; C₁₉H₂₂O₂, EIMS (*rel. int. %*): *m/z* 282 [M]⁺ (3), 254 (35), 239 (100), 224 (19), 197 (10), 181 (8), 105 (16), 141 (6), 115 (7). For NMR data, see Table S1.

1.4.4. 7 α -Acetoxyroyleanone (4): Yellow crystals; C₂₂H₃₀O₅, EIMS (*rel. int. %*): *m/z* 374 [M]⁺ (4.5), 332 (100), 314 (98), 299 (50), 271 (18), 245 (30), 231 (19), 83 (15), 69 (20). ¹H NMR (400 MHz, CDCl₃) δ 7.14 (s, 1H, OH-12), 5.93 (dd, *J* = 3.9, 1.6 Hz, 1H, H-7 β), 3.15 (hept, *J* = 7.0 Hz, 1H, H-15), 2.72 (ddd, *J* = 13.0, 3.1, 3.1 Hz, 1H, H-1_{eq}), 2.02 (s, 3H, CH₃CO), 1.93 (d, *J* = 14.6 Hz, 1H, H-6), 1.74 (dddd, *J* = 13.7, 13.7, 13.7, 3.3, 3.3 Hz, 1H, H-2_{ax}), 1.61 (m, 1H, H-6) – 1.52 (m, 1H, H-2), 1.49 (m, 2H, H-5, H-3), 1.24 (m, 2H, H-1, H-3), 1.23 (s, 3H, Me-20), 1.22 (d, *J* = 7.2 Hz, 3H, Me-17), 1.18 (d, *J* = 7.0 Hz, 3H, Me-16), 0.88 (s, 3H, Me-18), 0.87 (s, 3H, Me-19). ¹³C NMR (100 MHz, CDCl₃) δ 185.4 (C-14), 183.7 (C-11), 169.4 (CH₃CO), 150.7 (C-12), 149.9 (C-9), 139.4 (C-8), 124.7 (C-13), 64.5 (C-7), 46.1 (C-5), 40.9 (C-3), 39.0 (C-10), 35.8 (C-1), 32.9 (C-18), 24.6 (C-6), 24.1 (C-15), 21.6 (C-19), 21.0 (CH₃CO), 19.8 (C-17), 19.6 (C-16), 18.8 (C-2), 18.5 (C-20).

1.4.5. Labda-7,14-dien-13-ol (5): Yellow gummy material; C₂₀H₃₄O, EIMS (*rel. int. %*): *m/z* 290 [M]⁺ (0.5), 257 (4), 204 (100), 189 (17), 161 (25), 109 (51), 81 (33), 55 (42). ¹H NMR (500 MHz, CDCl₃) δ 5.93 (dd, *J* = 17.4, 10.7 Hz, 1H, H-14), 5.37 (s, 1H, H-7), 5.21 (d, *J* = 17.4 Hz, 1H, H-15_{trans}), 5.07 (d, *J* = 10.7 Hz, 1H, H-15_{cis}), 1.95, 1.85 (m, H-6), 1.86, 0.93 (m, H-1), 1.81, 1.47 (m, H-12), 1.67 (s, 3H, Me-17), 1.57 (m, 1H, H-9), 1.52, 1.45 (m, H-2), 1.42, 1.18 (m, H-3), 1.29 (s, 3H, Me-16), 1.18 (m, H-5), 1.22, 1.18 (m, H-11), 0.87 (s, 3H, Me-19), 0.85 (s, 3H, Me-18), 0.75 (s, 3H, Me-20). ¹³C NMR (125 MHz, CDCl₃) δ 145.1 (C-14), 135.34 (C-8), 122.2 (C-7), 111.8 (C-15), 73.7 (C-13), 55.1 (C-9), 50.1 (C-5), 44.9 (C-12), 42.3 (C-3), 39.2 (C-1), 37.1 (C-10), 33.2 (C-18), 33.0 (C-4), 27.5 (C-16), 23.8 (C-6), 22.2 (C-19), 21.9 (C-17), 21.2 (C-11), 18.78 (C-2), 13.6 (C-20).

Table S1. ^1H and ^{13}C NMR data of compounds **1** and **3** in CDCl_3 at 25°C . Chemical shifts are given in δ values. Multiplicities and J coupling constants in Hz are given in parentheses.

No.	Compound 1 ^a			Compound 3 ^b		
	^1H NMR	^{13}C NMR	HMBC	^1H NMR	^{13}C NMR	HMBC
1	3.04 (m)	30.2	C-2, C-3, C-5, C-10, C-9	3.16 (t, 6.4)	29.9	C-2, C-3, C-5, C-10, C-9
2	2.19 (ddd, 7.5, 7.5, 7.5)	27.5	C-1, C-3, C-4, C-10	1.78 (m ^c)	19.0	C-1, C-3, C-4, C-10
3	5.31 (tq, 7.5, 1.5)	123.8	C-18, C-19, C-2	1.63 (t, 5.4 ^c)	37.8	C-1, C-2, C-4, C-5, C-10, C-18, C-19
4	-	132.3	-	-	34.5	-
5	-	140.2	-	-	149.6	-
6	7.36 (d, 7.6)	136.6	C-7, C-8, C-10, C-20	7.58 (d, 7.9)	133.8	C-4, C-8, C-10, C-7
7	7.05 (d, 7.6)	128.1	C-5, C-8, C-9, C-20, C-14	7.16 (d, 7.9)	127.9	C-5, C-14, C-8
8	-	134.9	-	-	134.4	-
9	-	128.5	-	-	128.1	-
10	-	148.1	-	-	144.5	-
11	-	182.5	-	-	182.4	-
12	7.09 (s)	140.3	C-9, C-11, C-13, C-15	-	181.4	-
13	-	144.6	-	-	144.9	-
14	-	181.6	-	7.07 (s)	139.9	C-7, C-8, C-13, C-12, C-15
15	3.02 (m)	26.9	C-13, C-16, C-17	3.00 (hept. 6.9)	26.9	C-13, C-14, C-16, C-17
16	1.18 (d, 6.9)	21.5 ^d	C-13, C-15, C-17	1.15 (d, 6.9)	21.5 ^d	C-13, C-15, C-17
17	1.18 (d, 6.9)	21.5 ^d	C-13, C-15, C-16	1.15 (d, 6.9)	21.5 ^d	C-13, C-15, C-16
18	1.71 (s)	25.7	C-3, C-4, C-19	1.28 (s)	31.8 ^d	C-3, C-4, C-5, C-19
19	1.69 (s)	17.6	C-3, C-4, C-18	1.28 (s)	31.8 ^d	C-3, C-4, C-5, C-18
20	2.39 (s)	19.9	C-5, C-6, C-10	-	-	-

a) The ^1H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively.

b) The ^1H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively.

c) The signal's multiplicity were distorted. d) The assignments may be interchanged.