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Salvidorol, a nor-abietane diterpene with a rare carbon skeleton and two abietane diterpene derivatives from *Salvia dorrii*

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Abstract

Salvidorol (**1**), a irregular nor-abietane-type diterpene, was isolated from the aerial parts of *Salvia dorrii*, in addition to two epimeric abietane diterpenes (**2** and **3**). This is the first report of a nor-diterpene with an irregular skeleton. The structures were established by high-field NMR techniques (^{1}H–^{1}H COSY, DEPT, HMQC, HMBC, NOESY and HRMS) and in case of **2** was confirmed by X-ray analysis.

Keywords: *Salvia dorrii*; Lamiaceae; Nor-abietane diterpene; Salvidorol; 7α- and 7β-Methoxyrosmanol

1. Introduction

The genus *Salvia*, a member of the family Lamiaceae, consists of about 500 species distributed throughout the world. Some species of this genus have held a place of importance from ancient times, due to their medicinal properties (Penso, 1980). They are rich in flavonoids (Barberan, 1986), monoterpenes (Emboden et al., 1967) and diterpenes with abietane and clerodane skeletons (Luis, 1991; Rodriguez-Hahn et al., 1992). Many diterpenes were reported from *Salvia* have shown antioxidant (Nakanati, 1994) and antibacterial activities (Sosa et al., 1994). Recently, several nitrogen containing compounds were isolated from *S. miltiorrhiza* and were examined for cytotoxic and antimicrobial properties (Ming-Jaw et al., 2005). The flavonoid constituent of *S. dorrii* has been studied before (Wollenweber et al., 1992). In this paper we describe from *S. dorrii* (Kellog) Abrams the isolation and structural elucidation of salvidorol (**1**), a novel carbon skeletal nor-abietane diterpene and two diterpenes type abietane.

2. Results and discussion

The methylene chloride extract of the air-dried aerial parts of *S. dorrii* was chromatographed on silica gel and Sephadex LH-20 columns to give a novel nor-diterpene (**1**), for which the name salvidorol was given, and two epimeric abietane diterpenes (**2** and **3**) (new). Compound **1**, yellowish oil, $[\alpha]_D^{20} + 1.53°$ (*c* 0.98, $CHCl_3$), its IR spectrum showed absorption bands at 3409 cm^{-1} (OH) and 1719 cm^{-1} (C=O). The low resolution EIMS showed a molecular ion peak $[M]^+$ at *m/z* 318 (100%), followed by a fragment at *m/z* 300 $[M - H_2O]^+$. The high resolution mass spectrum exhibited a molecular ion peak $[M]^+$ at *m/z* 318.1824 (calcd. 318.1817), in accord with the molecular formula of $C_{19}H_{26}O_4$. The structure of salvidorol (**1**) was determined from careful investigation of the 1D and 2D NMR measurements. The ^{1}H NMR spectrum revealed

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the presence of two singlet signals at δ_H 1.12 (3H, H-18) and 1.11 (3H, H-19), an isopropyl group at δ_H 1.25 (3H, H-16), 1.26 (3H, H-17) and 3.27 (1H, H-15), a broad singlet at δ_H 5.80 (H-6) and a formyl proton at δ 10.0 (*s*, H-7). The most characteristic and important signal being a one-proton signal at δ_H 3.45 (1H, *ddd*, $J = 12.0$, 12.0, 3.0 Hz), which correlated in ^1H–^1H COSY with three signals at δ_H 1.15 (H-1α), 2.30 (H-1β) and 1.68 (H-5α). Therefore, this proton was assigned for H-10 and suggested the absence of H-20, which supported the presence of a nor-diterpene skeleton. The ^{13}C NMR spectrum showed 19 carbon signals were classified by DEPT experiments as follows: four methyl carbon signals at δ_C 22.1 (C-16), 22.2 (C-17), 20.8 (C-18) and 30.1 (C-19), three methylene carbon signals at δ_C 36.8 (C-1), 22.8 (C-2) and 42.6 (C-3), four methine carbon signals at δ_C 52.6 (C-5), 91.9 (C-6), 28.5 (C-10) and 27.0 (C-15). The formyl carbon signal appeared at δ 191.1, while the protonated aromatic carbon signal appeared at δ_C 125.3 (C-14). The downfield shift of C-6 at δ_C 91.9 in the ^{13}C NMR spectrum suggested the existence of a hemiacetal moiety in the structure. Moreover, all proton and carbon signals were determined by ^1H–^1H COSY, HMQC and HMBC (Table 1). The HMBC spectrum (Fig. 1) was used to place the aldehydic group at C-8 on the basis of the correlation between the aromatic proton at δ_H 7.37 (H-14) with the aldehydic carbon signal at δ_C 191.9 (C-7). Other important correlations were observed, namely, H-5 with C-10, H-6 with C-10 and C-11, H-10 with C-8 and C-11, H-18 and H-19 with C-3

Table 1
NMR data of **1** (600 MHz, $CDCl_3$, δ-values)

Position	δ_C	δ_H	HBMC (H–C)
1α	36.8	1.15 *m*	
1β		2.30 *dd* (12.0, 3.0)	
2α	22.8	1.65 *m*	
2β		1.78 *dt* (13.8, 4.0)	
3α	42.6	1.46 *m*	
3β		1.30 *dd* (13.8, 4.0)	
4	32.8 *s*		
5α	52.6	1.68 *dd* (12.0, 1.2)	C-4, C-10
6	91.9	5.80 *br s*	C-10, C-11
7	191.1	10.0 *s*	
8	127.0		
9	126.8		
10	28.5	3.45 *ddd* (12.0, 12.0, 3)	C-8, C-11
11	137.8		
12	148.0		
13	132.0		
14	125.3	7.37 *s*	C-7, C-8, C-12
15	27.0	3.27 septet (7.2)	C-12, C-13, C-14
16	22.1	1.25 *d* (7.2)	C-13
17	22.2	1.26 *d* (7.2)	C-13
18	20.8	1.12 *s*	C-3, C-5
19	30.1	1.11 *s*	C-3, C-5

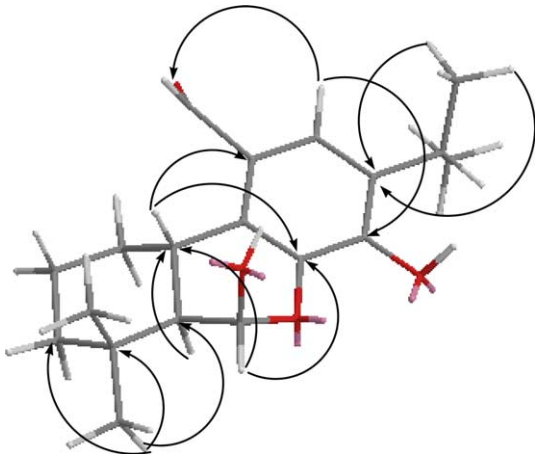
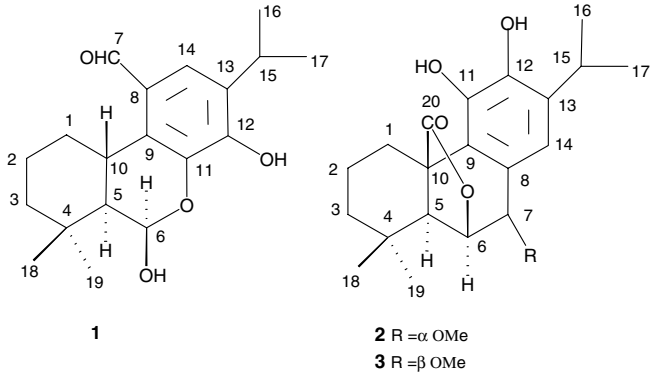


Fig. 1. Selective HMBC correlations of compound **1**.

and C-5 and H-15 with C-12, C-13 and C-14. The coupling constant between H-5 and H-6 was consistent with the β-configuration of hydroxyl group at C-6 (Gonzalez et al., 1989). Dreiding models demonstrated the angle between H-5 and H-6 was about 90, which was in agreement with the broad singlet observed for H-6. This stereochemistry was supported by a NOESY spectrum that exhibited effects between H-6 (δ 5.80) with H-1α (δ 1.15) and H-19 (δ 1.11), H-10 (δ 3.45) with H-1β (δ 2.30), H-2β (δ 1.78) and H-18 (δ 1.12). Also, it showed a clear effect between the formayl proton with H-10 (at δ 3.45) and H-1β (at δ 2.30). Although, few regular abietane diterpenes lacking the 20-methyl group were reported from the genus *Salvia* (Lee et al., 1987), this is the first irregular abietane diterpene which lacking the 20-methyl group.



1 **2** R =α OMe
3 R =β OMe

Compound **2** was isolated as colorless crystal. Its ^1H NMR spectrum showed an isopropyl moiety as one-proton septet at δ 3.07 ($J = 7$ Hz) and two geminal methyls doublets at δ 1.21 and 1.22 ($J = 7$ Hz). A singlet signal at δ 6.79 was assigned to an aromatic proton. Moreover, it revealed two doublets at δ 4.26 and 4.71 ($J = 3.0$ Hz) assigned for H-7 and H-6, respectively, while H-5 appeared as singlet signal at δ 2.24. Also, its ^1H NMR spectrum showed a sharp three-proton singlet at δ 3.66 in accord with **2** being a methoxylated derivative of rosmanol (Ahmed et al., 1995). The ^{13}C NMR and the multiplicities

of the individual signals were determined using DEPT as follows: five methyls (one oxygenated, δ 58.14), three methylenes, five methines (one aromatic, δ 120.81 and two oxygenated, δ 74.99 and 77.61) and eight quaternary carbons (one carbonyl and five aromatics). The relative stereochemistry of **2** could be deduced from NOESY experiment, where H-5, H-6, OMe and H-19 correlated with each other, indicating the α-orientation of these protons. Also, it showed correlations between the aromatic proton with H-7 and H-15. Additionally, the stereochemistry of **2** was confirmed by X-ray analysis (Fig. 2). Therefore, compound **2** was established to be 7α-methoxyrosmanol. Although, the NMR spectral data of **2** were identical with the previously reported data for 7α-methoxyrosmanol (Takenaka et al., 1997), compound **2** showed opposite optical rotation sign $[\alpha]_D^{22} + 6^\circ$ ($c = 0.35$, $CHCl_3$), while the previously reported optical rotation was $[\alpha]_D^{22} - 24.5^\circ$ ($c = 0.42$, $CHCl_3$), (Takenaka et al., 1997). Therefore, compound **2** could be enantiomer of the previously reported compound.

Compound **3** was isolated as yellow oil, its CIMS exhibited a molecular ion peak $[M + H]^+$ at *m/z* 361 and exact mass at *m/z* 361.20119 (calcd. 361.20150), established the elemental composition as $C_{21}H_{30}O_5$. Its IR spectrum showed absorption bands indicative of a γ-lactone group (1754 cm^{-1}) and aromatic hydroxyl groups (3360 cm^{-1}). The ^1H NMR and ^{13}C NMR spectral data of **3** were very similar to those of **2**, except the optical rotation sign which was opposite, $[\alpha]_D^{22} - 52$ ($c = 1.2$, $CHCl_3$), suggesting that **3** was an epimer of **2**. Comparison of the ^1H and ^{13}C NMR spectra of **3** with those of **2** showed some differences. The signals of H-6 (δ 4.92) and H-7 (δ 4.40) of **3** were detected at downfield shift ($\Delta\delta + 0.21$ and $\Delta\delta + 0.14$, respectively) in comparison with those of **2**. Moreover, the carbon signal at position 5 (δ_C 55.39) was shifted downfield. The positions of the methoxyl group, isopropyl group and lactone moiety were determined by HMBC spectrum. In this spectrum, H-C connectivity between the aromatic proton and C-7 (δ_C 78.2), C-9 (δ_C 123.5), C-11 (δ_C 142.5) and C-15 (δ_C 27.2); between the methoxyl and C-7 (δ_C 78.2), supported the location of the methoxyl group at C-7 and the isopropyl at C-12. Moreover, it displayed correlations between H-5 and C-7 (δ_C 78.2), C-9 (δ_C 123.5), C-10 (δ_C 47.9), C-18 (δ_C 21.9), C-19 (δ_C 31.8) and C-20 (δ_C 178.9); between H-6 and C-8 (δ_C 126.5) and C-20 (δ_C 178.9); between H-16, H-17 and C-15 (δ_C 27.2); between H-18, H-19 and C-3 (δ_C 37.9) and C-4 (δ_C 31.6). A NOESY experiment of **3** showed a cross-peak between H-5 and H-6 with H-7, indicated the α-orientation of H-7. Therefore, compound **3** was assigned to be 7β-methoxyrosmanol, a new epimer of **2**.

3. Experimental

3.1. General

NMR spectra were measured with a Bruker AMX-400 spectrometer and Varian Unity 600 MHz NMR spectrometry, with TMS as an internal standard. The IR spectra [oily film, $CHCl_3$] were taken on Perkin–Elmer FT-IR spectrometer. Optical rotations were measured with a Perkin–Elmer 241 Polarimeter operating at sodium D line. MS were recorded on a JEOL SX102A mass spectrometer (70 eV).

3.2. Plant material

Salvia dorrii (Kellog) Abrams (Lamiaceae) was collected in the flowering stage near Mitchell, Oregon (voucher # 195789 Oregon State University Herbarium).

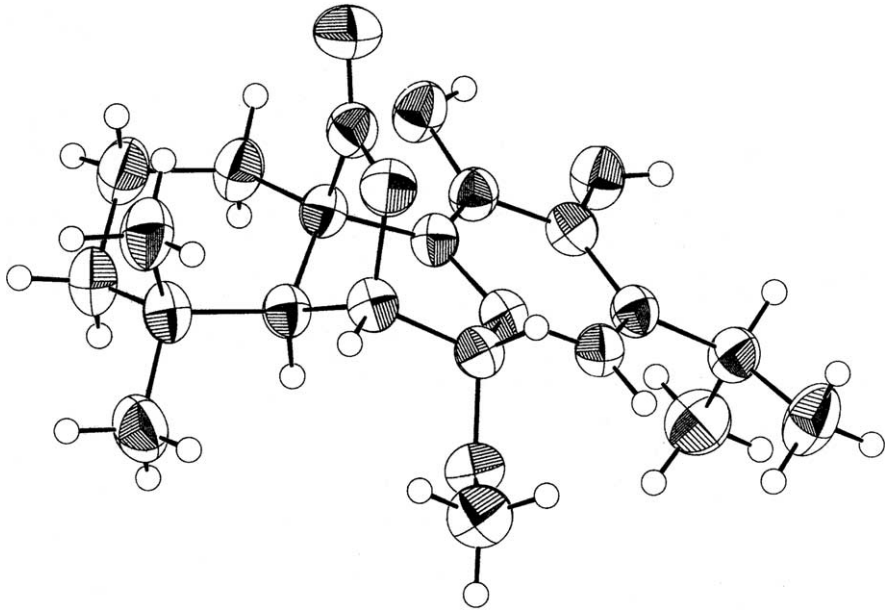


Fig. 2. ORTEP diagram of the crystal structure of **2**.

3.3. Extraction and isolation

The dichloromethane extract (20 mg) of the aerial parts (950 g) of *S. dorrii* was fractionated by flash column chromatography (5 × 55 cm) over silica gel (1 kg) eluting with *n*-hexane with an increasing amount of CH_2Cl_2. The fraction (100%, *n*-hexane 1 L) contained hydrocarbons and waxes. The second fraction (*n*-hexane–CH_2Cl_2 3:1, 2 L) gave a crude material which was purified by a Sephadex LH-20 (3 × 35 cm, *n*-hexane–CH_2Cl_2–MeOH 7:4:0.5, 300 mL) to give compounds **2** (14 mg), **3** (3 mg). The third fraction (CH_2Cl_2, 100%) was further purified by a Sephadex LH-20 (3 × 35 cm, *n*-hexane–CH_2Cl_2–MeOH 7:4:1, 500 mL) to afford compound **1** (2.5 mg).

3.3.1. Salvidorol (*1*)

Yellowish oil; $[\alpha]_D^{20} + 1.53°$ (*c* 0.98, $CHCl_3$); IR (ν_{max}^{KBr} cm^{-1}): 3409, 3019, 2927, 1719, 1680, 1606, 1571, 1436; EIMS $[M]^+ m/z$ 318 (100), $[M - H_2O]$ *m/z* 300 (30), 285 (40), 257 (60), 231 (30), 205 (40), 181(15); HREIMS *m/z* 318.1824 (calc. for $C_{19}H_{26}O_4$, 318.1817). ^1H and ^{13}C NMR (see Table 1).

3.3.2. 7α-Methoxyrosmanol (*2*)

Colorless crystal; $[\alpha]_D^{22} + 6°$ ($c = 0.35$, $CHCl_3$); IR (ν_{max}^{KBr} cm^{-1}): 3590, 2960, 1730, 1200; EIMS $[M]^+ m/z$ 360 (30), 316 (10), 285 (215); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.16$ (1H, br. *d*, $J = 14$ Hz, H-1β), 2.00 (1H, *m*, H-1α), 1.55 (1H, *m*, H-2β), 1.68 (1H, *m*, H-2α), 1.19 (1H, *m*, H-3β), 1.46 (1H, br. *d*, $J = 14$ Hz, H-3α), 2.24 (1H, *s*, H-5), 4.71 (1H, *d*, $J = 3.0$ Hz, H-6α), 4.26 (1H, *d*, $J = 3.0$ Hz, H-7β), 6.79 (1H, *s*, H-14), 3.07 (1H, septet, $J = 7$ Hz, H-15), 1.21 (3H, *d*, $J = 7$ Hz, H-16), 1.22 (3H, *d*, $J = 7$ Hz, H-17), 0.93 (3H, *s*, H-18), 1.01 (3H, *s*, H-19), 3.66 (3H, *s*, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 27.0$ (*t*, C-1), 19.0 (*t*, C-2), 38.0 (*t*, C-3), 31.3 (*s*, C-4), 51.1 (*d*, C-5), 75.0 (*d*, C-6), 77.6 (*d*, C-7), 125.9 (*s*, C-8), 124.8 (*s*, C-9), 47.2 (*s*, C-10), 143.5 (*s*, C-11), 142.0 (*s*, C-12), 135.6 (*s*, C-13), 120.8 (*d*, C-14), 27.1 (*d*, C-15), 22.2 (*q*, C-16), 22.5 (*q*, C-17), 22.0 (*q*, C-18), 31.5 (*q*, C-19), 179.9 (*s*, C-20), 58.2 (*q*, OMe).

3.3.3. 7β-Methoxyrosmanol (*3*)

Yellow material; $[\alpha]_D^{22} - 52°$ ($c = 1.2$, $CHCl_3$); IR (ν_{max}^{KBr} cm^{-1}): 3360, 2957, 1754, 1682, 1556, 1454; CIMS $[M + H]^+ m/z$ 361 (100), 329 (60), 315 (8), 301 (12), 183 (8); HRCIMS *m/z* 361.20119 (calc. for $C_{21}H_{28}O_5$, 361.20150). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.18$ (1H, br. *d*, $J = 14$ Hz, H-1β), 1.91 (1H, *m*, H-1α), 1.51 (1H, *m*, H-2β), 1.61 (1H, *m*, H-2α), 1.18 (1H, *m*, H-3β), 1.42 (1H, br *d*, $J = 14$ Hz, H-3α), 1.90 (1H, *s*, H-5α), 4.92 (1H, *d*, $J = 3.0$ Hz, H-6α), 4.40 (1H, *d*, $J = 3.0$ Hz, H-7α), 6.77 (1H, *s*, H-14), 3.00 (1H, septet, $J = 7$ Hz, H-15), 1.01 (3H, *d*, $J = 7$ Hz, H-16), 1.12 (3H, *d*, $J = 7$ Hz, H-17), 0.92 (3H, *s*, H-18), 0.97 (3H, *s*, H-19), 3.57 (3H, *s*, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.1 (*t*, C-1), 18.9 (*t*, C-2), 37.9 (*t*, C-3), 31.6 (*s*, C-4), 55.4 (*d*, C-5), 74.7 (*d*, C-6), 78.2 (*d*, C-7), 123.5 (*s*, C-8), 126.5 (*s*, C-9), 47.9 (*s*, C-10), 142.5 (*s*, C-11), 142.1 (*s*, C-12), 135.5 (*s*, C-13), 118.9 (*d*, C-14), 27.2 (*d*, C-15), 22.1 (*q*, C-16), 22.7 (*q*, C-17), 21.9 (*q*, C-18), 31.8 (*q*, C-19), 178.9 (*s*, C-20), 56.0 (*q*, OMe).

3.3.4. X-ray crystallography of compound *2*

Crystal data: $C_{21}H_{28}O_5$, formula wt. 362.466, orthorhombic, space group $P2_12_12_1$, $a = 8.7490$ (3) Å, $b = 12.5470$ (5) Å, $c = 17.1930$ (9) Å, $V = 1887.34$ (14) $Å^3$, $Z = 4$, $D_c = 1.276$ Mg m^{-3}. All diagrams and calculations were performed using maXus (Brucker Nonius, Delft & Mac Science, Japan), using graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). The structures were refined by full-matrix least-squares on F^2 using Brucker SHELEXL-97 (Sheldrick, 1997). The final *R* and R_w were 0.0504 and 0.1236, respectively. Crystallographic data for the structural analysis have been deposited with the Cambridge crystallographic data center. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving html (or from the CCDC, 250572 union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; e-mail: deposit@ccdc.camac.uk).

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