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TERPENOIDS AND FLAVONOIDS FROM THE AERIAL PARTS OF *SALVIA CANDIDISSIMA*

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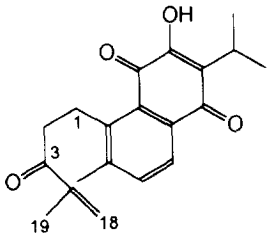
Key Word Index—*Salvia candidissima*; Labiatae; diterpenes; 3-oxosalvipisone; sesquiterpene; 11β-hydroxymanoyl oxide; 8,13-diepimanoyl oxide; flavonoids.

Abstract—From the aerial parts of *Salvia candidissima*, a new diterpene, 3-oxosalvipisone, was obtained together with 11β-hydroxymanoyl oxide, 8,13-diepimanoyl oxide, spathulenol, salvigenin, crysoeriol, diosmetin and *o,p*-dimethoxybenzoic acid. The structure of the new compound, 3-oxosalvipisone was established by spectroscopic means, and the structure of 11β-hydroxymanoyl oxide was reinvestigated by extensive 1D and 2D NMR studies and confirmed by X-ray analysis.

INTRODUCTION

In the present study with the aerial parts of *Salvia candidissima* Vahl. *occidentalis* Hedge we have isolated a rearranged abietane diterpene 3-oxosalvipisone (**1**) along with the known labdanes 11β-hydroxymanoyl oxide (**2**) [1], 8,13-diepimanoyl oxide [2], a sesquiterpene spathulenol [3] and the flavonoids salvigenin, crysoeriol, diosmetin and an aromatic compound, *o,p*-dimethoxybenzoic acid. In the previous studies with the roots of the same plant, we have isolated a group of abietane, rearranged abietane and pimarane diterpenes including manoyl oxide, salvipisone and 1-oxosalvipisone [4, 5].



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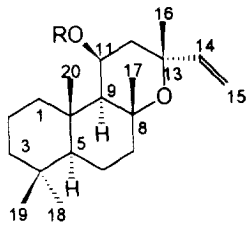


2 R=H
2a R=Ac

RESULTS AND DISCUSSION

The IR spectrum of the new diterpene **1** showed *p*-quinone (1665, 1645, 1590, 1560 cm^{-1}) and a conjugated ketone (1710 cm^{-1}) signals. The ^1H NMR spectrum (Table 1) showed an isopropyl group at δ 1.29 (6H, *d*, $J = 7.0$ Hz) (H-16 and H-17) and δ 3.40 (1H, *septet*, $J = 7.0$ Hz, H-15) which is characteristic for the side chain of abietane diterpenes [6]. In addition, an aromatic methyl signal at δ 2.33 (3H, *s*, H-20), an isopropylene methyl at δ 1.79 (3H, *br s*, H-19) along with exomethylene resonances at δ 4.77 and 4.72 (each 1H, *br s*, H-18a and H-18b) and aromatic proton signals at δ 8.06 and 7.59 (each 1H, *d*, $J = 8$ Hz) (H-7 and H-6, respectively) were observed indicating a rearranged abietane structure [7, 8]. The similarity between the ^1H NMR data of **1** and those of salvipisone and 1-oxosalvipisone was evident; however, there were small shift differences, particularly for H-7, H_2-18 and Me-20 [4, 7]. In the ^1H NMR spectrum of 1-oxosalvipisone, H-7 and δ 7.98 (*d*, $J = 8$ Hz), both exo-methylene signals at δ 4.82 (*br s*) and Me-20 at δ 2.33 were observed, along with some small shift differences for the aliphatic methylene signals. Compound **1** should bear one more oxo group than salvipisone as followed from its mass spectrum (HR-MS, $[M]^+$, *m/z* 326.1522), however, its spectral data was not identical with that of 1-oxosalvipisone, as also shown by TLC. Therefore, this oxo group could be placed either at C-2 or at C-3. In the case of 2-oxosalvipisone, in the ^1H NMR spectrum of **1**, isolated methylene protons for C-1 and C-3 should be observed. However, in the present case, two vicinal methylene signals were observed at δ 2.59–3.06 (see Experimental), therefore the location of the oxo group must be at C-3. Thus, the structure of **1** is 3-oxosalvipisone.

Table 1. ^1H–^1H correlations of compound **2** by DQF-COSY (double quantum filtered phase sensitive COSY) and TOCSY (total COSY)* (in $CDCl_3$, 500 MHz)

H	ppm	Correlated protons	
		DQF-COSY	TOCSY
1α	0.95 *ddd* (3.8, 12.8, 14.5)	H-1β	H-9,
1β	1.74 *dt* (3.2, 12)	H-1α	H-9, H-3α, H-3β, H-20
2α	1.38 *m*	H-1α, H-1β, H-3α, H-3β	
2β	1.64 *tt* (3.6, 13.8, 14.5)	H-1α, H-1β, H-3α, H-3β	
3α	1.07 *ddd* (4.1, 13.5, 14)	H-2α, H-2β	H-1β, H-1α
3β	1.34 *m*	H-2α, H-2β	H-1α, H-1β
5α	0.81 *dd* (2.6, 12.0)	H-6α, H-6β	H-1β, H-7α
6α	1.31 *m*	H-5, H-7α, H-7β,	
6β	1.58 *m*	H-5, H-7α, H-7β	
7α	1.41 *m*	H-6β, H-6α	H-5
7β	1.73 *dd* (2.5, 12.0)	H-6α, H-6β	H-5
9α	1.26 *d* (3.8)	H-11	H-5, H-12α, H-12β, H-20
11α	4.38 *ddd* (3.8, 5.0, 9.0)	H-12α, H-12β, H-9	
12α	1.79 *dd* (5.4, 14.2)	H-12β, H-11	H-9
12β	1.98 *dd* (6.0, 14.2)	H-12α, H-11	H-9
14	5.79 *dd* (10.0, 17.0)	H-15a, H-15b	
15a	5.07 *dd* (1.0, 17.0)	H-15b, H-14	
15b	4.86 *dd* (1.0, 10.0)	H-15a, H-14	
16	1.35 *s*		
17	1.53 *s*		H-20
18	0.79 *s*		H-3β
19	0.76 *s*		
20	1.11 *s*		H-1β

*Chemical shifts are reported in ppm relative to TMS and *J* values (Hz) in parentheses. Cross-peaks in TOCSY experiments are only given for correlations which were not observable in COSY experiments.

Table 2. HMQC (inverse phase HETCOR) and HMBC (inverse phase COLOC) correlations of compound **2** (in $CDCl_3$, 125.4 MHz)

C	APT	Direct correlated protons	Long range correlated protons
1	39.22	H-1α, H-1β	H-20
2	18.42	H-2α, H-2β	H-1α
3	41.92	H-3α, H-3β	H-1α, H-18
4	33.19	—	H-2α, H-2β, H-19
5	57.03	H-5α	H-1α, H-18, H-19, H-20
6	20.14	H-6α, H-6β	H-5, H-7α, H-7β
7	44.56	H-7α, H-7β	H-9, H-17
8	74.80	—	H-9, H-17
9	56.45	H-9α	H-1α, H-7α, H-12α, H-12β, H-17, H-20
10	37.76	—	H-9, H-20
11	65.24	H-11α	H-12α, H-12β
12	44.20	H-12α, H-12β	H-15b, H-16
13	72.38	—	H-12α, H-12β, H-14, H-15a, H-15b, H-16
14	147.77	H-14	H-12α, H-12β, H-15a, H-16
15	110.47	H-15a, H-15b	—
16	29.73	H-16	H-12α, H-12β
17	27.44	H-17	H-7, H-9
18	33.50	H-18	H-3α
19	21.38	H-19	H-3α, H-5, H-18
20	17.12	H-20	H-1α, H-5, H-9

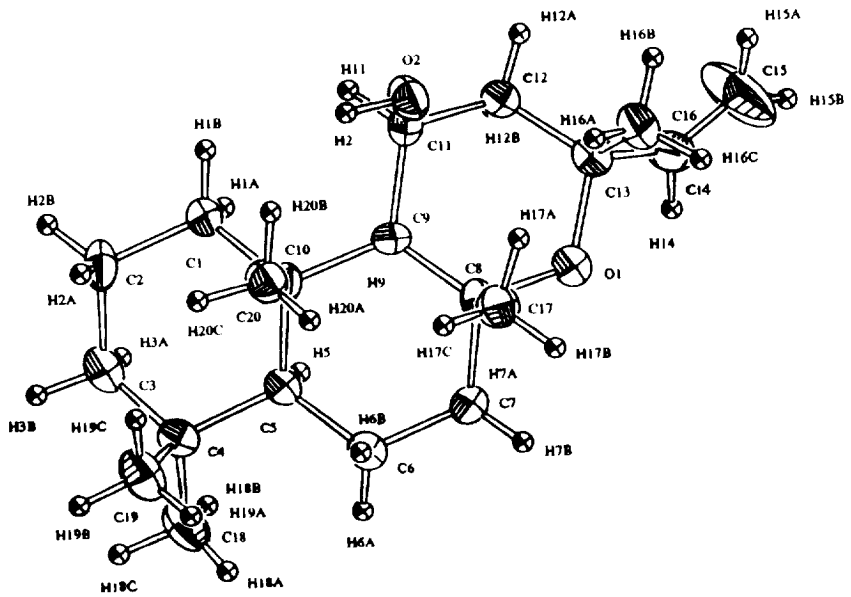
Since the spectral data of **2** given in the literature [1] were not sufficient, we have studied the structure by extensive ^{1}H and ^{13}C NMR data (Tables 1 and 2). From the ^{1}H and ^{13}C NMR spectra as well as from the acetylation of **1** (see Experimental) there should be one secondary hydroxyl group in the molecule. Based on the multiplicity and the *J* values of the carbinol methine at δ 4.38, the hydroxyl group which is between methine and methylene groups could be placed either at C-6 or C-11. The relationship between the carbinol methine and the double doublets at δ 1.98 (J = 6 and 14.2 Hz) and δ 1.79 (J = 5.4 and 14.2 Hz) was seen clearly in the COSY experiments. The DQF-COSY and particularly the TOCSY experiments (Table 1) showed an interaction between the carbinol methine (δ 4.38) and a proton at δ 1.26 (*d*, J = 3.8 Hz), with the latter signal attributed to either H-5 or H-9 depending upon the placement of the hydroxyl group at C-6 or C-11, respectively. Because of *J* values of this methine signal, a hydroxyl group should be more likely in β-position in one of these two locations. In fact, the ^{1}H NMR spectrum of 11α-hydroxymanoyl oxide isolated from *Kyllinga erecta* showed the 11-β proton at 3.94 with a coupling constant of J = 13.4 Hz unlike that of **2** [9]. Owing to the great similarity between the measured *J* values on Dreiding models of 11β-hydroxymanoyl oxide and 6β-hydroxymanoyl oxide, the following experiments were performed in order to decide between these two positions: DQF-COSY, TOCSY, HMQC, and HMBC.

If the hydroxyl group was at C-6, the ^{13}C NMR signal for C-7 would be observed around δ 50–53; however, if the hydroxyl was at C-11, the signal for C-12 would be around δ 40–45 which was observed in the present case. The HMBC expriments (Table 2) supported this finding showing long range correlations between C-9 at δ 56.45 and Me-20, Me-17, H_2-12, H-7α and H-1α, and between C-5 at δ 57.03 and Me-18, Me-19, Me-20 and H-1α. The assignment of all carbons and protons followed from HMQC and HMBC experiments (Table 2). As observed in the ^{13}C NMR (APT) spectrum there were three oxygenated C atoms, one was assigned to C-11 (δ 65.2) carrying the secondary hydroxyl group while the other two at δ 72.4 and 74.8 were quaternary C atoms, C-13 and C-8. Observation of the differentiation between these two carbons was possible by HMBC experiments, which showed long range correlations between the signal at δ 72.4 and H_2-12, H-14, H_2-15 and Me-16 while the signal at δ 74.8 showed a correlation with Me-17 and H-9 indicating that the former signal was C-13 and the latter was C-8.

The stereochemistry of the hydroxyl group at C-11 was established as β, based on *J* couplings of H-11 as well as NOESY experiments. NOESY experiments showed good NOEs between H-11α and the following protons: H-9α, H-1α, H-12α and H-12β. However, this experiment did not unambiguously define the stereochemistry for the methyl groups. In order to establish the absolute structure, an X-ray analysis was performed, thus, the structure of **2** was determined as 11β-hydroxymanoyl oxide (Fig. 1).

EXPERIMENTAL

General. Mp: Buchi 510; IR: Perkin Elmer 983 in $CHCl_3$. The spectra of **2** were run on a Bruker ARX 500 MHz for ^{1}H NMR and 125.694 MHz for ^{13}C NMR in $CDCl_3$ and the spectra of other compounds on a Bruker AC 200 L. HRMS: VG Zabspec. X-Ray crystallographic data were recorded on a Rigaku AFC6S diffractometer. Kieselgel 60F_{254} (E. Merck) plates were



used for prep. separation, Sephadex LH-20 (Fluka) for further purification.

Plant material. The aerial parts of *Salvia candidissima* were collected from Isparta (south western Turkey) in June 1992 and identified by Dr K. Alpinar (Istanbul), a voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, ISTE 63076.

Extraction and fractionation. The powdered plant (980 g) was extracted in a Soxhlet with Me_2CO and 35 g of a crude extract was obtained. The extract was fractionated in a silica gel column, and the compounds obtained in the following order: 11β-hydroxymanoyl oxide (**2**, 65.0 mg), 8,13-diepoxymanoyl oxide (100.0 mg), 3-oxo-salvipisone (**1**, 7.0 mg), 2,4-dimethoxybenzoic acid (95.5 mg), salvigenin (3.0 g), crysoeriol (1.0 g) and diosmetin (50.2 mg).

3-Oxosalvipisone (**1**). Amorphous compound, $UV\lambda_{max}^{MeOH}$ nm(log ε): 440 (3.5), 350 (3.1), 280 (3.3), 272 (3.1), 207 (4.2). $IR\nu_{max}^{CHCl_3}$ cm^{-1}: 3390, 1710, 1665, 1645, 1590, 1560, 1460, 1380, 1255, 1160. ^1H NMR (200 MHz, in $CDCl_3$): δ 8.06 (1H, *d*, $J = 8.0$ Hz, H-7), 7.59 (1H, *d*, $J = 8.0$ Hz, H-6), 4.77 and 4.72 (2H, each *br s*, H-18a and H-18b), 3.40 (1H, *septet*, $J = 7.0$ Hz, H-15), 3.06 (1H, *dd*, $J = 2.0$ and 11.5 Hz), 2.95 (1H, *br d*, $J = 14.5$ Hz), 2.67 (1H, *dd*, $J = 5.0$ and 11.0 Hz), 2.59 (1H, *dd*, $J = 5.0$ and 14.5 Hz) (C-1 and C-3 protons), 2.33 (3H, *s*, H-20), 1.79 (3H, *s*, H-19), 1.29 (6H, *d*, $J = 7.0$ Hz, H-16 and H-17). HRMS *m/z* (rel. int.): 326.1522 $C_{20}H_{22}O_4$ $[M]^+$ (4), 298 $[M - 28]^+$ (5), 258 $[M - C_5H_8]^+$ (26), 243 (14), 189 (100), 69 $[C_5H_9]^+$(51), 57 (64), 43 (58).

11β-Hydroxymanoyl oxide (**2**). Crystalline compound; mp 106–107. C; $[\alpha]_D = +21.2°$ ($CHCl_3$; *c* 0.1); $IR\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1640, 1460, 1440, 1385, 1375, 1120, 1080, 1040, 860. ^1H and ^{13}C NMR: given in Table 1. HRMS *m/z* (rel. int.): 306.2530 ($C_{20}H_{34}O_2$) $[M]^+$ (4), 305 $[M - 1]^+$ (7), 289 $[M - 1 - 16]^+$ (94), 271 (84), 221 (96), 193 (85), 97 (100), 71 (73), 58 (52).

11β-Acetoxymanoyl oxide (**2a**). Amorphous compound. $IR\nu_{max}^{CHCl_3}$ cm^{-1}: 1725, 1645, 1460, 1385, 1250, 1040, 825. ^1H NMR (200 MHz, in $CDCl_3$) δ 0.83 (6H, *s*, H-19 and H-18), 1.00 (3H, *s*, H-20), 1.35 (3H, *s*, H-16), 1.56 (3H, *s*, H-17), 2.05 (3H, *s*, OAc), 4.92 (1H, *dd*, $J = 1.5$ and 10.0 Hz, H-15 b), 5.15 (1H, *dd*, $J = 1.5$ and 17.5 Hz, H-15a), 5.42 (1H, *ddd*, $J = 4$, 5.5 and 10 Hz, H-11α), 5.86 (1H, *dd*, $J = 10$ and 17.5 Hz, H-14).

X-Ray crystallographic data of compound **2**. The compound was crystallized from EtOH giving prismatic crystals. A specimen with dimensions of $0.20 \times 0.25 \times 0.12$ mm was selected for X-ray analysis and was mounted on a glass fibre.

Crystal data. $C_{20}H_{34}O_2$, crystal system: orthorombic, space group $P2_12_12_1$ (#19), $Z = 4$, $D_c = 1.07$ g cm^{-3}. A total of 2280 unique reflections ($R_{int} = 0.0682$) were measured on a Rigaku AFC6S diffractometer (ω scan, scan speed 8/min, 2θ range: 3–157°, graphite monochromated CuK_α radiation). An absorption correction was applied (transmission factor range: 0.83–1.00). The structure was refined to a final *R* factor of 0.074. The atomic coordinates as well as distances and angles are deposited at the Cambridge Crystallographic Data Centre.

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REFERENCES

1. De Pascual Teresa, J., San Feliciano, A. and Miguel del Corral, Y. M. (1976) *Farm. Nueva* **41** (472), 343; *Chem. Abst.* (1977) **86**: 29949c.
2. Cheng, Y. S. and von Rudloff, E. (1970) *Tetrahedron Letters* **14**, 1131.
3. Bowyer, R. C. and Jefferies, P. R. (1963) *Chem. Ind. (Lond.)* 145.
4. Ulubelen, A., Topçu, G. and Tan, N. (1992) *Phytochemistry* **31**, 3637.
5. Ulubelen, A., Topçu, G. and Tan, N. (1992) *Tetrahedron Letters* **33**, 7241.
6. Hanson, J. R. (1991) *Nat. Prod. Rep.* **8**, 1.
7. Michavila, A., de la Torre, M. C. and Rodriguez, B. (1986) *Phytochemistry* **25**, 1935.
8. Rodriguez, B., Fernandez Gadea, F. and Savona, G. (1984) *Phytochemistry* **23**, 1805.
9. Mahmout, Y., Bessiere, J. M. and Dolmazon, R. (1993) *Phytochemistry* **34**, 865.