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NORABIETANE DITERPENOIDS AND OTHER TERPENOIDS FROM *SALVIA RECOGNITA*

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Key Word Index—*Salvia recognita*; Lamiaceae; triterpenoids; diterpenoids; norditerpenoids.

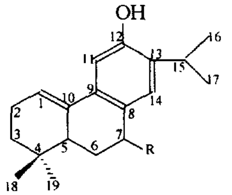
Abstract—Seven diterpenoids, three of them being new, salvirecognone, salvirecognine, 6α-hydroxy-11,12-dioxo-8,13-abieta-diene, were isolated from the roots of *Salvia recognita* together with three known triterpenoids. The structures of the known compounds were established by comparing their spectral data to those of authentic samples as well as by TLC comparisons. The structures of the new compounds were deduced by 1D and 2D NMR techniques including APT, DEPT, COSY and HETCOR. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

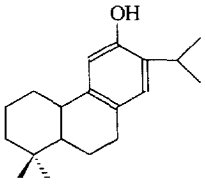
In continuation of our studies of *Salvia* species [1–5] we have investigated the roots of an endemic plant *Salvia recognita* Fisch. et Mey. collected from the highlands of southern Turkey. After a separation of the acetone extract on a silica gel column, the combined fractions were subjected to Chromatotron separations. Four known diterpenoids ferruginol [6], wiedelactone [7], sugiol [8], 12-methyl-5-dehydrohorminone [9], and three triterpenoids lupeone [10], 3α-acetylamyrin-28-al [11], 3-acetyloleanolic acid [12] were isolated together with the new compounds, salvirecognone (**1**), salvirecognine (**2**) and 6α-hydroxy-11,12-dioxo-8,13-abieta-diene (**3**).

RESULTS AND DISCUSSION

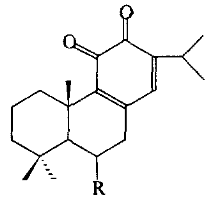
The first new compound salvirecognone (**1**) was obtained from the column fractions (38–51) on a silica gel rotor on a Chromatotron. From the same fractions ferruginol, wiedelactone and the second new compound salvirecognine (**2**) were also isolated together with 3α-acetylamyrin-28-al and 3-acetyloleanolic acid.



	R
1	=O
2	H



1a and 2a



	R
3	α-OH
4	H

Salvirecognone (**1**), a crystaline compound, the molecular formula $C_{19}H_{24}O_2$ was derived from its HREI mass spectrum (*m/z* 284.1869, calc. 284.1876) indicating a norditerpenoid structure. This was verified by its ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed the presence of three downfield protons two of which as sharp singlets, indicating H-11 at δ 7.00 (1H, *s*) and H-14 at δ 7.90 (1H, *s*), and one as a broad singlet at δ 6.42 ($w_{1/2} = 11$ Hz, H-1). The isopropyl group was observed at δ 3.20 (1H, *septet*, $J = 7$ Hz, H-15), together with methyl signals at δ 1.24 (3H, *d*, $J = 7$ Hz), 1.25 (3H, *d*, $J = 7$ Hz) (Me-16 and Me-17), two other methyl signals were at δ 1.01 (3H, *s*), 0.87 (3H, *s*) (Me-18 and Me-19). The signal for H-14 being at downfield (δ 7.90) indicated the presence of a car-

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Table 1. The ^{1}H NMR and ^{13}C NMR spectral data of compounds **1** and **2**

	1		**2**	
Position	^{1}H	^{13}C	^{1}H	^{13}C
1α	6.42 *br s*	125.7	6.26 *br s*	126.9
1β	—		—	
2α	2.30 *m*	23.3	2.00 *m*	23.4
2β	1.55 *dd*		1.70 *dd*	
3α	2.77 *dd*	40.0	2.75 *m*	36.9
3β	1.85 *dd*		1.30 *dd*	
4	—	37.8	—	38.9
5	2.52 *dd*	44.9	2.55 *dd*	45.9
6α	2.48 *dd*	35.4	2.00 *m*	19.4
6β	2.77 *dd*		1.75 *dd*	
7α	—	198.4	2.17 *m*	30.2
7β	—		2.23 *m*	
8	—	122.7	—	125.4
9	—	134.6	—	133.9
10	—	133.4	—	133.4
11	7.00 *s*	109.6	7.02 *s*	109.4
12	—	158.2	—	158.2
13	—	141.6	—	138.2
14	7.90 *s*	124.5	6.87 *s*	116.7
15	3.20 *septet*	26.9	3.15 *septet*	29.7
16	1.24 *d*	26.9	1.20 *d*	26.7
17	1.25 *d*	22.3	1.22 *d*	20.9
18	0.87 *s*	29.8	1.02 *s*	31.6
19	1.01 *s*	22.4	1.02 *s*	22.6

bonyl group at C-7 [13]. The relations between H-5 and H$_2$-6, observed at δ 2.52 (1H, *dd*, $J = 3$, 14 Hz, H-5α), δ 2.77 (1H, *dd*, $J = 3$, 14 Hz, H-6β) and δ 2.48 (1H, *dd*, $J = 14$, 14 Hz, H-6α) as well as between H-1 (δ 6.42) and H$_2$-2 (δ 2.3 *m*, 1.55 *dd*, $J = 4$, 10 Hz) were deduced by spin decoupling experiments. The ^{13}C NMR (APT and DEPT) spectra showed the presence of four methyl, three methylene, five methine and seven quaternary carbon signals for 19 carbon atoms. The signal at δ 198.4 correlated an aromatic ring conjugated with a carbonyl at C-7, five other downfield signals showed aromatic and unsaturated quaternary carbons (C-8, C-9, C-10, C-12, C-13), three downfield methine signals corresponded to C-1, C-11 and C-14. The proton bearing carbons were unambiguously assigned by a HETCOR experiment (Table 1). The UV maximum at 290 nm and the IR absorbance at 1650 cm^{-1} also correlated the presence of an aromatic ring conjugated with a carbonyl group. Hydrogenation of salvirecognone using Pd/C yielded **1a**. While expecting only the saturation of the double bond at $\Delta^{1(10)}$, the ^{1}H NMR and ^{13}C NMR spectra of **1a** showed that not only the double bond was saturated, but also the carbonyl at C-7 was reduced to a methylene group. The signal at δ_H 6.42 (*br s*), δ_C 125.7 disappeared and the signal at δ_H 7.90 (*s*), δ_C 124.5 shifted to δ_H 6.90, δ_C 115.4 in the NMR spectra. The formation of two additional CH$_2$ groups was observed in its ^{13}C NMR spectrum, at δ 31.7 and 30.1 and the presence of CH signal at δ 36.9 (C-10) indicated that a catalytic hydrogenolysis took place during this experiment. A literature survey showed that Pd/C hydrogenolysis in strongly acidic medium provides a useful alternative to Clemmensen reduction in the compounds having aromatic ring conjugated to carbonyl groups [14, 15]. Although in the present experiment no acid was used, the result was a hydrogenolysis. The mass spectrum of **1a** indicated a molecular formula $C_{19}H_{28}O$ (*m*/*z* 272.2129, calc. 272.2140) which was correlated with the ^{13}C NMR spectrum. All spectral data showed that salvirecognone (**1**) was 12-hydroxy-7-oxo-norabieta-1(10),8,11,13-tetraene.

The second new compound was designated as salvirecognine (**2**). The HREI mass spectrum of **2** gave a molecular formula $C_{19}H_{26}O$ (*m*/*z* 270.2118; calc. 270.2123). The ^{1}H NMR spectrum of **2** showed the presence of two aromatic protons at δ 7.02 (1H, *s*, H-11), 6.87 (1H, *s*, H-14), an olefinic proton at δ 6.26 (1H, *br s*, $w_{1/2} = 12$ Hz, H-1). The isopropyl group was at δ 3.15 (1H, *septet*, $J = 7$ Hz H-15) δ 1.20 (3H, *d*, $J = 7$ Hz) 1.22 (3H, *d*, $J = 7$ Hz) (Me-16 and Me-17), and two more methyl groups were observed at δ 1.02 (6H, *s*, Me-18 and Me-19). Spin decoupling experiments showed the relations between H-1 (δ 6.26) and H-2α (δ 2.00 *m*), H-2β (δ 1.70 *dd*, $J = 2$, 6 Hz) and between H$_2$-2 and H-3α (δ 2.75 *m*) and H-3β (δ 1.3 *dd*, $J = 5$, 9 Hz). The sequence from H-5 to H-7 also followed by spin decoupling and by the COSY experiments. The ^{13}C NMR (APT and DEPT) spectra showed the presence of four methyl, four methylene, five methine signals, three of them being in the downfield and six quaternary carbon signals for 19 carbon atoms of compound **2**. The COSY and HETCOR experiments led to the assignments of all of the vicinal protons and proton bearing carbons in the molecule. Hydrogenation of **2** yielded **2a**, the comparison of the spectral data of **1a** and **2a** showed that they are the same compounds.

Using a silica gel rotor in a Chromatotron a third norditerpene was isolated from the column fractions of 59–63, compound **3**. The same fractions also yielded sugiol, lupeone.

The third new compound **3** was established as the 6α-hydroxy derivative of a previously isolated compound, 11,12-dioxo-abieta-8,13-diene (**4**) [16]. The molecular formula $C_{20}H_{28}O_3$ derived from its HREI mass spectrum (*m*/*z* 316.2019; calc. 316.2028). The base peak at *m*/*z* 298 showed the loss of H$_2$O. In the ^{1}H NMR spectrum the signal at δ 3.65 (1H, *ddd*, $J = 3.5$,11,12 Hz, H-6β) indicated a proton between a methine and a methylene groups was observed in 6α-hydroxyambreinolide and in yosgadensenols [5]. The α-position of the hydroxyl group at C-6 was decided by measuring of the *J* values and studying a Dreiding model. Other ^{1}H NMR data were quite similar to those of the known compound **4**, as followed from the signals at δ 0.90 (6H, *s* Me-18 and Me-19), 1.10 (6H, *d*, $J = 7$ Hz, Me-16 and Me-17), 1.35 (3H, *s*, Me-20), 2.65 (1H, *dt*, $J = 14$,2,1.5 Hz, H-1β), 2.98 (1H, *septet*,

Table 2. ^{13}C NMR spectral data of compounds **3** and **4**

C	3	4
1	36.2	37.0
2	18.7	18.2
3	40.9	40.8
4	31.8	33.4
5	48.8	46.4
6	67.8	23.7
7	42.5	33.4
8	148.8	148.3
9	153.3	154.0
10	38.9	39.3
11	182.4	182.0
12	183.6	182.0
13	136.0	136.0
14	132.3	118.2
15	26.0	25.0
16	20.3	20.4
17	20.6	20.6
18	33.3	33.1
19	21.7	22.0
20	19.9	20.1

$J = 7$ Hz, H-15), 6.45 (1H, *br s*, H-14). The ^{13}C NMR (APT and DEPT) spectra indicated the presence of five methyl, four methylene, four methine and seven quaternary carbon signals for 20 C atoms of **3** (Table 2). Comparing the ^{1}H and ^{13}C NMR data of **3** to those of compound **4** clearly showed that **3** is the 6α-hydroxy derivative of **4**.

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ in 200 MHz and 50.32 MHz instrument. The mass spectra in VG Zab Spec instrument. Si gel columns, VLC and Chromatotron and when necessary prep. TLC plates were used for separation.

Plant material

The roots of *Salvia recognita* Fisch. et Mey. were collected from southern Turkey (Adana-Armutluk district) at an altitude 1950 m in June 1995. The plant was identified by Prof. Dr Kerim Alpinar (Istanbul) and a voucher specimen (ISTE 68304) is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul.

Extraction and isolation

The dried and powdered roots of the plant (1.820 kg) were extracted with Me_2CO in a Soxhlet, filtered and evaporated to dryness *in vacuo*. The residue (37 g) was fractionated on a Si gel column (5×80 cm) eluting with petrol, a gradient of EtOAc was added up to 100% followed by EtOH. Oily fractions were discarded and similar fractions were combined to be separated on VLC and on Chromatotron using Si gel rotors. Frs 38–51 (Pe:EtOAc 100:7 from Si gel column) upon separation on Chromatotron and further cleaned on preparative TLC plates, yielded the following: **1** (30 mg), 3α-acetylamyrin-28-al (10 mg), 3-acetyloleanolic acid (12 mg), lupeone (3 mg), **2** (6 mg), wiedelactone (8 mg). Frs 59–63 (Pe:EtOAc 90:10) yielded sugiol (7 mg), and **3** (7 mg).

Salvirecognone (**1**). Mp 225–230°C; $[\alpha]_D^{25} = +50.5°$ ($CHCl_3$; *c* 0.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200, 2880, 2860, 2870, 2865, 1650, 1635, 1595, 1560; UV λ_{max}^{MeOH} nm: 290 (log ε 4.0), 260 (log ε 3.0); ^{1}H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$) (see Table 1); HRMS *m/z* (rel. int.): 284.1869 $[M]^+$ ($C_{19}H_{24}O_2$) (100), 269 $[M-Me]^+$ (50), 228 (85), 213 (72), 200 (30), 186 (28), 149 (44).

Hydrogenated derivative of salvirecognone (**1a**). ^{1}H NMR ($CDCl_3$): see text; ^{13}C NMR ($CDCl_3$): C-1 (31.7 *t*), C-2 (22.8 *t*), C-3 (41.7 *t*), C-4 (38.8 *s*), C-5 (44.1 *d*), C-6 (18.9 *t*), C-7 (30.1 *t*), C-8 (124.3 *s*), C-9 (134.2 *s*), C-10 (36.9 *d*), C-11 (110.9 *d*), C-12 (156.5 *s*), C-13 (140.3 *s*), C-14 (115.4 *d*), C-15 (29.6 *d*), C-16 (26.8 *q*), C-17 (22.6 *q*), C-18 (33.3 *q*), C-19 (22.9 *q*). HRMS *m/z* (rel. int).: 272.2129 $[M]^+$ ($C_{19}H_{28}O$) (100), 257 $[M-Me]^+$ (82), 229 $[M-CH(CH_3)_2]^+$ (33), 215 $[229-CH_2]^+$ (10), 201 $[215-CH_2]^+$ (24).

Salvirecognine (**2**). Amorphous powder, $[\alpha]_D^{24} + 40°$ ($CDCl_3$; *c* 0.1); UV λ_{max}^{MeOH} nm: 306 (log ε 3.5), 260 (log ε 4.0); ^{1}H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$) (see Table 1); HRMS *m/z* (rel. int.): 270.2118 $[M]^+$ ($C_{19}H_{26}O$) (100), 253 $[M-OH]^+$ (90), 227 (72), 214 (85), 265 (57).

6α-Hydroxy-11,12-dioxo-8,13-abieta-diene (**3**). Amorphous powder, $[\alpha]_D^{25} + 6.9°$ ($CDCl_3$; *c* 0.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3525, 2959, 2916, 2848, 1725, 1645, 1594, 1462; UV λ_{max}^{MeOH} nm: 258 (log ε 5.0); ^{1}H NMR ($CDCl_3$): see text; ^{13}C NMR: Table 2; HRMS *m/z* (rel. int.): 316.2019 $[M]^+$ ($C_{20}H_{28}O_3$) (33), 298 $[M-H_2O]^+$ (100), 283 $[M-H_2O-Me]^+$ (76), 255 (28), 228 (35), 149 (52).

Hydrogenation. Compound **1** (10 mg) and 5 mg of **2** were dissolved in 2 ml EtOH respectively, 50 mg Pd/C (10%) were added and hydrogen gas was passed through the sols separately for 1 h. The sols were filtered, evaporated to dryness and the compounds were extracted, dissolved in CH_2Cl_2, cleaned on preperative TLC plates. The yields were 8 mg (80%) and 4.5 mg (90%) respectively.

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