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New Diterpenoids from the Aerial Parts of *Salvia pilifera*

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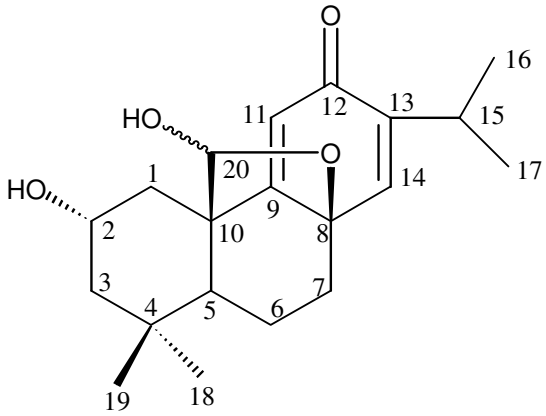
Three new diterpenoids, piliferol (2α,20-dihydroxy-12-oxo-abieta-9(11),13-dien-8,20-ether) (**1**), salvipiliferol (2α,10β-dihydroxy-12-oxo-norabieta-9(11),13-dien) (**2**), and piliferalactone (2α,8-dihydroxy-12-oxo-abieta-9(11),13-dien-20-oic acid-8,20-lactone) (**3**), together with 7 known compounds, ursolic acid, oleanolic acid, betulinic acid, α-amyrin, lupeol, β-sitosterol, and pectolinarigenin, have been isolated from the aerial parts of an endemic *Salvia* species, *S. pilifera*. Their structures were elucidated by spectral (UV, IR, 1D-, and 2D-NMR, and HRMS) methods.

Key Words: *Salvia pilifera*, Lamiaceae, abietane-type diterpenoids, triterpenoids, steroid, flavone.

Introduction

Salvia L. species (Lamiaceae) are used in traditional medicine all around the world due to their various pharmacological activities, such as antiseptic, sedative, carminative, and diuretic.[1] Although *Salvia* species (Sage) are distributed worldwide, they are mostly located in the Mediterranean, Southeast Asia, and Central and South America.[2] There are about 90 *Salvia* species in Turkey, half of which are endemic.[3] The aerial parts and/or roots of 50 *Salvia* species growing in Turkey have been studied by our group since 1968 for their chemical constituents and for some pharmacological activities.[4–6]

A literature survey showed that the composition of the essential oils of *S. pilifera* Montbret & Aucher ex Bentham[7,8] and their antibacterial activity[9] have been investigated. As a part of our research on *Salvia* species, the constituents of the aerial parts of *S. pilifera* collected from southern Turkey were studied. Three new diterpenoids, piliferol (**1**), salvipiliferol (**2**), and piliferalactone (**3**) (Figure), were isolated, in addition to 5 triterpenoids (ursolic acid,[10] oleanolic acid,[11] betulinic acid,[11] α-amyrin,[12] and lupeol[13]), a steroid (β-sitosterol,)[14] and a flavone (pectolinarigenin.)[15]



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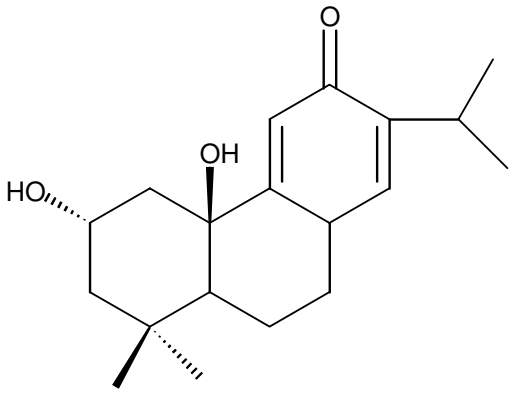

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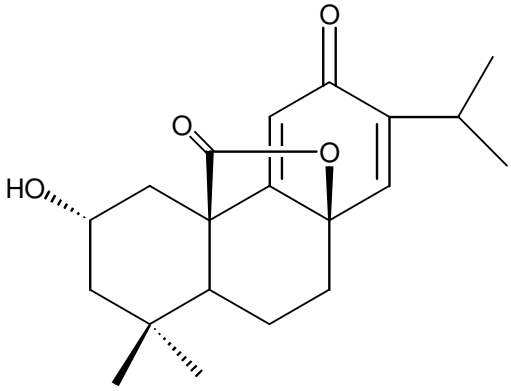

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Figure. New diterpenoids (**1-3**) isolated from *S. pilifera.*

The structures of the new diterpenoids were established by spectroscopic analyses (UV, IR, ^{1}H- and ^{13}C-NMR (APT), COSY, HMBC, HRMS) and those of the known isolates were determined by comparing their spectral data to those given in the literature and by TLC comparison with standard samples.

Experimental

General Experimental Procedures

The UV spectra (λ_{max}) were recorded on a Shimadzu UV-1601 and the IR spectra (υ_{max}) were recorded on a Perkin-Elmer Model 983. Optical rotations were determined in an Opt. Act. Ltd. AA-5 polarimeter. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were recorded on a Varian Mercury-Vx instrument. EIMS and HRMS were recorded on a ZabSpec (Micromass) mass spectrometer. Silica gel columns (Merck Art. 7734), a Chromatotron apparatus on silica gel radial plates (Merck Art. 7749), ready-made silica gel 60 $PF_{254+366}$ (Merck Art. 7748, 1 mm thick), and ready-to-use plates (silica gel 60 F_{254}, Merck Art. 5554, 0.25 mm thick) were used for chromatographic separations.

Plant Material

The aerial parts of *S. pilifera* & "Montbret & Aucher ex Bentham" were collected from southern Turkey (Kahramanmaraş, Berit Mountain) in May 2000 and identified by Dr. Gamze Kökdil. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, Ankara University (AEF 21134).

Extraction and Isolation

The dried and powdered aerial parts of *S. pilifera* (500 g) were macerated with EtOH (3 times). The solvent was removed by a rotary evaporator (Büchi R200) and the crude extract (55 g) was fractionated using a silica gel column (3.5 × 72 cm). The column was eluted with petroleum ether (10 × 200 mL) and a gradient of CH_2Cl_2 was added in 10-mL increments into 150 mL of petroleum ether until reaching 100%; thus, 15 × 150 mL was used, followed by EtOAc in 10-mL increments up to 100% (15 × 150 mL) and by ethanol in 1-mL increments up to 10% (10 × 100 mL). Similar fractions were combined by using TLC plates (**A**-**D**). The fractions (**A**-**D**) were applied to 1-mm thick silica gel rotors of a Chromatotron and were eluted with petroleum ether (10 × 25 mL). Gradients of CH_2Cl_2 (20 × 25 mL) were added up to 100%, followed by ethanol (5 × 25 mL). After Chromatotron separation, final purification was carried out on preparative TLC plates using the following solvent systems: for fraction **B**, ursolic acid (21 mg) and oleanolic acid (23 mg) (CH_2Cl_2:Et_2O, 9:2), α-amyrin (10 mg) and lupeol (18 mg) (CH_2Cl_2), betulinic acid (14 mg) (toluene:Et_2O, 33:10), and β-sitosterol (14 mg) (toluene:Et_2O, 22:5); for fraction **C**, piliferol (**1**, 10 mg) and salvipiliferol (**2**, 15 mg) (CH_2Cl_2:Et_2O, 3:1), piliferalactone (**3**, 5 mg) (toluene:Et_2O, 2:1), and pectolinarigenin (8 mg) (toluene: Et_2O, 3:1).

Piliferol (1)

Amorphous diterpenoid, $[\alpha]_D^{20}$ –62° ($CHCl_3$; c 0.4); UV (MeOH) λ_{max}: 242 nm; IR ($CHCl_3$) ν_{max}: 3418, 2926, 2279, 1731, 1682, 1633, 1462, 1369, 1262, 1079, 999, 915, 875, 791, 666 cm^{-1}; EIMS 70 eV, m/z (rel. int.): 332 $[M]^+$ (47), 314 $[M\text{-}H_2O]^+$ (58), 303 (19), 296 (32), 285 $[314\text{-}COH]^+$ (100), 268 $[285\text{-}OH]^+$ (36), 253 $[268\text{-}Me]^+$ (32), 243 (18), 225 (13), 215 (17), 201 (22), 194 (23), 187 (21), 178 (17), 165 (87), 149 (23), 135 (12), 129 (13), 121 (14), 109 (15), 95 (17), 83 (18), 69 (23), 57 (20).^{1}H-NMR (400 MHz, $CDCl_3$) data: see Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$) data: see Table 2. HRMS: m/z 332.1978 (calcd for $C_{20}H_{28}O_4$, 332.1987).

Salvipiliferol (2)

Amorphous diterpenoid, $[\alpha]_D^{20}$ –55° ($CHCl_3$, c 0.2); UV (MeOH) λ_{max}: 239 nm; IR ($CHCl_3$) ν_{max}: 3407, 2929, 2855, 1716, 1680, 1627, 1463, 1389, 1216, 1036, 758, 666 cm^{-1}; EIMS 70 eV, m/z (rel. int.): 304 $[M]^+$ (47), 286 $[M\text{-}H_2O]^+$ (100), 271 $[286\text{-}Me]^+$ (22), 243 $[271\text{-}CO]^+$ (30), 230 $[243\text{-}CH]^+$ (25), 203 (20), 165 (79), 121 (27), 95 (10), 81 (7), 69 (9).^{1}H-NMR (400 MHz, $CDCl_3$) data: see Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$) data: see Table 2. HRMS: m/z 304.2029 (calcd for $C_{19}H_{28}O_3$, 304.2038).

Table 1. ^{1}H-NMR (400 MHz) data of diterpenoids **1-3** in $CDCl_3$.

	1	**2**	**3**
	δ_H (m, J Hz)	δ_H (m, J Hz)	δ_H (m, J Hz)
1α	1.85 dt (3.0,13.0)	1.88 dt (3.0,13.0)	1.86 dt (4.5,12.0)
1β	2.20 dd (3.0,13.0)	2.25 dd (3.0,13.0)	2.22 dd (4.5,12.0)
2β	3.87 tt (3.0,13.0,13.0)	3.90 tt (3.0,13.0, 13.0)	4.53 tt (4.5,12.0,12.0)
3α	1.28 dd (3.0,13.0)	1.32 dd (3.0,13.0)	1.26 dd (4.5,12.0)
3β	1.70 dt (3.0,13.0)	1.74 dt (3.0,13.0)	1.70 dd (4.5,12.0)
5α	1.55 dd (5.0,12.0)	1.65 dd (5.1,12.2)	1.51 dd (5.1,12.2)
6α	1.90 ddd (5.0,6.0,11.0)	1.70 ddd (5.1,6.0,11.1)	1.94 ddd (5.1,6.0,11.2)
6β	1.60 dddd (5.0,11.0,12.0,12.0)	1.45 dddd (5.1,11.1,12.2,12.2)	1.65 dddd (5.8,11.2,12.2,12.3)
7α	1.40 ddd (6.0,12.0,13.0)	1.10 ddd (6.0,12.2,13.0)	1.48 ddd (6.0,12.3,13.6)
7β	2.30 dd (5.0,13.0)	2.07 brd (13.0	2.45 dd (5.8,13.6)
8	—	2.68 brt (12.0, 13.0)	—
11	5.97 s	5.95 d (1.0)	5.98 s
14	6.70 s	6.53 d (1.0)	6.75 d (1.0)
15	2.96 sept. (7.0)	2.90 sept. (7.0)	3.04 sept. (7.0)
16	1.05 d (7.0)	1.05 d (7.0)	1.04 d (7.0)
17	1.09 d (7.0)	1.05 d (7.0)	1.08 d (7.0)
18	1.07 s	0.98 s	1.15 s
19	1.02 s	0.98 s	1.12 s
20	5.60 s	—	—

Table 2. ^{13}C-NMR (100 MHz) data of diterpenoids **1-3** in $CDCl_3$.

	1	**2**	**3**
	δ_C	δ_C	δ_C
1	50.6 t	51.2 t	44.2 t
2	64.7 d	67.2 d	63.5 d
3	40.8 t	39.5 t	45.7 t
4	36.3 s	35.6 s	36.5 s
5	52.0 d	52.7 d	52.6 d
6	19.7 t	20.7 t	21.4 t
7	36.0 t	38.6 t	38.1 t
8	78.2 s	37.3 d	79.6 d
9	167.5 s	164.9 s	161.6 s
10	53.3 s	68.7 s	50.3 s
11	116.5 d	121.9 d	116.0 d
12	185.9 s	185.7 s	181.5 s
13	145.5 s	143.7 s	147.2 s
14	138.5 d	144.3 d	139.8 d
15	26.8 d	26.3 d	26.9 d
16	22.5 q	22.0 q	23.1 q
17	23.4 q	22.2 q	23.1 q
18	33.2 q	31.1 q	32.3 q
19	21.8 q	21.8 q	22.2 q
20	99.8 d	—	176.0 s

Piliferalactone (3)

Amorphous diterpenoid, $[\alpha]_D^{20}$ +112° ($CHCl_3$; c 0.5); UV (MeOH) λ_{max}: 245, 330; IR ($CHCl_3$) ν_{max}: 3423, 2925, 2854, 1786, 1734, 1687, 1652, 1463, 1370, 1271, 1160, 1124, 1068, 1031, 990, 939, 755 cm^{-1}; EIMS 70 eV, m/z (rel. int.): 330 $[M]^+$ (5), 313 $[M\text{-}H_2O+H]^+$ (8), 299 (7), 284 $[M\text{-}H_2O\text{-}CO]^+$ (17), 268 $[284\text{-}O]^+$ (12), 253 $[268\text{-}Me]^+$ (8), 236 (37), 222 (11), 208 (11), 185 (10), 167 (10), 152 (15), 137 (30), 123 (24), 111 (29), 97 (53), 81 (62), 69 (100), 57 (71).^{1}H-NMR (400 MHz, $CDCl_3$) data: see Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$) data: see Table 2. HRMS: m/z 330.1822 (calcd for $C_{20}H_{26}O_4$, 330.1831).

Results and Discussion

The powdered aerial parts were macerated with EtOH, filtered, and evaporated to dryness. The EtOH extract was separated using column and preparative thin layer chromatographic methods, as well as Chromatotron separation. Three new and 7 known isolates were obtained from *S. pilifera*.

The HRMS of the first new diterpenoid (**1**) indicated the molecular formula $C_{20}H_{28}O_4$ m/z 332.1978 (calcd for $C_{20}H_{28}O_4$, 332.1987), which showed 7 degrees of unsaturation as a double bond equivalent, of which 3 were accounted for by a tricyclic skeleton, 1 by a ketone, 2 by double bonds, and 1 by a hemiketalic ring between C-8 and C-20. The IR spectrum exhibited absorbency at 3418 cm^{-1} for a hydroxyl and at 1682 cm^{-1} for a conjugated ketone. The ^{13}C-NMR (APT) spectrum of **1** includes 4 methyl, 4 methylene, 6 methine, and 6 quaternary carbon signals. In the ^{1}H-NMR spectrum (see Table 1), the signals of an abietane-type skeleton were observed; a methine proton signal at δ_H 2.96 (1H, septet, J = 7.0 Hz, H-15) together with 2 methyl doublets at δ_H 1.05 (3H, d,J = 7.0 Hz, Me-16) and 1.09 (3H, d, J = 7.0 Hz, Me-17) showed the presence of an isopropyl group, and the signals at δ_H 1.07 (3H, s) and 1.02 (3H, s) showed 2 other methyl groups, Me-18 and Me-19, respectively; however, the fifth methyl group of an abietane skeleton was not present. Two signals at δ_H 5.97 (1H, s, H-11) and δ_H 6.70 (1H, s, H-14) indicated dienone protons. The APT spectrum (see Table 2) exhibited the presence of 2 secondary hydroxyl groups in the molecule; one signal was at δ_C 64.7 d (C-2). The proton signal at δ_H 3.87 (J = 3.0; 13.0; 13.0) was analyzed as a triple triplet and showed that the secondary hydroxyl group was equatorial and coupled with 2 axial protons and 2 equatorial protons located on 2 neighboring carbon atoms at C-1 (δ_C 50.4) and C-3 (δ_C 40.7).[16] In order to correlate the position of the hydroxyl group, a COSY experiment was carried out. The COSY relationships between H-2β (δ_H 3.87) and H-3α (δ_H 1.28), and between H-2β and both C-1 protons (δ_H 2.20 and 1.85) were observed. In addition, the 2-bond away correlations between H-2β and C-1, and H-2β and C-3, the 3-bond away correlations between H-3α and C-1, and H-3α and C-18, and between H-1β and C-3 were observed in the HMBC spectrum showed that the secondary hydroxyl group (δ_C 64.7) should be placed at C-2. Since the ^{13}C-NMR value of the second hydroxyl signal (δ_C 99.9d) is quite high, the C atom should be placed between 2 oxygen functions; therefore, the second hydroxyl group should be at C-20. As mentioned above, in the ^{1}H-NMR spectrum, the methyl signal at C-20, which is generally present in abietane-type diterpenoids, was not found. Due to the presence of a singlet at δ_H 5.60 (1H, H-20) that showed a correlation to C-20 in the HMBC spectrum, and the existence of the signals at δ_C 78.2s (C-8) and δ_C 99.9d (C-20), the hemiketalic ring could only be placed between C-8 and C-20.[17] The structure of piliferol (**1**) was established as 2α,20-dihydroxy-12-oxo-abieta-9(11),13-dien-8,20-ether.

The molecular formula of the new norditerpene salvipiliferol (**2**), $C_{19}H_{28}O_3$ m/z 304.2029 (calcd for $C_{19}H_{28}O_3$, 304.2038) in HRMS, indicated 6 double bond equivalents, of which 3 were accounted for by a tricyclic skeleton, 1 by a ketone group, and 2 by double bonds. The ^{13}C-NMR (APT) (see Table 2) spectrum exhibited 4 methyl, 4 methylene, 6 methine, and 5 quaternary carbon signals. The HRMS as well as ^{13}C-NMR indicated that compound **2** is a norditerpenoid. The IR spectrum indicated a hydroxyl (3407 cm^{-1}) and a conjugated ketone (1680 cm^{-1}). The ^{1}H-NMR (see Table 1) spectrum exhibited 4 methyl signals, 2 of them at δ_H 0.98 (6H, s), indicating Me-18 and Me-19, and the 2 other methyl groups both observed at δ_H 1.05 (6H, d, J = 7.0 Hz, Me-16 and Me-17) together with the signal at δ_H 2.90 (1H, septet, J = 7.0 Hz, H-15) indicated the presence of an isopropyl group. Dienone proton signals were observed at δ_H 5.95 (1H, d, J = 1.0 Hz, H-11) and 6.53 (1H, d, J = 1.0 Hz, H-14), while a methine signal (H-8β) was observed at δ_H 2.68 (1H, brt, J = 12.0, 13.0 Hz, H-8β). A W coupling between H-8 and H-11 followed a COSY experiment. In addition, another W coupling between H-14 and H-15 was also observed. In the ^{13}C-NMR (APT) spectrum, the signals at δ_C 67.2 d and 68.7 s were assigned to the 2 hydroxyl groups. The splitting pattern of the signal at δ_H 3.90 (1H, tt, J = 3.0, 13.0, 13.0 Hz) was very similar to that of compound **1**. The HMBC correlations observed between H-2β and C-1, as well as H-2β and C-3, indicated that the secondary hydroxyl group (δ_C 67.2) was placed at C-2 in α position. Since the fifth methyl signal, generally present in abietane-type diterpenoids, is lacking the tertiary hydroxyl group, it could only be located at C-10. The structure of salvipiliferol **2** was established as 2α,10β-dihydroxy-12-oxo-norabieta-9(11),13-dien.

The spectral data of the third new diterpenoid piliferalactone (**3**) indicated a molecular formula, $C_{20}H_{26}O_4$ m/z 330.1822 (calcd for $C_{20}H_{26}O_4$, 330.1831), in HRMS, showing 8 degrees of unsaturation, of which 3 were accounted for by a tricyclic skeleton, 1 by a ketone, 2 by double bonds, and 2 by a lactone ring between C-8 and C-20. Its IR spectrum indicated bands for a hydroxyl group (3423 cm^{-1}), a lactone group (1786 cm^{-1}), and a conjugated ketone group (1687 cm^{-1}). The ^{1}H-NMR (see Table 1) and ^{13}C-NMR (APT) (see Table 2) spectra of **3** are quite similar to those of 8-hydroxy-12-oxo-abieta-9(11),13-dien-20-oic acid-8,20-lactone obtained from *Salvia wiedemannii* Boiss,[18] with the exception of the signals at δ_H 4.53 tt (J= 4.5; 12.0; 12.0 Hz) and δ_C 63.5d, which indicated a secondary hydroxyl group. The hydroxyl group was located at C-2 in equatorial orientation, based on a characteristic multiplicity of the geminal proton. The chemical shift difference from compounds **1** and **2** in the ^{1}H-NMR spectrum could be explained by the presence of the lactonic carbonyl group between C-8 and C-20. Studying with a Dreiding model, the α-hydroxyl at C-2 and the lactone carbonyl at C-20 are very close to each other, explaining the chemical shift difference. The spectral data indicated a 2α,8-dihydroxy-12-oxo-abieta-9(11),13-dien-20-oic acid-8,20-lactone structure for piliferalactone (**3**).

Conclusion

Our investigations on Turkish *Salvia* species showed that their aerial parts and roots contain mainly abietane-type, labdane-type, and, rarely, pimarane- diterpenoids.[19] In this study, 3 new abietane-type diterpenoids (**1**-**3**) were isolated from *S. pilifera*, together with 7 known compounds. We obtained 8,20-hemiketale- (**1**) and 8,20-lactone-type (**3**) structures, as well as a norabietane-type (**2**) diterpenoid, which are rarely encountered in Turkish *Salvia* species.

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