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SESTERTERPENES AND OTHER CONSTITUENTS OF *SALVIA YOSGADENSIS*

GÜLAÇTI TOPCU,*† AYHAN ULUBELEN,†‡ TIMOTHY C.-M. TAM§ and CHUN TAO-CHE§

†TUBITAK, Marmara Research Centre, Research Institute for Basic Sciences, Department of Chemistry, P.O. Box 21, 41470 Gebze, Kocaeli, Turkey; ‡Faculty of Pharmacy, University of Istanbul, 34452 Beyazit, Istanbul, Turkey; §Department of Chemistry, The Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong

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Key Word Index—*Salvia yosgadensis*; Lamiaceae; sesterterpenes; sesquiterpene; sesquiterpene lactones; flavonoids; aromatic compounds.

Abstract—From the aerial parts of *Salvia yosgadensis* we have obtained two new sesterterpenes, yosgadensolide A (6α,14-dihydroxymanoyloxide-15,17-dien-16,19-olide) and yosgadensolide B (6α,16-dihydroxymanoyloxide-14,17-dien-16,19-olide) and their epimers. In addition, a known sesquiterpene, three known sesquiterpene lactones, eight known flavonoids and two known aromatics were isolated.

INTRODUCTION

In a previous study of *Salvia yosgadensis* Freyn et Bornm. we obtained two norditerpenes and two norsesterterpenes [1]. Further studies of the same plant extract provided two new sesterterpenes and their epimers, together with the sesquiterpene spathulenol [2] and the sesquiterpene lactones, istanbulin D [3], 1β-acetoxy-8β-hydroxyeudesm-4(15),7-dien-8,12-olide and 1β-acetoxyeudesm-4(15),7-dien-8,12-olide [4]. Although sesquiterpenes are rather rare in *Salvia* species, some sesquiterpenes have been obtained from *S. sclarea* [2], *S. potentillifolia* [5], *S. divaricata* [6] and *S. palaefolia* [7, 8]. The flavonoids luteolin, apigenin, apigenin 7-methyl ether, apigenin 4′-methyl ether, apigenin 7,4′-dimethyl ether, apigenin 6,4′-dimethyl ether, salvigenin, kaempferol 3-methyl ether in addition to *p*-hydroxyquinone and *p*-acetoxyphenol were isolated from the same extract. The structures of the new and the known compounds were established by spectral analysis, particularly one- and two-dimensional NMR techniques and mass spectra.

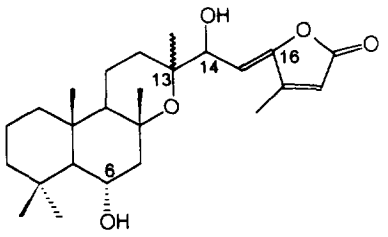
RESULTS AND DISCUSSION

In this study, we have isolated two new sesterterpene lactones, yosgadensolide A (**1**) and yosgadensolide B (**2**) along with their epimers. The CI mass spectrum of yosgadensolide A (**1**) gave a [M + 1] ion peak at *m/z* 419 corresponding to the molecular formula $C_{25}H_{38}O_5$, and the HREI mass spectrum confirmed this formula (*m/z* 418.2700, calcd 418.2719). The ^{13}C NMR spectrum (Table 1), which supported the presence of a sesterterpene structure, showed 25 carbon atoms consisting of six methyl, six methylene, six methine and seven quaternary carbons. The IR spectrum showed a

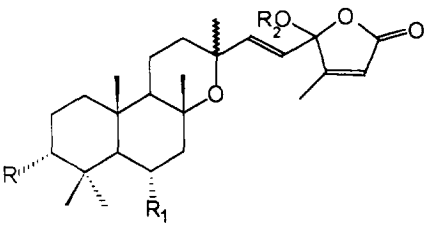
Table 1. ^{13}C NMR data of compounds **1**, **1a Ac** and **2** (in $CDCl_3$)

C	1	1a Ac	2
1	39.0	39.8	39.4
2	18.3	18.2	18.7
3	43.5	43.2	43.3
4	30.7	33.3	33.2
5	61.6	61.7	59.6
6	69.0	70.7	70.6
7	54.1	49.4	53.3
8	73.2	74.1	73.6
9	58.0	58.8	60.4
10	33.8	33.3	34.0
11	14.9	14.8	14.2
12	37.6	36.0	37.6
13	75.3	75.0	75.2
14	72.9	75.4	147.2
15	109.3	106.6	119.7
16	151.0	152.2	107.0
17	154.7	154.7	164.0
18	117.3	117.7	118.2
19	176.7	172.2	171.3
20	11.7	11.9	12.8
21	25.9	25.9	30.9
22	24.6	23.9	24.0
23	36.4	36.0	35.9
24	21.8	21.1	21.6
25	17.2	16.8	14.9
C=O	—	170.1	—
CH_3	—	21.8	—
C=O	—	169.8	—
CH_3	—	21.9	—

*Author to whom correspondence should be addressed.



1 a and b (E and Z isomers) 13 ß Me
1c 13α Me



2 R=H, R_1=OH, R_2=H 13 ß Me
3 R=OH, R_1=H, R_2= CH_3
4 R=H, R_1=OH, R_2=H13α Me

hydroxyl group at 3400 cm^{-1}, a five-membered lactone ring at 1780 cm^{-1} and unsaturation at 1615 cm^{-1}. The UV spectrum correlated with the IR spectrum in exhibiting a maximum at 274 nm for an α,β-unsaturated five-membered lactone ring. The ^{1}H NMR spectrum (Table 2) exhibited five methyl singlets at δ 0.81, 1.00, 1.18, 1.24, 1.38 and a vinylic methyl doublet at δ 2.16 (J = 1.5 Hz). A carbinol methine signal was at δ 3.88 (1H, *ddd*, J = 3.8, 11.2, 12 Hz) indicating a hydroxyl group situated between a methine and a methylene group. Such a hydroxyl group could only be placed either at C-6 or C-11. The ^{13}C NMR spectrum helped to decide between these two locations. In the case of a C-11 hydroxyl group, C-12 should be shifted to 40–45 ppm while with a hydroxyl group at C-6, C-7 is above 50 ppm, as was observed (δ 54.1) in the present case. No other methylene carbon exhibiting a shift near 50 ppm was present. The resonance of the second carbinol methine proton was at δ 4.45 (1H, *d*, J = 9.5 Hz, H-14) coupled with a vinylic proton at δ 5.20 (1H, *d*, J = 9.5 Hz, H-15), while another vinylic proton was observed at δ 5.96 as a narrow doublet (1H, *d*, J = 1.5 Hz, H-18). Spin decoupling experiments showed the relation between H-6 and H-5, H-6 and H_2-7 and also H-14 and H-15. In the ^{13}C NMR spectrum, among methine signals, two were assigned to carbinol carbons at δ 72.9 and δ 69.0, C-14 and C-6, respectively. Among the quaternary carbons, two were seen at δ 75.3 and 73.2 which corresponded to C-8 and C-13, respectively, and a third was a lactone carbonyl seen at δ 176.7. HETCOR and HMBC experiments provided unambiguous assignment of the protons and carbons. Long-range correlations were observed between H-14 and C-15, C-16 as well as between H-15 and C-16, C-17, the correlations between the vinylic methyl (Me-20) and C-16, C-17, C-18, and between Me-21 and C-15. After acetylation, compound **1** yielded two acetyl derivatives (**1a Ac** and **1b Ac**) which were separated by preparative TLC, thus indicating that compound **1** is a mixture of two stereoisomers. The spectral data of **1a Ac** and **1b Ac** were quite similar; only the signals of protons and carbons for C-14, C-15, C-18 and C-20 showed differences indicating the possible presence of *E*- and *Z*-stereoisomers. Based on the spectral data, the structures of **1a** and **1b** were established as 6α,14-dihydroxymanoyloxide-15,17-dien-15(*Z*)-16,19-olide and 6α,14-dihydroxymanoyloxide-15,17-dien-15(*E*)-16,19-olide.

Table 2. ^{1}H NMR assignments of compounds **1**, **1a Ac**, **1b Ac** and **1c**

Proton	**1** (**1a** + **1b**)	**1a Ac**	**1b Ac**	**1c**
H-6	3.88 *ddd*	5.08 *ddd*	5.08 *ddd*	3.88 *ddd*
H-14	4.45 *d*	5.57 *d*	5.68 *d*	4.31 *d*
H-15	5.20 *d*	5.32 *d*	5.51 *d*	5.30 *d*
H-18	5.96 *d*	5.97 *br s*	6.04 *br s*	5.96 *d*
H-20	2.16 *d*	2.18 *br s*	2.08 *br s*	2.17 *d*
H-21	1.38 s	1.37 s	1.38 s	1.36 s
H-22	1.24 *s*	1.28 *s*	1.28 *s*	1.17 *s*
H-23	1.18 *s*	1.00 *s*	1.01 *s*	1.17 *s*
H-24	1.00 *s*	0.85 *s*	0.84 *s*	1.01 *s*
H-25	0.81 *s*	0.85 *s*	0.84 *s*	0.82 *s*
OAc	—	2.10 *s*	2.08 *s*	—
OAc	—	2.02 *s*	2.05 *s*	—

Another epimer of the same gross structure was also isolated separately (**1c**). The ^{1}H NMR spectrum of **1c** was quite similar to that of **1** (Table 2), the main differences being in the resonances of H-14 and H-15 as well as those of two methyl groups (Me-21 and Me-22). This suggested the presence of a C-13 epimer, but the small quantity of **1c** did not allow further investigation of the stereochemistry.

The CI-mass spectrum of yosgadensolide B (**2**) gave a $[M]^+$ peak at m/z 419 corresponding to a molecular formula $C_{25}H_{38}O_5$. The HREI-mass spectrum confirmed this formula (m/z 418.2705, calcd 418.2719). The IR spectrum of **2** showed a five-membered lactone ring at 1780 cm^{-1} and a hydroxyl at 3450 cm^{-1} while the UV spectrum exhibited a shoulder at 274 nm and a maximum at 222 nm. The ^{1}H NMR spectrum of **2** (Table 3) displayed five tertiary methyl signals at δ 0.82, 1.00, 1.17, 1.28 and 1.36 and a vinylic methyl at δ 2.04. The resonance of a carbinol methine was at δ 3.85 (1H, *ddd*, J = 3.8, 11.2 and 12 Hz) which was assigned to H-6β as in the case of compounds **1a–1c**. There were also three olefinic protons, one at δ 5.79 (1H, *d*, J = 1.5 Hz, H-18) and the other two at δ 6.15

and 5.72 ($J = 16$ Hz) which indicated the presence of a *trans* double-bond between C-14 and C-15. The ^{13}C NMR spectrum of **2** displayed resonances of six methyl quartets, six methylene triplets, six methine doublets and seven quaternary singlets (Table 1). One of the quaternary carbons was at δ 107.3, indicating a hydroxyl group next to another oxygen function which should be situated at C-16. This compound, like yosgadensolide A (**1**), also had a sesterterpene structure derived from manoyloxide, but the side-chain differed from that of **1**. Compound **2** showed similarities both in the side chain and in the main skeleton to that of salviaethiopisolide (**3**) and its 13-epimer isolated from *S. aethiopis* [9], the only differences between **2** and **3** being presence of one hydroxyl group instead of a methoxyl, and the placement of the secondary hydroxyl group at C-6 instead of at C-3. Based on the spectral and literature [9] data, yosgadensolide B was established as 6α,16-dihydroxymanoyloxide-14,17-dien-16,19-olide.

Epimer **4** exhibited very similar spectral data to those of **2**. The IR, UV and mass spectra were exactly the same while the ^1H NMR data showed some chemical shift differences, particularly for H-14 (6.35), H-15 (5.52) and for some methyl groups. The two methyl groups of **4**, which are next to oxygenated carbons, were observed at δ 1.22 (Me-21) and 1.15 (Me-22) while in **2** they were at δ 1.36 and 1.28, indicating that the substance might be a C-13 epimer, a conclusion which was verified by an NOE. After acetylation, the H-6 resonance of both compounds was shifted to δ 5.08 while the other protons resonated more or less at the same values, with only the methyl groups exhibiting some chemical shift differences (Table 3). Based on the spectral data, compound **4** was established as 6α,16-dihydroxy-13-epi-manoyloxide-14,17-dien-16,19-olide.

This is the first isolation of sesterterpenes from a Turkish *Salvia* species. Sesterterpenes are not common in the genus *Salvia*, although several of them have been isolated from *S. aethiopis* [9], *S. hypoleuca* [10], *S. syriaca* [11], and *S. sahendica* [12].

Table 3. ^1H NMR assignments of compounds **2**, **4**, **2 Ac** and **4 Ac**

Proton	2	4	2 Ac	4 Ac
H-6	3.85 *ddd*	3.85 *ddd*	5.08 *ddd*	5.08 *ddd*
H-11	2.18 *dd*	2.11 *dd*	2.12 *dd*	2.12 *dd*
H-14	6.15 *d*	6.35 *d*	6.19 *d*	6.17 *d*
H-15	5.72 *d*	5.52 *d*	5.49 *d*	5.40 *d*
H-18	5.79 *d*	5.82 *br s*	5.90 *s*	5.90 *s*
H-20	2.04 *d*	2.01 *br s*	1.99 *s*	1.97 *s*
H-21	1.36 *s*	1.22 *s*	1.19 *s*	1.17 *s*
H-22	1.28 *s*	1.15 *s*	1.32 *s*	1.24 *s*
H-23	1.17 *s*	1.15 *s*	1.26 *s*	1.14 *s*
H-24	1.00 *s*	0.98 *s*	1.22 *s*	1.00 *s*
H-25	0.82 *s*	0.74 *s*	0.74 *s*	0.85 *s*
OAc	—	—	2.10 *s*	2.03 *s*

EXPERIMENTAL

General procedures. UV: Varian Techtron 685 in EtOH; IR: Perkin Elmer 980 in $CHCl_3$; ^1H and ^{13}C NMR: 200 and/or 400 MHz Bruker (for HMQC, HMBC and NOE experiments) with TMS as int. standard; CI- and HREI-MS: VG ZabSpec GC-MS instruments.

Plant material. The aerial parts of *S. yosgadensis* were collected from Central Turkey (near Sultanhani, Konya) and identified by Dr E. Tuzlaci and deposited in the Herbarium of faculty of Pharmacy, University of Istanbul (ISTE 50876).

Extraction and isolation. Powdered plant material (680 g) was extracted with distilled Me_2CO in a Soxhlet, the extract was evapd *in vacuo* to give 40 g of a residue, the latter was fractionated on a silica gel column (5.5 × 70 cm) and eluted with petrol, followed by a gradient of $CHCl_3$ up to 100% and then EtOH. After TLC application, similar frs were combined and further sepd on smaller silica gel columns and purified on Sephadex LH-20 columns using petrol–$CHCl_3$–MeOH (7:4:1) or on prep. TLC plates. The following compounds were obtained: **1** (25 mg), **1c** (6 mg), **2** (9 mg), **4** (8 mg), spathulenol (12 mg), Istanbulin D (9 mg), 1β-acetoxy-8β-hydroxyeudesm-4(15),7(11)-dien-8,12-olide (13 mg), 1β-acetoxyeudesm-4(15),7(11)-dien-8-12-olide (8 mg) and luteolin (10 mg), apigenin (14 mg), apigenin 7-methyl ether (12 mg), apigenin 4′-methyl ether (15 mg), apigenin 7,4′-dimethyl ether (14 mg), apigenin 6,4′-dimethyl ether (10 mg), salvigenin (18 mg), kaempferol 3-methyl ether (7 mg), *p*-hydroxyquinone (9 mg), *p*-acetoxyphenol (11 mg).

6α,14 - *Dihydroxymanoyloxide* - 15,17 - *dien* - 16,19 - *olide* (**1**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1780, 1615, 1480, 1385, 1280, 1230, 1140, 1030, 960, 850, 760. UV λ_{max}^{MeOH} (log ε) nm: 274 (3.8). ^1H and ^{13}C NMR (400 MHz, in $CDCl_3$, see Tables 1 and 2). HREI-MS *m/z* (rel. int.): 418.2700 $[M]^+$ ($C_{25}H_{38}O_5$) (4.1), 401 $[M - OH]^+$ (80.9), 383 $[401 - H_2O]^+$ (85.5), 365 $[383 - H_2O]^+$ (25.0), 355 (8.9), 291 (7.6), 277 (12.3), 261 (100), 251 (10.4), 243 (27.3), 217 (11.6), 203 (7.3), 191 (12.1), 177 (14.8), 165 (11.6), 151 (9.0), 139 (15.1), 109 (8.4), 99 (7.3).

Diacetyl derivative of **1a** (**1a Ac**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2927, 1780, 1740, 1612, 1462, 1380, 1242, 1030, 965, 860, 840, 760. ^1H and ^{13}C NMR (400 MHz, in $CDCl_3$, see Tables 1 and 2). EI-MS *m/z* (rel. int.): 460 $[M - HOAc]^+$(0.6), 442 $[460 - H_2O]^+$ (1.5), 427 $[442 - Me]^+$ (4.5), 383 $[M + 1\text{-}2HOAc]^+$ (11.2), 367 $[383 - Me]^+$ (13.3), 321 (27.4), 262 (87.6), 243 (42.3), 217 (13.9), 203 (90.2), 191 (47.3), 177 (92.4), 165 (37.6), 151 (33.5), 140 (100), 109 (87.3), 95 (46.8), 85 (34.0), 69 (42.2).

Diacetyl derivative of **1b** (**1b Ac**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3010, 1780, 1740, 1615, 1465, 1380, 1242, 1030, 965, 860, 840, 765: ^1H NMR (400 MHz, in $CDCl_3$, see Table 2). EI-MS *m/z* (rel. int.): 460 $[M - HOAc]^+$ (1.6), 442 $[460 - H_2O]^+$ (2.5), 427 $[442 - Me]^+$ (5.0),

383 $[M + 1\text{-}2HOAc]^+$ (14.3), 367 $[383 - Me]^+$ (15.3), 262 (93.6), 217 (12.9), 203 (91.8), 191 (49.1), 177 (94.5), 151 (33.5), 140 (100), 123 (49.4), 109 (87.3), 95 (46.8), 85 (36.0), 69 (43.2).

6α,14 - *Dihydroxy* - 13 - *epi* - *manoyloxide* - 15,17 - *dien* - 16,19 - *olide* (**1c**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1780, 1740, 1615, 1465, 1380, 1242, 1030, 965, 860, 840, 765. ^1H NMR (200 MHz, in $CDCl_3$, see Table 1). EI-MS *m/z* (rel. int.): 460 $[M - HOAc]^+$ (1.6), 442 $[460 - H_2O]^+$ (2.5), 427 $[442 - Me]^+$ (5.0), 383 $[M + 1\text{-}2HOAc]^+$ (14.3), 367 $[383 - Me]^+$ (15.3), 262 (93.6), 217 (12.9), 203 (91.8), (49.1), 177 (94.5), 151 (33.5), 140 (100), 123 (49.4), 109 (87.3), 95 (46.8), 85 (36.0), 69 (43.2).

6α,16 - *Dihydroxymanoyloxide* - 14,17 - *dien* - 16,19 - *olide* (**2**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1780, 1655, 1630, 1475, 1390, 1285, 1230, 1180, 1090, 1070, 985, 960, 900, 850. UV λ_{max}^{MeOH} (log ε) nm: 274 (3.8), 222 (4.2). ^1H and ^{13}C NMR (400 MHz, see Tables 1 and 3). HR-MS *m/z* (rel. int.): 418.2705 ($C_{25}H_{38}O_5$), CIMS: 419 $[M + 1]$ (3.8), 401 $[M - H_2O]^+$ (18.5), 383 (16.2), 367 (90.1), 349 (18.7), 261 (38.4), 203 (57.6), 190 (100), 135 (70.1), 119 (86.3), 69 (83.2).

Acetyl derivative of **2** (**2** Ac). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1790, 1735, 1475, 1390, 1260, 1180, 1090, 1070, 985, 960, 900, 840. ^1H NMR (200 MHz, see Table 3). EI-MS *m/z* (rel. int.): 460 (not observed), 446 $[M - Me - 1]^+$ (10.1), 426 $[M - Ac]^+$ (22.3), 383 $[M - OAc - OH]^+$ (24.2), 354 (76.0), 339 (40.1), 325 (36.2), 297 (26.4), 199 (37.0), 173 (56.3), 133 (63.1), 147 (98.5), 119 (80.8), 97 (100), 81 (78.3), 67 (49.2).

6α,16 - *Dihydroxymanoyloxide* - 13 - *epi* - 14,17 - *dien* - 16,19 - *olide* (**4**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1780, 1660, 1630, 1475, 1390, 1280, 1230, 1180, 1090, 985, 960, 900, 850. UV λ_{max}^{MeOH} (log ε) nm: 274 (2.8), 222 (4.1). ^1H NMR (400 MHz, see Table 3). HR-MS *m/z* (rel. int.): 418.2705 ($C_{25}H_{38}O_5$), CI-MS 419 $[M + 1]^+$ (3.8), 401 $[M - H_2O]^+$ (18.5), 383 (16.2), 367 (90.1), 349 (18.7), 261 (38.4), 203 (57.6), 190 (100), 135 (70.1), 119 (86.3), 69 (83.2).

Acetyl derivative of **4** (**4Ac**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1790, 1730, 1475, 1390, 1255, 1180, 1090, 1070, 985, 960, 900, 840. ^1H NMR (400 MHz, see Table 3). CI-MS *m/z* (rel. int.): 460 (not observed), 427 $[M - H_2O - Me + 1]^+$ (22.1), 383 $[M - OAc - OH]^+$ (16.2), 367 (89.0), 349 (20.1), 301 (6.2), 287 (8.4), 203 (57.9), 190 (100), 175 (53.3), 147 (48.6), 135 (70.8), 119 (85.8), 107 (62.3), 81 (64.3), 69 (82.6).

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