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NORSESTERTERPENES AND DITERPENES FROM THE AERIAL PARTS OF *SALVIA LIMBATA*

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Key Word Index—*Salvia limbata*; Lamiaceae; sesquiterpene; diterpenes; dinorsesterterpenes; triterpenes; flavonoids; 6-dehydroxy-yosgadensonol; 6-dehydroxy-13-*epi*-yosgadensonol.

Abstract—From the aerial parts of *Salvia limbata*, two new diterpenes (limbinal and acetyllimbinol) and two new dinorsesterterpenes (6-dehydroxy-yosgadensonol and 6-dehydroxy-13-*epi*-yosgadensonol) were isolated in addition to eight known terpenoids and four flavonoids. The structures of the new and the known compounds were established by spectral data. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In a previous study with the roots of *Salvia limbata* C. A. Meyer [1] we have isolated six new rearranged abietane diterpenoids, 12-hydroxysapriparaquinone, 3,12-dihydroxysapriparaquinone-1-ene, 2-hydroxysaprorthoquinone, limbinol, salvilimbinol and 4-dehydrosalvilimbinol. The present study with the aerial parts of the plant led to the isolation of four new compounds; two are epimeric dinorsesterterpenes (**1** and **2**) and the other two are rearranged abietane diterpenoids (**3** and **4**). In addition to the new compounds, one sesquiterpene, spathulenol [2], four diterpenes, ferruginol [3], abieta-8,11,13-triene [4], sclareol [5] and manool [6], and sitosterol and ursolic and oleanolic acids, as well as four flavonoids (salvigenin, luteoline, eupatilin and quercetin 3-methyl ether), were isolated.

RESULTS AND DISCUSSION

Aerial parts of *S. limbata* were extracted with acetone in a Soxhlet apparatus, evaporated to dryness and the residue fractionated in a silica gel column. The crude fractions yielded four new and 12 known compounds after VLC separation and preparative TLC. The new compounds were 6-dehydroxy-yosgadensonol (**1**), 6-dehydroxy-13-*epi*-yosgadensonol (**2**), limbinal (12-hydroxysapriparaquinone-16-al) (**3**) and acetyllimbinol (4,5-*seco*-5,10-friedo-2-acetyl-12-hydroxyabieta-3,5(10),6,8,11,13-hexaene-1-one) (**4**).

The HR mass spectrum of compound **1** indicated a molecular formula $C_{23}H_{38}O_2$ (*m/z* 346.2820, calc. 346.2871). The UV spectrum of **1** showed a maximum at 242 nm, indicating an enone group on the side chain as we observed in yosgadensenol (**5**) [7].

In the IR spectrum, the presence of an α,β-unsaturated ketone group was confirmed with the signal at 1698 cm^{-1}. The ^1H NMR spectrum of **1** showed the signals for unsaturation at δ 6.76 (1H, *d*, $J = 16$ Hz, H-14) and 6.24 (1H, *d*, $J = 16$ Hz, H-15) indicating a *trans* double bond, a methyl triplet at δ 1.10 (3H, *t*, $J = 7$ Hz, H-18) and a methylene quartet at δ 2.59 (2H, *q*, $J = 7$ Hz, H_2-17) which was assigned to a terminal ethyl group as observed in compound **5**. Other methyl signals were at δ 0.80, 0.85, 1.18, 1.27 and 1.34 (each 3H, *s*). Although the ^1H NMR data resembles those of **5**, an important signal for the proton next to the secondary hydroxyl group at δ 3.87 (1H, *ddd*, H-6β) was missing. The ^{13}C NMR signals were quite similar to those of **5** except for the signal at C-6; instead of a doublet at δ 69.2 there was a triplet at δ 18.2 in the present case. The correlation between carbons and protons were deduced by an HETCOR experiment (Table 1). The NMR spectral data, as well as the mass spectrum, indicated 6-dehydroxy-yosgadensonol as the structure for **1**.

The UV and IR spectra of **2** were similar to those of **1**. However, its ^1H NMR spectrum showed differences for H-14 and H-15 (δ 6.95 and 6.00), both doublets with $J = 16$ Hz values, as well as slight differences in some methyl signals observed at δ 0.78, 0.85, 1.15, 1.27 and 1.32 (each 3H, *s*), which indicated that **2** was the C-13 epimer of **1**. The ^{13}C NMR spectrum of **2** was

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Table 1. ^1H and ^{13}C NMR data for compounds **1–4**

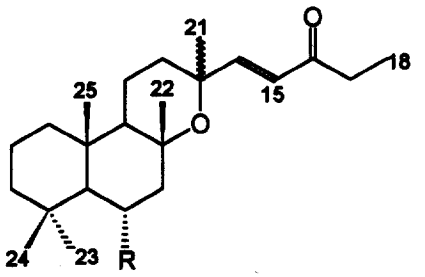
	1		**2**		**3**		**4**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1α	1.03 *ddd*	39.8 *t*	1.02 *ddd*	39.2 *t*	2.95 *m*	30.2 *t*	–	196.0 *s*
1β	1.65 *dt*		1.64 *dt*		–	–	–	
2α,β	1.50 *m*	18.2 *t*	1.50 *m*	18.2 *t*	2.30 *dd*	27.8 *t*	5.38 *d*	65.3 *d*
3α	1.17 *ddd*	43.5 *t*	1.18 *ddd*	43.6 *t*	5.45 *br d*	123.7 *d*	5.50 *dt*	125.2 *d*
3β	1.42 *m*		1.40 *m*					
4	—	32.9 *s*	–	32.7 *s*	–	126.3 *s*	–	126.3 *s*
5	1.00 *dd*	62.0 *d*	1.00 *dd*	61.9 *d*	–	144.4 *s*	–	144.6 *s*
6α	1.38 *m*	18.6 *t*	1.40 *m*	18.7 *t*	7.52 *d*	136.2 *d*	6.96 *d*	135.6 *d*
6β	1.60 *m*		1.60 *m*					
7α	1.45 *m*	42.2 *t*	1.48 *m*	42.6 *t*	7.82 *d*	126.4 *d*	7.12 *d*	130.4 *d*
7β	1.80 *dd*		1.82 *dd*					
8	–	74.7 *s*	–	75.6 *s*	–	136.3 *s*	–	135.6 *s*
9	1.19 *dd*	54.7 *d*	1.25 *dd*	57.6 *d*	–	132.5 *s*	–	130.8 *s*
10	–	37.5 *s*	–	37.9 *s*	–	143.2 *s*	–	125.2 *s*
11α	0.94 *m*	16.0 *t*	1.05 *m*	16.0 *t*	–	184.2 *s*	7.10 *d*	127.1 *s*
11β	1.57 *m*		1.54 *m*					
12α	2.11 *m*	34.3 *t*	2.20 *m*	36.4 *t*	–	153.2 *s*	–	136.3 *s*
12β	2.32 *m*		2.43 *m*					
13	–	73.0 *s*	–	72.5 *s*	–	132.5 *s*	–	125.2 *s*
14	6.76 *d*	154.2 *d*	6.95 *d*	154.6 *d*	–	183.8 *s*	6.90 *d*	126.3 *d*
15	6.24 *d*	125.3 *d*	6.00 *d*	125.2 *d*	2.95 *m*	24.4 *d*	2.95 *dsept*	27.1 *d*
16	–	195.3 *s*	–	199.3 *s*	10.69 *br s*	201.9 *d*	1.15 *d*	19.8 *q*
17	2.59 *q*	33.8 *t*	2.61 *q*	35.1 *t*	1.32 *d*	19.8 *q*	1.15 *d*	20.1 *q*
18	1.10 *t*	8.3 *q*	1.11 *t*	8.1 *q*	1.78 *s*	26.9 *q*	1.77 *s*	26.8 *q*
19	–	–	–	–	1.83 *s*	17.6 *q*	1.80 *s*	17.6 *q*
20	–	–	–	–	2.40 *s*	20.3 *q*	2.20 *s*	20.3 *q*
21	1.27 *s*	28.8 *q*	1.27 *s*	25.3 *q*	–	–	–	–
22	1.34 *s*	26.8 *q*	1.32 *s*	28.8 *q*	–	–	–	–
23	1.18 *s*	36.4 *q*	1.15 *s*	37.5 *q*	–	–	–	–
24	0.85 *s*	21.8 *q*	0.85 *s*	21.8 *q*	–	–	–	–
25	0.80 *s*	16.6 *q*	0.78 *s*	17.2 *q*	–	–	–	–
C=O |	–	–	–	–	–	–	–	170.1 *s*
Me	–	–	–	–	–	–	2.18 *s*	23.1 *q*

J (Hz). **1** and **2**: 1α, 1β = 13; 1α, 1β = 15; 1α,2α = 4; 2α,3β = 14; 3α,3β = 13.5; 5,6β = 12; 5,6α = 2.5; 6β, 7β = 2.3; 7α, 7β = 12; 9α, 11α = 2.5; 9α, 11β = 12; 17, 18 = 7. **3**: 2α, 2β = 11, 2, 3 = 8; 6, 7 = 8; 15, 17 = 7. **4**: 2,3 = 10; 6, 7 = 8; 14, 15 = 1; 15, 16 = 15, 17 = 7.

also similar to that of **1**; carbon and proton correlations were decided by an HETCOR experiment (Table 1). In a previous study with *S. yosgadensis* we isolated norsesterterpenes [7] and this is now the second Turkish *Salvia* species in which norsesterterpenes are found.

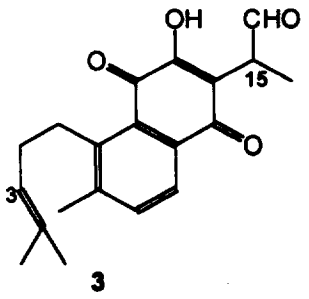
The HR mass spectrum of **3** (limbinal) indicated a molecular formula $C_{20}H_{22}O_4$ (*m/z* 326.1510, calc. 326.1517). The UV spectrum exhibited a long conjugation at 400 (sh), 339 and 230 nm, showing the quinoid character of the compound. The IR absorptions were at 3405 cm^{-1} (OH), 1694, 1685, 1650 and 1594 cm^{-1}, confirming the *p*-quinoid structure. The ^1H NMR spectrum displayed methyl signals at δ 1.32, 1.78, 1.83 and 2.40 (each 3H, *s*), indicating Me-17, Me-18, Me-19 and Me-20, respectively. However, instead of a further methyl signal, an aldehyde signal was present at δ 10.69 (1H, *br s*), which should be assigned to C-16. Other signals typical for 12-hydroxysapriparaquionone were observed at δ 7.82 (1H, *d*, *J* = 8 Hz, H-7), 7.52 (1H, *d*, *J* = 8 Hz, H-6), 5.45 (1H, *dd*, *J* = 2 and 8 Hz, H-3) and 2.95 (3H, *m*, H-15 and C-1 protons). The ^{13}C NMR spectrum of **3** confirmed the presence of an aldehyde group by giving a signal at δ 201.9; the carbonyls of the *p*-quinone group were at δ 184.2 and 183.8. Other signals were more or less similar to those of 12-hydroxysapriparaquinone [1] (Table 1). The spectral data indicated that **3** is a rearranged abietane type compound and it was identified as 12-hydroxysapriparaquinone-16-al.

Compound **4** had a molecular formula $C_{22}H_{26}O_4$ (*m/z* 354.1815, calc. 354.1831) as decided from its HR mass spectrum. The IR spectrum indicated the presence of hydroxyl (3426 cm^{-1}), acetyl (1720 and 1255 cm^{-1}) and conjugated carbonyl (1693 cm^{-1}) groups. The UV spectrum exhibited a conjugated aromatic system giving a maximum at 370 nm. The ^1H NMR spectrum of **4** displayed the structure quite clearly with the signals at δ 7.12 (1H, *d*, *J* = 8 Hz, H-7), 7.10 (1H, *s*, H-11), 6.96 (1H, *d*, *J* = 8 Hz, H-6), 6.90 (1H, *d*, *J* = 1 Hz, H-14), 5.50 (1H, *dt*, *J* = 1 and 10 Hz, H-3), 5.38 (1H, *d*, *J* = 10 Hz, H-2), 2.95 (1H, *d septet*, *J* = 1 and 7 Hz,



1 and 2 R=H

5 R=OH



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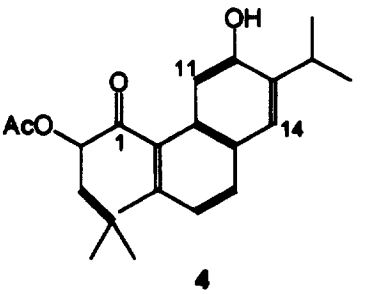

4

H-15), 2.20 (3H, *s*, Me-20), 2.18 (3H, *s*, OAc), 1.80 (3H, *d*, $J = 1$ Hz), 1.77 (3H, *s*) (Me-18 and Me-19) and 1.15 (6H, *dd*, $J = 1$ and 7 Hz) (Me-16 and Me-17). The ^{13}C NMR spectrum of **4** showed the presence of six methyl quartets, seven methine doublets and nine carbon singlets, the carbonyl being at δ 196.0, the acetyl carbonyl at δ 170.1 and four methine protons in the lower field at δ 135.6, 130.4, 126.3 and 127.1 and two in the upper field at δ 65.3 (C-2) and 27.1 (C-15), which supported a rearranged abietane skeleton with aromatic B and C rings (Table 1).

EXPERIMENTAL

General. Spectra were recorded with the following instruments. UV: Varian Techtron 635. IR: Perkin-Elmer 983. ^1H and ^{13}C NMR: Bruker AC 200 L. HRMS: VG ZabSpec. Silica gel 40 (E. Merck) and Sephadex LH 20 (Fluka) were used for CC sepns; for prep. sepns ready made plates (E. Merck) and for VLC Kieselgel 60 PF 254+366 were used.

Plant material. The aerial parts of *S. limbata* C. A. Meyer were collected from eastern Turkey (Ilıca-Erzurum) at elevations of 1800–2000 m in July 1994. The plant was identified by Dr Kerim Alpınar (Istanbul). A voucher specimen is deposited at the Herbarium of the Faculty of Istanbul (ISTE 66394).

Extraction and isolation. The dried and powdered plant material (960 g) was extracted with Me_2CO in a Soxhlet apparatus to yield a residue (55 g). After preliminary sepn in a silica (5 × 70 cm) column similar frs were combined and these were then sepd on VLC columns while some frs were sepd on Sehadex LH 20 using petrol–$CHCl_3$–MeOH (7:4:1) with final purification of the compounds by prep. TLC. The following compounds were obtained: 8,11,13-abietatrien (22 mg), ferruginol (18 mg), spathulenol (10 mg), **1** (12 mg), **2** (5 mg), **3** (10 mg), **4** (17 mg), sitosterol (25 mg), ursolic acid (37 mg), luteolin (12 mg), eupatilin (15 mg), quercetin 3-methyl ether (10 mg) and salvigenin (8 mg).

6-Dehydroxyyosgadensonol (**1**). UV λ_{max}^{MeOH} (log ε) nm: 242 (3.6). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940, 2866, 1698, 1674, 1456, 1413, 1388, 1150, 1120, 1090, 1030, 860, 800. ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): Table 1. HRMS *m/z* (rel. int.): 346.2820 $[M]^+$ ($C_{23}H_{38}O_2$) (23), 331 $[M-Me]^+$ (100), 313 $[M-Me-H_2O]^+$ (93), 261 [M–side chain–2H]$^+$ (55), 221 (37), 192 (78), 177 (65), 123 (72), 95 (77), 82 (82), 69 (83).

6-Dehydroxy-13-epi-yosgadensonol (**2**). UV λ_{max}^{MeOH} (log ε) nm: 242 (3.6). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2942, 2866, 1698, 1675, 1456, 1410, 1387, 1150, 1120, 1090, 1030. ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): Table 1. HRMS *m/z* (rel. int.): 346.2822 $[M]^+$ ($C_{23}H_{38}O_2$) (30), 331 $[M-Me]^+$ (100), 313 $[M-Me-H_2O]^+$ (80), 261 $[M-\text{side chain}-2H]^+$ (40), 221 (50), 205 (18), 192 (78), 177 (65), 109 (62), 95 (75), 69 (80).

Limbinal (**3**). UV λ_{max}^{MeOH} (log ε) nm: 400 (sh), 339 (3.2), 230 (4.0). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3405, 2980, 2840, 1694, 1685, 1650, 1594, 1470, 1380, 1050, 880. ^1H

NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): Table 1. HRMS *m/z* (rel. int.): 326.1510 $[M]^+$ ($C_{20}H_{22}O_4$) (32), 311 $[M - Me]^+$ (14), 242 [M − side chain − H]$^+$ (88), 185 $[242 - C_3H_4O]^+$ (26), 176 (32), 115 (30), 83 (35), 69 (100), 57 (72).

Acetyllimbinol (**4**). UV λ_{max}^{MeOH} (log ε) nm: 370 (3.0), 338 (3.2), 240 (4.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3426, 2960, 2870, 1720, 1693, 1462, 1383, 1278, 1255, 1175, 1074, 1040, 910, 816. ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): Table 1. HRMS *m/z* (rel. int.): 354.1815 $[M]^+$ ($C_{22}H_{26}O_4$) (25), 339 $[M - Me]^+$ (20), 311 $[M - Ac]^+$ (30), 295 $[M - OAc]^+$ (48), 267 (80), 227 (100), 211 (48), 183 (40), 165 (25), 128 (28), 83 (45), 69 (90).

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REFERENCES

1. Topcu, G., Eriş, C. and Ulubelen, A. (1996) *Phytochemistry* **41**, 1143.
2. Bowyer, R. C. and Jeffries, P. R. (1963) *Chem. Ind. (Lond.)* 145.
3. Cambie, R. C., Madden, R. J. and Parnell, J. C. (1971) *Aust. J. Chem.* **24**, 217.
4. Kitadani, M., Yoshikoshi, A., Kitahara, Y., de Paira Campello, J., McChesney, J. D., Watts, D. J. and Wenkert, E. (1970) *Chem. Pharm. Bull. (Jpn)* **18**, 402.
5. Janot, M. M. (1932) *Ann. Chem.* **17**, 5.
6. Barrero, A. F., Sanchez, J. F., Alvarez-Manzeneda, E. F., Dorado, M. M. and Haidour, A. (1993) *Phytochemistry* **32**, 1261.
7. Topcu, G., Ulubelen, A., Tam, T. C.-M. and Che, C.-T. (1996) *J. Nat. Prod.* **59**, 113.