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# TERPENOIDS FROM *SALVIA CANDIDISSIMA* SUBSP. *CANDIDISSIMA*

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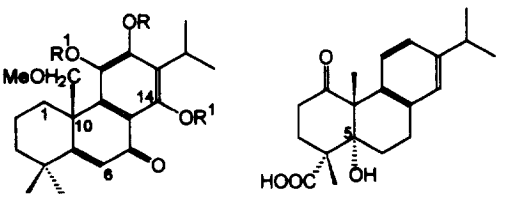
**Key Word Index**—*Salvia candidissima* subsp. *candidissima*; Lamiaceae; abietane; rearranged abietane; pimarane diterpenoids; steroidal ester.

**Abstract**—From the roots of *Salvia candidissima* Vahl subsp. *candidissima*, in addition to diterpenoids, two new diterpenes, a new steroidal ester and α-amyrin acetate were isolated. The structures were established by spectral data and by some chemical reactions. © 1997 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

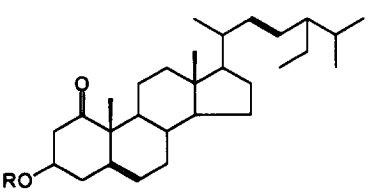
The roots of *Salvia candidissima* Vahl subsp. *candidissima* afforded 20 diterpenoids, two of them being new, along with a new steroidal ester, the triterpene α-amyrin acetate, and the flavone salvigenine. Eight of the diterpenes had been isolated from *S. candidissima* subsp. *occidentalis* Hedge [1–3]: 11-hydroxy-12-methoxy-abieta-8,11,13-triene, salvipisone, microstegiol, ferruginol [1], candidissiol, manoyl oxide, pachystazone [2] and 11β-hydroxymanoyl oxide [3]. The additional diterpenes obtained from this subsp. were 7,20-epoxyroyleanone, 11,12-dioxo-abieta-8,13-dien [4], 2,3-dehydrosalvipisone [5], salvinolone [6], salvinolone 12-methyl ether [7], cryptojaponol [8], manool [9], 1-oxo-salvibretol [10] and *ent*-sclareol [11] together with the new diterpenes (**1**, **2**) and the new steroidal ester (**3**). The structures of the new and the known compounds were established by using spectral data and by some chemical reactions. The known compounds were also compared on TLC plates with authentic samples.



**1** R=R¹=H
**1a** R=Ac, R¹=H
**1b** R=R¹=Ac



**2**



**3** R=OCO(CH2)14CH3
**3a** R=H

## RESULTS AND DISCUSSION

The HREI mass spectrum of the first new diterpenoid (**1**) indicated a molecular formula $C_{21}H_{28}O_5$ (*m/z* 360.1927, calc. 360.1934). The $^1H$ NMR spectrum of **1** showed signals at δ 7.06, 6.70 and 5.96 (each 1H, *s*), 3.72 (3H, *s*, OMe), 3.56 (1H, *d*, $J = 14$ Hz), 3.60 (1H, *d*, $J = 14$ Hz) ($CH_2$-20), 3.15 (1H, *sept*, $J = 7$ Hz, H-15), 3.10 (1H, *br d*, $J = 13$ Hz, H-1β), 1.22 and 1.20 (each 3H, *d*, $J = 7$ Hz, Me-16 and Me-17), and 0.90 and 0.92 (each 3H, *s*, Me-18 and Me-19). A signal at δ 13.25 (1H, *br s*) indicated a hydrogen bond between the carbonyl group at C-7 and the hydroxyl group at C-14. $D_2O$ exchange showed that the two signals at δ 6.70 and 7.05 (corresponding to three hydroxyl groups) disappeared while the signal at δ 5.96 remained unchanged and was placed to C-6 from the $^1H$ (5.96 *s*) and $^{13}C$ NMR (145.5 *s* C-5, 126.9 *d* C-6) spectral data. Spin decoupling experiments showed the relationships between H-1β (δ 3.10) and H-1α (δ 2.08), between H-1α and H-2a (δ 1.45), and between H-2a and H-3 (δ 2.40). The signal at δ 3.10 for H-1β indicated the presence of a hydroxyl group at C-11 [12]. The other four oxygen functions were placed at C-12, C-14 as two hydroxyl groups and at C-7 as an oxo group (IR, 1640 $cm^{-1}$, $^{13}C$ NMR δ 184.2), with the last oxygen function at C-20 as a hydroxymethylene

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Table 1. $^{13}C$ NMR of compounds **1**, **2** and **3**

| | | | |
|---|---|---|---|
| 1 | 35.9 *t* | 204.2 *s* | 206.0 *s* |
| 2 | 19.3 *t* | 37.8 *t* | 38.2 *t* |
| 3 | 41.4 *t* | 36.6 *t* | 73.6 *d* |
| 4 | 33.3 *s* | 29.9 *s* | 36.6 *t* |
| 5 | 145.5 *s* | 74.2 *s* | 139.1 *s* |
| 6 | 126.9 *d* | 18.5 *t* | 122.6 *d* |
| 7 | 184.2 *s* | 21.7 *t* | 31.9 *t* |
| 8 | 133.0 *s* | 133.4 *s* | 34.7 *d* |
| 9 | 139.2 *s* | 138.6 *s* | 56.7 *d* |
| 10 | 39.9 *s* | 36.7 *s* | 36.1 *s* |
| 11 | 157.4 *s* | 123.8 *d* | 19.8 *t* |
| 12 | 155.0 *s* | 124.1 *d* | 39.7 *t* |
| 13 | 139.0 *s* | 139.0 *s* | 42.3 *s* |
| 14 | 157.6 *s* | 126.8 *d* | 50.1 *d* |
| 15 | 33.2 *d* | 44.6 *d* | 27.8 *t* |
| 16 | 22.3 *q* | 21.7 *q* | 21.1 *t* |
| 17 | 22.0 *q* | 23.9 *q* | 56.0 *d* |
| 18 | 15.7 *q* | 180.2 *s* | 18.7 *q* |
| 19 | 26.4 *q* | 33.4 *q* | 14.1 *q* |
| 20 | 64.1 *t* | 16.2 *q* | 45.6 *d* |
| 21 | — | — | 18.7 *q* |
| 22 | — | — | 129.9 *d* |
| 23 | — | — | 129.7 *d* |
| 24 | — | — | 50.0 *d* |
| 25 | — | — | 24.3 *t* |
| 26 | — | — | 11.6 *q* |
| 27 | — | — | 34.7 *d* |
| 28 | — | — | 19.8 *q* |
| 29 | — | — | 21.0 *q* |
| OMe | 61.3 *q* | — | — |
| C=O 1′ | — | — | 173.3 *s* |
| $CH_2$ (2′-14′) | — | — | 29.6 *t* |
| $CH_2$ 15′ | — | — | 22.6 *t* |
| Me 16′ | — | — | 11.9 *q* |

group. Acetylation under normal conditions yielded a monoacetate (**1a**), while drastic acetylation gave a triacetylated compound (**1b**). These findings correlate with the $D_2O$ experiment. After acetylation the chemical shift of the signal of $CH_2$-20 did not change indicating the presence of the methoxyl group at that position. In the $^1H$ NMR spectrum of **1a** the signal at $\delta$ 6.70 disappeared and an acetyl signal was observed at $\delta$ 2.32 (3H, *s*), while in **1b** both signals at $\delta$ 6.70 and 7.05 disappeared and the signals for three acetyl groups were observed at $\delta$ 2.35 (3H, *s*) and 2.50 (6H, *s*). The $^{13}C$ NMR (APT) spectrum showed C-20 at $\delta$ 64.1 (*t*) and OMe at $\delta$ 61.3 (*q*). The other signals were in agreement with the suggested structure of compound **1** (7-oxo-11,12,14-trihydroxy-20-methoxyhydroxy-methylene-abieta-5,8,11,13-tetraene) (Table 1).

The second new diterpenoid (**2**) was the 5-hydroxyl derivative of the known compound 1-oxo-abieta-8,11,13-trien-18-oic acid (**4**) [13]. The HREI mass spectrum of **2** indicated a molecular formula $C_{20}H_{26}O_4$ (*m/z* 330.1958, calc. 330.1970). The $^1H$ NMR spectrum was the same to that of **4** at $\delta$ 7.17 (1H, *d*, $J = 8$ Hz, H-11), 7.0 (1H, *dd*, $J = 2$ and 8 Hz, H-12) and 6.88 (1H, *d*, $J = 2$ Hz), 2.8 (1H, *septet*, $J = 7$ Hz, H-15), 1.28 (3H, *s*, Me-20), 1.22 (6H, *d*, $J = 7$ Hz, Me-16 and Me-17) and 1.20 (3H, *s*, Me-19). The IR spectrum of **2** indicated the presence of a carbonyl at 1720 $cm^{-1}$ and a carboxyl at 1695 $cm^{-1}$. The $^{13}C$ NMR spectrum showed the carbonyl group at $\delta$ 204.2 and the carboxyl group at $\delta$ 180.2. The carbonyl group could be placed at one of the following positions: C-1, C-2, C-3, C-6 and C-7. Since the chemical shift of H-14 was at $\delta$ 6.88 its presence at C-7 was unlikely, if the carbonyl group was at C-6 the benzylic protons of C-7 should be seen at about $\delta$ 2.5 and 3.0 as well divided doublets, there were no such signals in its $^1H$ NMR spectrum. If the carbonyl group was at C-2, two isolated methylene signals for C-1 and C-2 protons should be observed separately, also as well divided doublets. From the two remaining positions, C-1 and C-3, the first was more likely due to the chemical shift of H-11 to $\delta$ 7.17 instead of being at about $\delta$ 6.6–6.8. The carboxylic acid had to be situated either at C-4 or at C-10, if it was at C-10 the signals of the two methyl groups at C-4 would come close together which was not the case. The difference between compounds **2** and **4** followed from the hydroxyl group which was observed in the IR (3450 $cm^{-1}$) and the $^{13}C$ NMR ($\delta$ 74.2 *s*) spectra, but it did not show a geminal hydrogen in its $^1H$ NMR spectrum, therefore it should be tertiary. The only plausible place for the hydroxyl was at C-5. The spectral evidence indicated that compound **2** was 1-oxo-5-hydroxy-abieta-8,11,13-trien-18-oic acid.

The new steroidal compound was established as 1-oxostigmasterol 3$\beta$-palmitate (**3**). The HREI mass spectrum indicated a molecular formula $C_{45}H_{76}O_3$ (*m/z* 664.5789, calc. 664.5794). The structure of **3** was deduced mainly from the $^1H$ and $^{13}C$ NMR spectral data as well as by hydrolysis and comparison its acid part with an authentic sample. The $^1H$ NMR spectrum indicated the following signals at $\delta$ 5.35 (1H, *d*, $J = 4$ Hz, H-6), 5.30 (1H, *dd*, $J = 5$, 11 Hz, H-23), 5.42 (1H, *dd*, $J = 4$, 11 Hz, H-24), 4.60 (1H, *m*, H-3$\alpha$). Two methyl singlets were observed at $\delta$ 0.67 (3H, *s*, Me-19) and 1.05 (3H, *s*, Me-18), three methyl doublets at $\delta$ 0.92, 0.88 and 0.84 (each 3H, *d*, $J = 7$ Hz), a methyl triplet at $\delta$ 0.82 ($J = 7$ Hz) and the signals for the palmitic acid part were at $\delta$ 1.24 [26H, *br s*, $(CH_2)_{13}$], 1.57 (2H, *q*, $J = 7$ Hz, $CH_2$-2′), 0.75 (3H, *t*, $J = 7$ Hz, Me-16′). Spin decoupling experiments indicated the relationships between H-3 and H-2a ($\delta$ 2.75) and H-2b ($\delta$ 1.30), between H-6 ($\delta$ 5.35) and H-7 ($\delta$ 2.2). The IR spectrum of **3** showed an ester carbonyl at 1736 $cm^{-1}$, no hydroxyl signal was present. The $^{13}C$ NMR (APT) spectrum showed the six-membered ring carbonyl at $\delta$ 206.0 (*s*) and the ester carbonyl at $\delta$ 171.3 (*s*). Unsaturation signals were observed at $\delta$ 139.1 (*s*, C-5), 122.6 (*d*, C-6), 129.7 (*d*, C-24) and 129.9 (*d*, C-23). C-3 was observed at $\delta$ 73.6 as a doublet, and all other signals were in agreement with the suggested structure (Table 1). Hydrolysis of compound **3** yielded the aglycone **3a**, the $^1H$ NMR spectrum of which was quite similar to that of **3**, except the signal for H-3

was shifted upfield to $\delta$ 3.48 (1H, *m*) and the long chain signal of palmitic acid disappeared. After methylation, the palmitic acid part on GS-mass spectrometry gave a $[M]^+$ peak at *m/z* 270.2542 (calc. for $C_{17}H_{34}O_2$ 270.2558), and the fragmentation pattern was in agreement with the suggested formula.

## EXPERIMENTAL

*Plant material.* Roots of *Salvia candidissima* subsp. *candidissima* syn. *S. candidissima* Vahl var. *cordifolia* were collected from southern Turkey (Isparta-Eğirdir) in July 1991. They were identified by Dr Kerim Alpinar, and a voucher specimen is deposited in the Herbarium of Faculty of Pharmacy, University of Istanbul ISTE 63077.

*Extraction and isolation.* Powdered roots of the plant (700 g) were extracted with redistilled $Me_2CO$ in a Soxhlet, the extract was evapd *in vacuo* to yield 10.5 g of a residue which was fractionated on a silica gel column (3 × 70 cm) and eluted with petrol followed by EtOAc up to 100% and with EtOH. Similar frs were combined and sepd on a chromatotron. Compounds were obtained in the following order: 11-hydroxy-12-methoxy-abieta-8,11,13-trien (20 mg), microstegiol (25 mg), manoyl oxide (15 mg), 11,12-dioxo-abieta-8,13-dien (7 mg), **3** (23 mg), candidissiol (9 mg), salvipisone (10 mg), 2,3-dehydrosalvipisone (13 mg), paschytazone (20 mg), **1** (12 mg), 7,20-epoxy-royleanone (9 mg), 11$\beta$-hydroxymanoyl oxide (15 mg), *ent*-sclareol (30 mg), salvinolone 12-methyl ether (14 mg), $\alpha$-amyrin acetate (12 mg), crytojaponol (5 mg), ferruginol (10 mg), manool (15 mg), salvinolone (10 mg), salvigenine (25 mg), 1-oxo-salvibretol (6 mg) and **2** (15 mg).

*Compound* **1**. $[\alpha]_D$ +8.4° ($CHCl_3$ *c*, 0.1); UV $\lambda^{MeOH}$ (log $\varepsilon$) nm: 345 (3.6), 280 (2.2), 222 (3.8); IR $\nu^{CHCl_3}$ $cm^{-1}$: 3500, 3440, 2960, 2920, 2870, 1640, 1610, 1460, 1420, 1330, 1240, 1100, 1080; $^1H$ NMR: text; $^{13}C$ NMR: Table 1; HREIMS *m/z* (rel. int.): 360.1927 $[M]^+$ (93), 316 $[M-CH_2OMe+H]^+$ (100), 299 (62), 267 (50), 229 (68), 199 (22), 177 (22), 115 (10), 83 (18), 69 (22).

*Compound* **2**. UV $\lambda^{MeOH}$ nm: 267 (log $\varepsilon$), 220 (log $\varepsilon$); IR $\nu^{CHCl_3}$ $cm^{-1}$: 3450, 3050, 2960, 2920, 2860, 1720, 1695, 1605, 1580, 1510, 1380, 1270, 1150, 1060, 880; $^1H$ NMR ($CDCl_3$): text; $^{13}C$ NMR: Table 1; HREIMS *m/z* (rel. int.): 330.1958 $[M]^+$ (52), 300 $[M-2xMe]^+$ (60), 285 $[M-CO_2H]^+$ (100), 239 (95), 197 (53), 141 (30).

*Compound* **3**. $[\alpha]_D$ −5.5° ($CHCl_3$ *c*, 0.2); UV $\lambda^{MeOH}$ (log $\varepsilon$) nm: 251 (3.2); IR $\nu^{CHCl_3}$ $cm^{-1}$: 2922, 2851, 1736, 1720, 1465, 1378, 1011, 924; $^1H$ NMR: text; $^{13}C$ NMR: Table 1; HREIMS *m/z* (rel. int.) 664.5789 $[M]^+$ (6), 525 $[M-C_{10}H_{19}]^+$ (2), 409 $[M-C_{16}H_{31}O_2]^+$ (12), 394 $[409-Me]^+$ (100), 382 (35), 289 (7), 255 (15), 218 (46), 109 (13), 95 (15), 81 (15).

*Hydrolysis of* **3**. 10 mg of **3** was refluxed with 5% NaOH for 2 hr. The aglycone (**3a**) part was extracted with $Et_2O$. The IR and $^1H$ NMR spectra of **3a** were found to be quite similar to those of **3** with the lack of 1736 $cm^{-1}$ peak in the IR and with the shift of H-3 to $\delta$ 3.48. The acid part, obtained after acidification of the soln with HCl and extraction with $CHCl_3$, was dissolved in MeOH and methylated with $CH_2N_2$. GS-MS *m/z* (rel. int.): 270 $[M]^+$ (36), 239 $[M-OMe]^+$ (22), 211 $[M-COOMe]^+$ (6), 199 $[M-CH_2COOMe+2H]^+$ (10), 185 $[199-CH_2]^+$ (15), 171 $[185-CH_2]^+$ (13), 157 $[171-CH_2]^+$ (6), 143 $[157-CH_2]^+$ (45), 129 $[143-CH_2]^+$ (20), 87 $[C_6H_{13}]^+$ (100), 74 (60).

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