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# SALVIANDULINE C, A 5,6-SECOCLERODANE DITERPENOID FROM *SALVIA LAVANDULOIDES**

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**Key Word Index**—*Salvia lavanduloides*; Labiatae; diterpene; 5,6-secoclerodane.

**Abstract**—The structure and configuration of salvianduline C, a novel 5,6-secoclerodane diterpenoid isolated from *Salvia lavanduloides*, have been established by high resolution NMR spectroscopy and X-ray crystallographic analysis.

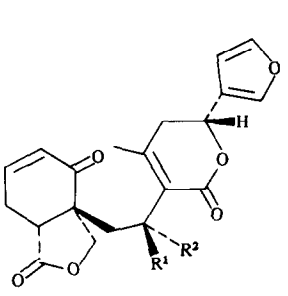
## INTRODUCTION

Previously [1], we described the characterization of salviandulines A (**1**) and B (**2**), two 9,10-secoclerodane diterpenoids from the aerial parts of *Salvia lavanduloides* L. We now describe the isolation and structural elucidation of a further secoditerpenoid, salvianduline C (**3**), from the same plant.

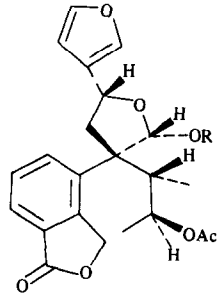
## RESULTS AND DISCUSSION

The new compound (**3**) had the molecular formula $C_{22}H_{24}O_7$ by mass spectrometry ($[M]^+$ at *m/z* 400). Its IR spectrum showed absorptions for a hydroxyl group, an $\alpha$-$\beta$ unsaturated-$\gamma$-lactone, an ester and a $\beta$-substituted furan ring. The $^1$H–H and $^1$H$^{13}$C COSY and COLOC spectra allowed us to propose the secoclerodanic structure **3** for salvianduline C.

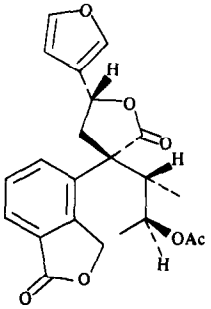
The $^{13}$C NMR spectrum (Table 1) revealed the presence of a trisubstituted benzene. Thus it contained six aromatic carbons signals, three as singlets at $\delta$126.0 (C-4), $\delta$145.2 (C-5) and $\delta$136.5 (C-10) and three as doublets at $\delta$135.4 (C-1), $\delta$128.7 (C-2) and $\delta$124.2 (C-3). The last signal correlates in the $^1$H-$^{13}$C COSY spectrum with a double doublet signal at $\delta$7.82 ($J$=6 and 3 Hz, H-3) which is *ortho* to H-2 ($\delta$7.46 *t*, $J$=6 Hz) and *meta* to H-1 ($\delta$7.4–7.5), so establishing the substitution pattern of the aromatic ring as vicinal trisubstituted. This aromatic ring should correspond to the ring A of a clerodane diterpene in which the aromatization of ring A proceeds with cleavage of the C-5/C-6 linkage, but retention of the C-18 C-19-$\gamma$-lactone ring system (IR 1762 cm$^{-1}$; $^{13}$C: $\delta$170.2 (C-18), $\delta$71.3 (C-19); $^1$H NMR $\delta$5.57 *d*, $J$=15 Hz (H-19), $\delta$5.46 *d*, $J$=15 Hz (H-19'), the presence of which is frequently present in clerodanes from Mexican *Salvia* species [2]. Furthermore, the above assumptions also account for the presence of a primary and secondary methyl groups in salvianduline C. The primary methyl group was responsible for the doublet at $\delta$1.23 ($J$=6.5 Hz, H-6) and the signal at $\delta$20.1 (C-6) in the $^1$H and $^{13}$C NMR spectra. It was located at C-7 and geminal to an oxygenated function, because of the chemical shift of the H-7 signal, which appeared as a quartet at $\delta$5.28 ($J$=6.5 Hz). The secondary methyl group was located at C-8, as the H-8 signal was also a quartet ($\delta$1.93, $J$=7 Hz)



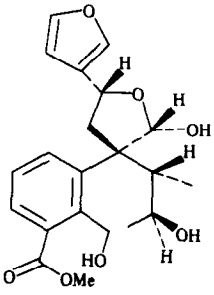
| | R¹ | R² |
|---|---|---|
| **1** | H | OAc |
| **2** | OH | H |



| | R |
|---|---|
| **3** | H |
| **4** | OAc |



**5**



**6**

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and the $^{13}C$ signal for this methyl (C-17) was observed at $\delta$10.0. It is interesting that there was no coupling between H-7 and H-8, indicating a rigid conformation of the chain (C-6/C-9) bonded to C-10. This can be explained if the acetoxy group (IR 1728 $cm^{-1}$; $^{13}C$ $\delta$170.2 and 20.7) is located at C-7. At this position, the ester group can settle over the aromatic ring, which induces a strong diamagnetic shift upon the acetyl methyl group ($\delta$1.38) [3, 4]. The NMR spectra also showed signals attributable to a $\beta$-substituted furan at $\delta$6.44 (H-14), 7.38 (H-15) and 7.44 (H-16), and $\delta$126.9, 109.0, 143.9 and 140.2 (C-13 to C-16, respectively).

The remaining signals correspond to a $\gamma$-lactol closed to C-12 ($\delta$72.2). The H-12 signal appeared as a double doublet at $\delta$5.18 coupled with H-11$\beta$ ($\delta$2.92) and H-11$\alpha$ ($\delta$2.61) in an axial-equatorial and axial-axial relationship. The hemiacetalic proton, H-20, appeared as a doublet at $\delta$5.77 by interaction with the hydroxylic proton ($\delta$2.88). From the above data structure **3** was proposed for salvianduline C.

Acetylation of **3** confirmed the presence of the C-20 hydroxyl group, because in the $^1H$ NMR spectrum of the acetoxy derivative **4**, the H-20 signal was shifted downfield, appearing at $\delta$6.82 and the signals for the methyl acetates at C-7 and C-20 were observed at $\delta$1.35 and 1.77, respectively. In this spectrum, the signals for the aromatic protons H-1, H-2 and H-3 were clearly observed at $\delta$7.3 (*dd* $J = 7$ and 2 Hz), $\delta$7.48 (*t*, $J = 7$ Hz) and $\delta$7.82 (*dd*, $J = 7$ and 2 Hz), thus confirming the existence of a vicinal trisubstituted benzene ring.

The formation of the dilactone **5** on oxidation of **3**, gave further support to the proposed structure. In the $^1H$ NMR spectrum of **5**, the H-20 signal was not observed and the H-12 signal was shifted downfield ($\delta$5.54), while the $^{13}C$ NMR spectrum (Table 1) showed an additional carbonyl signal at $\delta$175.6 (C-20). In this compound, the signal for the ester methyl group appeared at lower field ($\delta$1.92) probably due to a conformational change in the molecule.

Table 1. $^{13}C$ NMR spectral data of compounds **3** and **5** ($CDCl_3$, 90 MHz)

| C | 3 | 5 |
|---|---|---|
| 1 *d* | 135.4 | 133.3 |
| 2 *d* | 128.7 | 129.5 |
| 3 *d* | 124.2 | 125.6 |
| 4 *s* | 126.0[a] | 127.3 |
| 5 *s* | 145.2 | 144.0 |
| 6 *q* | 20.1 | 20.3 |
| 7 *d* | 68.7 | 69.2 |
| 8 *d* | 42.3 | 45.4 |
| 9 *s* | 60.9 | 55.4 |
| 10 *s* | 136.5 | 136.0 |
| 11 *t* | 39.8 | 40.4 |
| 12 *d* | 72.2 | 71.7 |
| 13 *s* | 126.9[a] | 124.1 |
| 14 *d* | 109.0 | 107.8 |
| 15 *d* | 143.9 | 144.3 |
| 16 *d* | 140.2 | 139.8 |
| 17 *q* | 10.0 | 9.9 |
| 18 *s* | 170.2 | 170.5 |
| 19 *t* | 71.3 | 70.4 |
| 20 | 99.4 | 175.6 |
| OAc *s* | 170.2 | 170.2 |
| *q* | 20.7 | 21.1 |

[a] Values may be interchangeable.

Basic hydrolysis of **3** followed by methylation of the resulting product gave **6**. Its $^1H$ NMR spectrum showed the H-7 signal at $\delta$3.54 as a quartet of doublets as result of interaction with H-6 and H-8. In the IR spectrum, $\gamma$-lactone and the acetyl absorptions were replaced by an aromatic carbomethoxy band at 1688 $cm^{-1}$.

In order to establish the relative stereochemistry of **3**, NOE differential spectra were determined. These allowed us to postulate that H-12, H-11$\beta$, H-20 and the chain on C-9 were at the same side of the lactol ring (Fig. 1). However, this information was not enough to establish the orientation of the substituent at C-7 and C-8. Therefore, X-ray analysis of **3** was performed. It showed that the isobenzofuran-18(19 H)-one and the furan ring are essentially planar (maximum deviation of C-19 is 0.055 Å) and almost perpendicular (84.7° between least-squares planes). The $\gamma$-lactol ring displays an $\beta$-envelop conformation, with C-9 as the flap, in which the hydroxyl at C-20 in $\alpha$-orientation holds an *anti* relation to the side chain. In this conformation the acetoxy group at C-7 lies then almost coplanar to the isobenzofuranone in order to minimize steric repulsion, confirming the upfield signal for this group observed in the $^1H$ NMR spectrum. Application of the Karplus equation to the solid state conformation ($\theta$[H-7/C-7/C-8/H-8] = 70.3 (1)° in order to estimate the vicinal coupling constants expected for H-7 and H-8 and comparison with the observed value, confirms the extreme rigidity of the side chain.

The 5,6-secoclerodane diterpene structure for salvianduline C (**3**) was thus confirmed to be as as shown in Fig. 1 [stereochemistry relative to C-12 (*R*)]. To our knowledge, the diterpenoid **3** is only the second one of this type to be found so far in nature [5].

## EXPERIMENTAL

*General.* $^{13}C$ NMR: 90 MHz; $^1H$ NMR: 80 and 360 MHz 1D spectra: 16 K time domain addresses and processed with 32 K addresses (accuracy 0.24 Hz/point). The $CDCl_3$ proton residual signal was taken as reference (7.24 ppm). Connectivities between protons were established by 2D correlated spectroscopy (COSY) using pulse sequences $D_1$-90°-$D_0$-45°-FID. COSY was obtained through 512 $t_1$ values and, prior to Fourier transformation, the $t_2$ and $t_1$ domain $FID_S$ were multiplied with a non-shifted sine-bell window function. All spectra are available on request.

*Isolation of compound* **3**. Ground, dried aerial parts of *Salvia lavanduloides* (2460 g) collected in Morelos, México (voucher deposited in the Herbarium of the Instituto de Biología, U.N.A.M. AOH 214) were extracted with $Me_2CO$ and MeOH to give 296.1 and 70.5 g of extracts, respectively. The $Me_2CO$ extract was partitoned between MeOH–$H_2O$ (4:1) and hexane–$C_6H_6$ (1:1) to give 185.2 and 88.3 g of residues of the polar and non-polar phases, respectively.

The polar phase was percolated through activated charcoal. The decoloured material was fractioned by CC (silica gel Merck G; hexane–EtOAc gradient elution). Frs eluted with hexane–EtOAc (7:3) contained 3.61 g of compound **3**, which was purified by recrystallization from EtOAc–hexane, mp 96–98° (occludes EtOAc in the crystalline lattice) mp 177–179° from $Et_2O$–hexane, $[\alpha]_D$ +31.87 ($CHCl_3$; *c* 0.251); UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$): 227 (6599), 274 (1537), 280 (1510); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3589, 3420,

Table 2. $^1$H NMR spectral data of compounds **3**–**6**, (80 MHz, $CDCl_3$, TMS as int. standard)

| H | **3** (360 MHz) | **4** | **5** (360 MHz) | **6** |
|---|---|---|---|---|
| 1 | 7.4–7.5 | 7.30 *dd*<br>7, 2 | 7.89 *d*<br>7.6 | |
| 2 | 7.46 *t*<br>6 | 7.48 *t*<br>7 | 7.53 *t*<br>7.6 | |
| 3 | 7.82 *dd*<br>6, 3 | 7.82 *dd*<br>7, 2 | 7.89 *d*<br>7.6 | 7.9 *dd*<br>6, 3 |
| 6 | 1.23 *d*<br>6.5 | 1.23 *d*<br>7 | 1.20 *d*<br>6.5 | 1.04 *d*<br>7 |
| 7 | 5.28 *q*<br>6.5 | 5.17 *q*<br>7 | 5.03 *qd*<br>6.5, 1.2 | 3.54 *qd*<br>7, 1 |
| 8 | 1.93 *q*<br>7 | 1.98 *q*<br>7 | 2.25 *qd*<br>7.1, 1.2 | 2.11 *qd*<br>7, 1 |
| 11 | 2.61 *dd*<br>13, 10 | 2.55 *dd*<br>12, 10 | 3.05 *dd*<br>13.5, 7.5 | 2.49 *dd*<br>13, 10 |
| 11 | 2.92 *dd*<br>13, 7 | 2.90 *dd*<br>12, 6 | 2.66 *dd*<br>13.5, 7 | 2.19 *dd*<br>13, 5 |
| 12 | 5.18 *dd*<br>10, 7 | 5.29 *dd*<br>10, 6 | 5.54 *ddd*<br>7.5, 7, 0.8 | 4.56 *dd*<br>10, 5 |
| 14 | 6.44 *dd*<br>2, 1 | 6.34 *m*<br>$W/2=4$ | 6.20 *dd*<br>1.8, 0.8 | 6.34 *m*<br>$W/2=4$ |
| 15 | 7.38 *t*<br>2 | 7.37 *m* | 7.35 *dd*<br>1.8, 1.7 | |
| 16 | 7.44 *m* | 7.37 *m* | 7.28 *dt*<br>1.7, 0.8 | |
| 17 | 1.23 *d*<br>7 | 1.23 *d*<br>7 | 1.17 *d*<br>7.1 | 1.20 *d*<br>7 |
| 19 | 5.57 *d*<br>15 | 5.47 *d*<br>14 | 5.39 *s*<br>(2 H) | 5.35 *d*<br>16 |
| 19′ | 5.46 *d*<br>15 | 5.25 *d*<br>14 | | 5.12 *d*<br>16 |
| 20 | 5.77 *d*<br>1.1 | 6.82 *s* | | 5.84 *s* |
| $MeCO_2$ | 1.38 *s* | 1.35 *s*<br>1.77 *s* | | |
| OH or OMe | 2.88 *d* (OH)<br>1.1 | | | 3.37 *s* (OMe) |

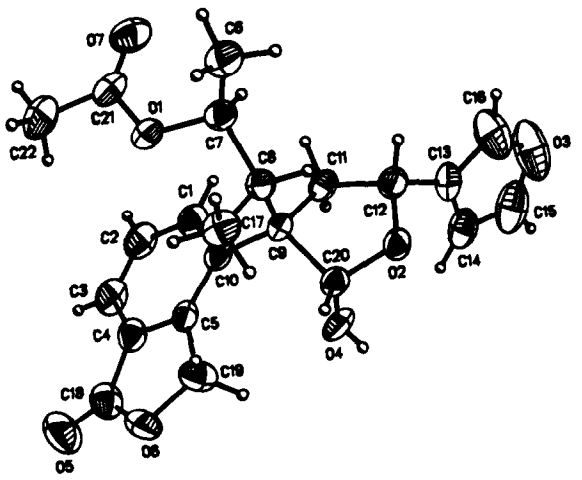


Fig. 1. The molecular conformation of salvianduline C (**3**), showing atom numbering.

1762, 1729, 1529, 1592, 872; EIMS *m/z* (rel. int.); 400 $[M]^+$ (23), 340 (35), 294 (15), 279 (19), 265 (24), 239 (28), 195 (18), 115 (29), 95 (37), 81 (36), 43 (100); HR MS 400.1853 (calc. for $C_{22}H_{24}O_7$: 400.1864).

*Acetylation of compound* **3**. A soln of **3** (100 mg) in pyridine (1 ml) and $Ac_2O$ (1 ml) was left to stand by 4 hr and worked-up as usual to give 83.1 mg of **4** as an oil: IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$; 1765, 1735, 942, 871. EIMS *m/z*: 442 $[M]^+$ (2.5); 400 (1), 382 (0.2); 358 (0.2); 340 (1); 322 (3); 294 (3); 279 (4); 267 (7); 239 (4); 221 (3); 115 (8); 95 (15); 81 (19); 43 (100). HR MS 442.1596 (calc. for $C_{24}H_{26}O_8$: 442.1620).

*Oxidation of compound* **3**. Jones reagent was added dropwise to a soln of **3** (101.3 mg) in $Me_2CO$ (10 ml) at 0°. After the usual work-up 96 mg of **5** were obtained: mp 191–194° (EtOAc–hexane), IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 1768, 1732, 928, 873; EIMS *m/z* (rel. int.): 398 $[M]^+$ (10); 356 (6); 338 (3); 282 (39); 245 (14); 236 (12); 221 (9); 115 (22); 95 (79); 81 (28); 43 (100). HR MS 398.1700 (calc. for $C_{22}H_{22}O_7$: 398.1716).

*Synthesis of compound* **6**. Compound **3** was added to an aq. soln of NaOH (40%, 15 ml). The suspension was stirred until complete solubilization, then neutralized with aq. HCl (10%),

extracted with EtOAc and dried over $Na_2SO_4$. The residue was dissolved in MeOH, cooled (0°) and stirred. Then ethereal $CH_2N_2$ was added dropwise until the reaction was complete. After solvent evapn, a residue obtained, which after CC afforded 34 mg of **6** as a gum, IR $\nu_{max}^{film}$ $cm^{-1}$: 3472, 1714, 1688, 1591, 991, 875; EIMS *m/z* (rel. int.): 390 $[M]^+$ ($C_{21}H_{26}O_7$; unobserved); 372 (10); 354 (4); 341 (5); 260 (17); 243 (23); 221 (14); 171 (65); 143 (49); 128 (45); 115 (43); 97 (90); 95 (92); 69 (63); 58 (100); 41 (72).

*X-Ray crystallography of* **3**. A crystal of salvianduline C (**3**) was mounted on a R3M Nicolet automated diffractometer. Crystal size: $0.20 \times 0.21 \times 0.40$ mm. Unit cell dimensions: $a = 10.300$ (5), $b = 12.803$ (6), $c = 19.250$ (7) Å, (orthorhombic $P_{2_12_12_1}$) were determined by least-squares refinement of the best angular positions for 25 independent reflections in the range $5° < 2\theta < 14°$ using Mo$K_\alpha$ radiation ($\lambda = 0.71073$ Å). Data (2558 reflections) were collected at room temp., using $\theta/2\theta$ scan mode to a maximum $2\theta$ value of 50°. The intensities of two standard reflections were measured every 50 reflections and as the intensities of these reflections showed less than 3% variation corrections for decay were deemed unnecessary. Intensities were corrected for Lorentz and polarization effects, but no absorption correction was made. A total of 1932 reflections were considered [$F > 3\ \sigma(F)$]. The structure was solved by direct methods included in SHELXTL package [6] to located 28 of the 35 non-hydrogen atoms having the structure. Successive cycles of least-squares blocked-cascade refinements, followed by difference Fourier synthesis, allowed location of the remainder non-H atoms. Refinements of scale factor, positional and anisotropic thermal parameters for all-non-hydrogen atoms was carried out to convergence minimizing the function $\Sigma w\ (|Fo| - |Fc|)^2$. All H-atoms attached to C-atoms were located at geometric positions with constant $U = 0.06$ Å$^2$. The hydroxylic H-atom was located on a difference Fourier synthesis at advanced stage and refined isotropically. The final cycle of refinements led to a final agreement factor $R = 0.06$ ($R_W = 0.06$) with maximum residual density in the final difference map of $\pm 0.24$.

Atomic scattering factor as for International Tables for X-ray Crystallography [7], were calculated on a NOVA 4S computer using the SHELXTL system.

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