# 8-Hydroxysalviarin and 7,8-Didehydrorhyacophiline, Two New Diterpenes from *Salvia reflexa*

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From the leaves of *Salvia reflexa* the known neoclerodanes salviarin (**1**) and 6$\beta$-hydroxysalviarin (**2**) have been isolated, together with two new diterpenes, 15,16-epoxy-8α-hydroxyneocleroda-2,13(16),14-triene-17,12*R*:18,19-diolide (**3**), and the 5,6-secoclerodane, 7,8-didehydrorhyacophiline (**5**). The structures of **3** and **5** were deduced from their spectroscopic data.

*Salvia* constitutes the largest genus of the family Labiatae with more than 500 species distributed around the world, which grow wild or are cultivated in both temperate and tropical regions. Several diterpenes have been isolated from this genus, with abietane,[1] neoclerodane,[2] or secoclerodane skeletons.[3,4]

Although certain *Salvia* species are used in folk medicine, *S. reflexa* Hornem. is reported to be toxic for livestock in Argentina and Australia.[5] As part of a search for new neoclerodane diterpenes,[6,7] we report herein the results of a phytochemical study on this species. From the aerial parts of this plant we have isolated the previously known diterpenoids salviarin (**1**)[8] and 6$\beta$-hydroxysalviarin (**2**),[4] together with two new compounds, the neoclerodane **3** and the 5,6-secoclerodane **5**, whose structures were deduced from their spectral data. From the roots of *S. reflexa*, oleanolic acid and $\beta$-sitosterol were isolated.

The HREIMS of **3** showed a $[M]^+$ ion at *m/z* 358.1408 ($C_{20}H_{22}O_6$ requires *m/z* 358.1416). Its IR spectrum showed absorptions for $\gamma$-lactone (1755 $cm^{-1}$), $\delta$-lactone (1730 $cm^{-1}$), $\beta$-substituted furan ring (1505, 875 $cm^{-1}$), olefinic double bond (1630 $cm^{-1}$), and hydroxyl (3500 $cm^{-1}$) functionalities. The $^1$H-NMR and $^{13}$C-NMR spectra of **1** and **3** were very similar; the principal difference was in the presence of an additional quaternary carbon-bearing oxygen. The $^1$H-NMR spectrum of compound **3** (Table 1) showed signals at $\delta$ 7.42 (dd, $J$ = 2.0, 1.0 Hz), 7.49 (d, $J$ = 1.0 Hz), and 6.48 (dd, $J$ = 2.0, 1.0 Hz), ascribable to a $\beta$-substituted furan ring as confirmed by the fragments observed at *m/z* 81 and 95 in the EIMS. Three one-proton signals clearly coupled in the COSY spectrum at $\delta$ 5.95 (ddd, $J$ = 10.0, 4.0, 3.0 Hz), 5.60 (ddd, $J$ = 10.0, 8.0, 1.0 Hz) and 2.80 (ddd, $J$ = 8.0, 1.5, 1.0 Hz), and were assigned, respectively, to H-2, H-3, and H-4.

The one-proton signal at $\delta$ 5.25 (dd, $J$ = 13.0, 4.0 Hz) together with signals as double doublets at 2.40 ($J$ = 15.0, 4.0 Hz) and 1.80 ($J$ = 15.0, 13.0 Hz) ppm, were consistent with an ABX system formed by H-12 and the C-11 methylene group in the molecule of **3**. The angular methyl group showed a resonance at $\delta$ 0.98 as a singlet, and the C-19 protons appeared at $\delta$ 4.19 (d, $J$ = 8.8 Hz) and 4.15 (dd, $J$ = 8.8, 1.5 Hz). This last signal was assigned to the H-19 *pro-S* diasterotopic proton, which showed long-range *W* coupling ($^4J$ = 1.5 Hz) with H-6$\beta$.

**Table 1.** NMR Spectral Data of Compounds **3** and **5** in $CDCl_3$

| | compound | | | |
|---|---|---|---|---|
| | **3** | | **5** | |
| position | $\delta_H$[a] | $\delta_C$[b] | $\delta_H$[c] | $\delta_C$[d] |
| 1 | 2.10 m | 22.0 t | 7.58 m | 129.4 d |
| 2 | 5.95 ddd (10.0, 4.0, 3.0) | 128.6 d | 7.58 m | 131.4 d |
| 3 | 5.60 ddd (10.0, 8.0, 1.0) | 120.9 d | 7.88 dd (9.0, 3.0) | 124.7 d |
| 4 | 2.80 ddd (8.0, 1.5, 1.0) | 51.9 d | | 126.0 s |
| 5 | | 40.9 s | | 149.7 s |
| 6α | 1.95 m | 33.1 t | | 11.4 q |
| 6$\beta$ | 1.30 ddd (13.8, 9.3, 3.0) | | 1.95 br s | |
| 7α | 1.80 ddd (14.0, 9.3, 4.0) | 27.2 t | | 136.8 s |
| 7$\beta$ | 2.40 dt (14.0, 3.5) | | | |
| 8 | | 75.3 s | | 103.5 s |
| 9 | | 39.2 s | | 65.5 s |
| 10 | 1.95 m | 37.9 d | | 145.5 s |
| 11α | 1.80 dd (15.0, 13.0) | 33.9 t | 2.45 dd (12.0, 10.0) | 39.8 t |
| 11$\beta$ | 2.40 dd (15.0, 4.0) | | 2.60 dd (12.0, 5.7) | |
| 12 | 5.25 dd (13.0, 4.0) | 71.1 d | 5.12 dd (10.0, 5.7) | 73.5 d |
| 13 | | 124.4 s | | 123.7 s |
| 14 | 6.48 dd (2.0, 1.0) | 108.5 d | 6.50 dd (2.0, 1.0) | 108.5 d |
| 15 | 7.42 dd (2.0, 1.0) | 143.9 d | 7.45 dd (2.5, 1.0) | 143.6 d |
| 16 | 7.49 d (1.0) | 139.9 d | 7.51 br s | 140.0 d |
| 17 | | 172.3 s | 1.35 br s | 8.1 q |
| 18 | | 175.6 s | | 170.9 s |
| 19 *pro*-R | 4.19 d (8.8) | 70.1 t | 5.35 d (13.5) | 69.0 t |
| 19 *pro*-S | 4.15 dd (8.8, 1.5) | | 5.15 d (13.5) | |
| 20 | 0.98 s | 17.2 q | 5.90 s | 110.2 d |

[a] Run at 500.13 MHz. [b] Run at 127.7 MHz. [c] Run at 200.13 MHz. [d] Run at 50.23 MHz.

These observations were indicative of the axial orientation for C-19 and of the absence of a $\beta$-substituent at C-6.[9,10] The C-1 allylic protons appeared as a complex multiplet centered at 2.10 ppm coupled through five bonds with H-4.

All unambiguous $^{13}$C-NMR assignments of **3** (Table 1) were resolved by a combination of 1D- and 2D-NMR techniques comprising DEPT, HMBC, and HMQC. The HMQC and HMBC spectra, measured on an instrument equipped with self-shielded gradients, provided additional long-range C−H correlations. Thus, the C-8 signal at $\delta$ 75.3 showed HMBC correlations with signals for H-7α, H-7$\beta$, and H-20. In addition, the resonance at $\delta$ 22.0 (C-1) showed a correlation with the signal at $\delta$ 1.95 (m) assigned to H-10. These observations confirmed the attachment of the hydroxyl group at C-8.

The $^{13}$C-NMR chemical shift of the C-20 methyl group ($\delta$ 17.2) was in accordance with an A/B trans ring junction, with C-20 axially oriented.[11] The relative stereochemistry of **3** was established from its $^1$H−$^1$H NOESY spectrum. Interactions among H-20, H-19 (two protons), H-1α, and H-11α indicated that these are all on the same face of the molecule (Figure 1). Similar

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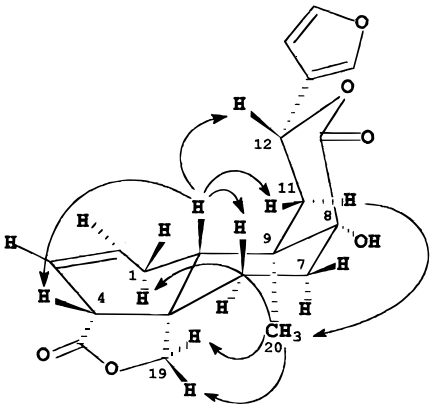


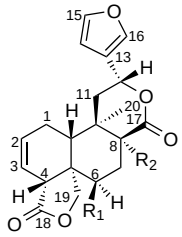
**Figure 1.** NOESY interactions observed for **3**.

interactions among H-10, H-4, H-6$\beta$, H-11$\beta$, and H-12 supported their close proximity. The NOE between H-10 and H-12 in the NOESY spectrum, together with the coupling constants of the latter proton ($J$ = 13.0, 4.0 Hz), suggested that the C-12 stereocenter has an *R* configuration.

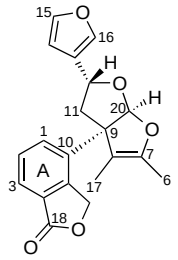
The stereochemistry at the C-8 position with the hydroxyl group attached in an α-orientation was confirmed from the NOESY spectrum of the acetyl derivative **4**, which revealed that the acetyl protons showed NOEs with the H-19 and H-20 protons. All of these data were in agreement with compound **3** being based on a neoclerodane skeleton similar to salviarin (**1**) and to 6$\beta$-hydroxysalviarin (**2**),[4] with the structure of **1** supported by X-ray crystallographic analysis.[8] Finally, the *m/z* 220 (base peak) [**A**] fragment in the EIMS of **1** may be proposed as a consequence of the loss of $CO_2$ and the furan side chain (*m/z* 81 and 94) from the molecular ion. Accordingly, the structure of **3** was assigned as 8-hydroxysalviarin.

The second new compound, **5**, isolated from the aerial parts of *S. reflexa* showed a $[M]^+$ ion at *m/z* 338, and the IR spectrum exhibited an absorptions for an α,$\beta$-unsaturated-$\gamma$-lactone (1760 $cm^{-1}$) and bands at 1500 and 875 $cm^{-1}$ due to a $\beta$-furyl moiety, as well as typical aromatic absorption at 1605 $cm^{-1}$. The $^{13}$C-NMR data (Table 1) showed that the molecule contained 13 unsaturated carbons; two methyl groups attached to $sp^2$ carbons; two methylene groups, with one of these bearing an oxygen atom; and two oxygenated methine groups, with that at $\delta$ 73.5 similar to C-12 in compounds **1-4** and the other at $\delta$ 110.2, suggesting an acetal methine carbon (C-20).

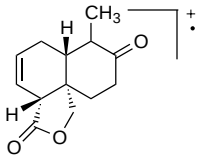
The $^1$H-NMR spectrum (Table 1) of **5**, analyzed with the aid of 2D COSY, showed resonances for aromatic protons at $\delta$ 7.88 (1H, dd, $J$ = 9.0, 3.0 Hz) due to H-3, while H-1 and H-2 appeared as a complex multiplet centered at 7.58 ppm near the H-16 ($\delta$ 7.51 br.s) and H-15 ($\delta$ 7.45, dd, $J$ = 2.0, 1.0 Hz) furan protons. The absence of the C-20 methyl proton signal together with the one-proton singlet at $\delta$ 5.90, as well as the observed downfield shift of the C-19 methylene protons as compared to **1-3**, were indicative of the rhyacophane skeleton for **5**,[3,4] with an aromatic A ring. The C-19 methylene protons resonated as doublets ($J$ = 13.5 Hz) at $\delta$ 5.35 and 5.15, superimposed with the H-12 resonance ($\delta$ 5.12, dd, $J$ = 10.0, 5.7 Hz), which was clearly coupled with H-11α and H-11$\beta$ . Two three-proton singlets at 1.95 and 1.35 ppm were ascribed to H-6 and H-17, respectively, which showed homo-allyllic coupling



**1**, $R_1 = R_2 = H$
**2**, $R_1 = OH$, $R_2 = H$
**3**, $R_1 = H$, $R_2 = OH$
**4**, $R_1 = H$, $R_2 = OAc$



**5**



[**A**]
*m/z* 220

through the C-7, C-8 double bond. The XH−CORR and COLOC (Table 2) experiments were in agreement with the given structure **5**.

The application of NOESY NMR made it possible to determine the relative stereochemistry of **5**. The observed NOE between H-19 and H-20 is only possible if C-20 has a *S* configuration, while interactions between H-14 and H-19 were in agreement with an *R* configuration at C-12 as in compounds **1-3**. All these NOESY spectral data revealed that the ring junction in the furofuran moiety should be cis. The above results are in accordance with structure **5** for the diterpene under study, which showed the 5,6-secofuranclerodane skeleton typical of *Salvia* species. Accordingly, compound **5** was assigned as 7,8-didehydrorhyacophiline.

## Experimental Section

**General Experimental Procedures.** Mps were obtained on a Leitz hot-plate microscope and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 141 polarimeter. The $^1$H-NMR spectra were recorded in $CDCl_3$ at 200.13 and 500.13 MHz on a Bruker AC-200 and a Bruker AMX-500 spectrometer, respectively. The $^{13}$C-NMR spectra were obtained with the same instruments at 50.23 and 125.7 MHz. COSY, HMQC, HMBC, NOESY, XH−CORR, and COLOC experiments were obtained using standard Bruker software. IR spectra were recorded on a Bruker IFS-25 spectrometer. EIMS were collected at 70 eV on a VG Trio-2 instrument, and HREIMS were obtained with a VG−ZAB−BEQQ spectrometer at LANAIS−EMAR−CONICET, University of Buenos Aires. Column chromatography was performed on Si gel G 70−230 mesh and Kieselgel 60 H; TLC was carried out on Si gel 60 $F_{254}$ (0.2-mm thick plates) using $C_6H_6$−dioxane−AcOH (30:5:1) as solvent.

**Plant Material.** *Salvia reflexa* was collected in Juana Koslay, San Luis, Argentina, in the flowering stage on March 1991 (voucher Del Vitto-Petenatti no. 6250, Herbario Universidad Nacional de San Luis).

**Extraction and Isolation.** The dried aerial parts (2.4 kg) of *S. reflexa* were extracted twice with $Me_2CO$ at room temperature for 10 days, and the resulting residue (130 g) was chromatographed over Si gel using a hexane−$CHCl_3$ gradient. After several column chro-

**Table 2.** COLOC Experiments on Compound **5**[a]

| carbon[b] | correlated protons | carbon[b] | correlated protons |
|---|---|---|---|
| 4 | H-1, H-19 | 9 | H-17, H-20, H-1 |
| 5 | H-2, H-3, H-19 | 10 | H-2, H-19, H-20 |
| 7 | H-6, H-17, H-20 | 13 | H-16 |
| 8 | H-6, H-17 | 18 | H-19 |

[a] 200 MHz, in $CDCl_3$, $J_{CH}$ = 10 Hz. [b] Quaternary carbons only.

matography separations, salviarin (**1**) (2.85 g), 6$\beta$-hydroxysalviarin (**2**) (0.043 g), 8-hydroxysalviarin (**3**) (0.310 g), and 7,8-didehydrorhyacophiline (**5**) (0.135 g) were obtained.

The dried roots (75 g) of *S. reflexa* were triturated and extracted with boiling $Me_2CO$. The residue (8 g) was purified over Si gel using a hexane−EtOAc gradient, and after several column chromatography separations, oleanolic acid (0.980 g) and $\beta$-sitosterol (0.080 g) were obtained.

**Compound 3:** white needles after crystallization from $Me_2CO$−*n*-hexane, mp 256−257 °C; $[\alpha]^{25}_D$ −50.4° (*c* 1.29, $CHCl_3$); IR (KBr) $\nu$ max 3500, 1755, 1730, 1600, 1505, 875 $cm^{-1}$; $^1$H-NMR and $^{13}$C-NMR data, see Table 1; HREIMS *m*/*z* 358.1408 $[M]^+$ ($C_{20}H_{22}O_6$) (4), 314 [M − $CO_2$] (4), 220 [**A**] (100), 144 (16), 122 (62), 105 (10), 94 (19), 95 (19), 91 (33), 81 (16).

**Compound 4.** Treatment of **3** (80 mg) with $Ac_2O$ (2 mL), pyridine (2 mL), and dimethylaminopyridine (30 mg) at room temperature for 2 d, followed by the usual workup and column chromatography purification, yielded 45 mg of **4** as an amorphous solid: IR (KBr) $\nu$ max 1770, 1760, 1730, 1620, 1450, 875 $cm^{-1}$; $^1$H NMR ($CDCl_3$, 200.13 MHz) $\delta$ 7.50 (1 H, br s, H-15), 7.40 (1 H, dd, *J* = 2.0, 1.0 Hz, H-16), 6.51 (1 H, br s, H-14), 6.01 (1 H, ddd, *J* = 10.0, 4.0, 3.0 Hz, H-2), 5.50 (1 H, dd, *J* = 10.0, 3.0 Hz, H-3), 5.38 (1 H,dd, *J* = 12.0, 5.0 Hz, H-12), 4.28 (1H, d, *J* = 10.0 Hz , H-19 *pro-R*), 4.20 (1 H, d br, *J* = 10.0 Hz, H-19 *pro-S*), 2.82 (1 H, t, *J* = 2 Hz, H-4), 2.11 (3 H, s, OAc), 1.01 (3 H, s, H-20); $^{13}$C NMR ($CDCl_3$, 50.23 MHz) $\delta$ 18.2 (q, C-20), 21.3 (q, OAc), 22.2 (t, C-1), 26.5 (t, C-7), 32.6 (t, C-6), 35.1 (d, C-10), 37.6 (t, C-11), 40.6 (s, C-9 or C-5), 40.9 (s, C-5 or C-9), 51.9 (d, C-4), 69.7 (t, C-19), 71.6 (d, C-12), 81.5 (d, C-8), 108.8 (d, C-14), 121.1 (d, C-3), 124.1 (s, C-13), 128.2 (d, C-2), 140.3 (d, C-16), 143.6 (d, C-15), 167.3 (s, OAc), 169.0 (s, C-18), 175.0 (s, C-17); EIMS *m*/*z* 400 $[M^+]$ (7), 357 (4), 356 (6), 340 (20), 220 (3), 122 (25), 121 (3), 95 (80), 81 (16), 43 (100).

**Compound 5:** white needles from $Me_2CO$−*n*-hexane, mp 180−181 °C; $[\alpha]^{25}_D$ −16.0° (*c* 1.5, $CHCl_3$); IR (KBr) $\nu$ max 1760, 1605, 1500, 1480, 1021, 875 $cm^{-1}$; $^1$H-NMR and $^{13}$C-NMR data, see Table 1; EIMS *m*/*z* 338 $[M^+]$ (5), 310 (7), 294 (32), 239 (100), 221 (6), 195 (28), 95 (37), 81 (26); HREIMS *m*/*z* 338.1158 $[M^+]$ ($C_{20}H_{18}O_5$).

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