Phytochemistry, Vol. 22, No. 3, pp. 784–786, 1983.
Printed in Great Britain.

0031–9422/83/0784–03$03.00/0

SALVIFARIN AND SALVIFARICIN, NEO-CLERODANE DITERPENOIDS FROM *SALVIA FARINACEA*

GIUSEPPE SAVONA, DEMETRIO RAFFA, MAURICIO BRUNO and BENJAMÍN RODRÍGUEZ*

Istituto di Chimica Organica dell'Università, Archirafi 20, 90123 Palermo, Italy; *Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, Madrid-6, Spain

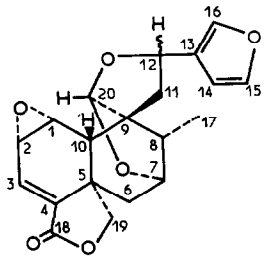
(*Received* 4 *August* 1982)

Key Word Index—*Salvia farinacea*; Labiatae; diterpenoids; new neo-clerodane derivatives; salvifarin; salvifaricin.

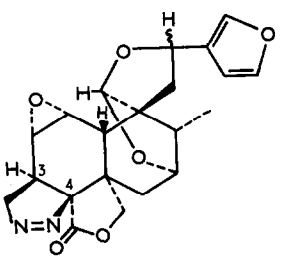
Abstract—From the aerial part of *Salvia farinacea* two new neo-clerodane diterpenoids, salvifarin and salvifaricin, have been isolated. The structures of these diterpenoids have been established mainly by spectroscopic means and by comparison with closely related compounds.

INTRODUCTION

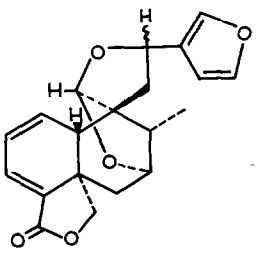
In a continuation of our studies on the terpenoid compounds from *Salvia* spp. [1–3], we have now investigated *S. farinacea* Benth., a species originating from Mexico. From this plant we have isolated two new neo-clerodane diterpenoids, salvifarin (**1**) and salvifaricin (**3**), whose structures have been established mainly by spectroscopic studies.



1



2



3

RESULTS AND DISCUSSION

Combustion analysis and mass spectrometry indicated the molecular formula $C_{20}H_{20}O_6$ for salvifarin (**1**). Its IR spectrum was consistent with the presence of a furan ring (3155, 3135, 1605, 1508, 880 cm^{-1}), an α,β-unsaturated-γ-lactone (3085, 1755, 1675 cm^{-1}) and an epoxide (3050 cm^{-1}). The presence of a furan ring and an α,β-unsaturated-γ-lactone group were also revealed by the UV spectrum of compound **1**, which showed typical absorptions for these chromophores [λ_{max} nm (log ε): 208 (3.70) (furan ring), 236.5 (3.45) (α,β-unsaturated-γ-lactone)], and the existence of an α,α'-disubstituted oxirane ring was confirmed by the ^1H and ^{13}C NMR spectra of salvifarin (**1**) (δ 3.56, 2H, *m*, $W_{\frac{1}{2}}$ = 3 Hz, and carbon atom reasonances at δ 58.7 *d* and 46.2 *d*) (Tables 1 and 2).

However, it was the ^1H NMR spectrum of salvifarin, together with extensive spin decoupling and NOE experiments, that established the structure as **1** for this new diterpenoid. Effectively, all the protons of salvifarin were clearly observed in its ^1H NMR spectrum, which showed typical signals from a β-substituted furan ring, a secondary methyl group, a β-proton of an exocyclic α,β-unsaturated-γ-lactone and an acetalic proton (*s*, δ 5.28) (Table 1). Two one-proton signals at δ 4.49 (*br d*) and 5.37 (*t*) revealed the closure of the acetalic group. In addition, double reasonance experiments showed the following facts: the β-proton of the α,β-unsaturated-γ-lactone was coupled with the epoxide protons (at δ 3.56), the signal at δ 5.37 appeared as the X part of an ABX system (δ_A 2.91, δ_B 2.00), the signal at δ 4.49 was part of another ABX system with the signals at δ 2.17 and 1.44, and the protons of the closure of the α,β-unsaturated-γ-lactone grouping appeared as an AB system (δ_A 4.90, δ_B 3.94), the B proton of which was in turn coupled with the proton at δ 1.44. On the basis of these results, and taking into account that neo-clerodane diterpenoids are common constituents of *Salvia* spp. [3, 4], only structure **1** can be envisaged for salvifarin, because among other data which will not be described in detail (see Table 1), the value of $J_{19B,6\beta}$ = 2 Hz has been previously observed in related substances [3–5] and a value of $J_{7\beta,8\beta}$ = 0 Hz (Table 1) was in agreement with the reported data of some C-20,C-7α lactonic or hemiacetalic clerodanes, such as isodiasin [6] and auropolin [7], since in these compounds, and also in salvifarin (**1**), the H-7β–H-8β dihedral angle is close to 90°.

Ethereal diazomethane treatment of salvifarin (**1**) yield-

Table 1. ^{1}H NMR spectral data of compounds **1**–**3** ($CDCl_3$, TMS as int. standard)

	1 (100 MHz)	**2*** (360 MHz)	**3** (90 MHz)
H-1	3.56 *m*, $W_{\frac{1}{2}} = 3$ Hz	3.25 *dd*, $J_{1,2} = 3.90$ Hz $J_{1,10} = 0.66$ Hz	5.83 *dd*, $J_{1,2} = 10$ Hz $J_{1,10} = 2$ Hz
H-2	3.56 *m*, $W_{\frac{1}{2}} = 3$ Hz	3.30 *dd*, $J_{2,3} = 3.90$ Hz	6.24 *ddd*, $J_{2,3} = 4.5$ Hz $J_{2,10} = 3$ Hz
H-3	7.03 *m*, $W_{\frac{1}{2}} = 5$ Hz	2.92 *ddd*, $= J_{3,2} = 3.90$ Hz	6.87 *d*
H-6α	2.17 *dd*, $J_{6\alpha,6\beta} = 14$ Hz $J_{6\alpha,7\beta} = 4$ Hz	1.93 *dd*, $J_{6\alpha,6\beta} = 14.24$ Hz $J_{6\alpha,7\beta} = 3.86$ Hz	2.07 *dd*, $J_{6\alpha,6\beta} = 13.5$ Hz $J_{6\alpha,7\beta} = 4$ Hz
H-6β	1.44 *br dd*, $J_{6\beta,19B} = 2$ Hz $J_{6\beta,7\beta} < 1$ Hz	1.22 *br d*, $J_{6\beta,19B}$ $+ J_{6\beta,7\beta} < 0.75$ Hz	1.26 *br dd*, $J_{6\beta,19B} = 2$ Hz $J_{6\beta,7\beta} < 0.8$ Hz
H-7	4.49 *br d*, $J_{7\beta,8\beta} = 0$ Hz	4.36 *br d*, $J_{7\beta,8\beta} = 0$ Hz	4.35 *br d*, $J_{7\beta,8\beta} = 0$ Hz
H-8	2.67 *q*, $J_{8,17} = 7.5$ Hz	2.62 *q*, $J_{8,17} = 7.10$ Hz	†
H-10	2.10 *br s*	2.31 *br s*	2.76 *dd*
H_A-11	2.91 *dd*, $J_{11A,11B} = 13.5$ Hz $J_{11A,12} = 8$ Hz	2.82 *dd*, $J_{11A,11B} = 13.0$ Hz $J_{11A,12} = 7.95$ Hz	2.77 *dd*, $J_{11A,11B} = 13.5$ Hz $J_{11A,12} = 8.5$ Hz
H_B-11	2.00 *dd*, $J_{11B,12} = 8$ Hz	1.95 *dd*, $J_{11B,12} = 7.95$ Hz	1.88 *dd*, $J_{11B,12} = 8.5$ Hz
H-12	5.37 *t*	5.24 *t*	5.28 *t*
H-14	6.35 *m*, $W_{\frac{1}{2}} = 4$ Hz	6.33 *m*, $W_{\frac{1}{2}} = 3.7$ Hz	6.29 *m*, $W_{\frac{1}{2}} = 4.5$ Hz
H-15	7.42 *m*, $W_{\frac{1}{2}} = 4$Hz	7.42 *t*, $J_{15,14} = J_{15,16} = 1.55$ Hz	7.35 *m*, $W_{\frac{1}{2}} = 4$ Hz
H-16	7.42 *m*, $W_{\frac{1}{2}} = 4$ Hz	7.38 *m*, $W_{\frac{1}{2}} = 4$ Hz	7.35 *m*, $W_{\frac{1}{2}} = 4$ Hz
Me-17	1.38 *d*	1.32 *d*	1.37 *d*, $J_{17,8} = 7.5$ Hz
H_A-19	4.90 *d*, $J_{19A,19B} = 8$ Hz	4.90 *d*, $J_{19A,19B} = 10.23$ Hz	4.93 *d*, $J_{19A,19B} = 8$ Hz
H_B-19	3.94 *dd*	4.76 *br d*, $J_{19B,6\beta} < 0.5$ Hz	4.03 *dd*
H-20	5.28 *s*	5.78 *s*	5.19 *s*

*Pyrazoline protons: δ_A 5.19 *dd*, δ_B 4.47 *dd*, $J_{AB} = 17.8$ Hz, $J_{A,3} = 9.30$ Hz, $J_{B,3} = 8.70$ Hz.
†Could not be identified.
All these assignments have been confirmed by double resonance experiments.

Table 2. ^{13}C NMR spectral data of compounds **1** and **3** (20.15 MHz, $CDCl_3$, TMS as int. standard)

C	**1**	**3**	C	**1**	**3**
1	58.7 *d**†	126.9 *d*†	11	38.8 *t*	38.8 *t*
2	46.2 *d*†	123.5 *d*†	12	75.6 *d*	75.1 *d*
3	130.0 *d*	132.3 *d*	13	127.5 *s*	128.1 *s*
4	137.8 *s*	129.7 *s*	14	108.3 *d*	107.8 *d*
5	58.6 *s*	57.8 *s*	15	143.8 *d*	143.3 *d*
6	40.6 *t*	37.9 *t*	16	138.6 *d*	138.1 *d*
7	87.3 *d*	83.9 *d*	17	14.8 *q*	14.4 *q*
8	39.2 *d*	41.5 *d*	18	169.1 *s*	164.9 *s*
9	38.3 *s*	34.3 *s*	19	79.8 *t*	80.2 *t*
10	45.7 *d*	48.9 *d*	20	110.2 *d*	109.3 *d*

*SFORD multiplicity.
†These assignments may be interchanged, but those given here are considered to be most likely.

ed the pyrazoline derivative **2**, the ^{1}H NMR spectrum of which (Table 1) confirmed all the above conclusions and established that the C-19 methylene protons and the acetalic proton were very close, because a strong NOE (20%) was observed in the C-19 protons when the signal at δ 5.78 (acetalic proton) was irradiated, and vice versa. This fact also established that the C-10 hydrogen atom of salvifarin (**1**) was β-oriented, because in an AB *cis*-clerodane structure no NOE is expected between these protons. Moreover, the α-configuration of the epoxide ring of compound **1** was revealed by the small $J_{10\beta,1\beta}$ and $J_{3,2\beta}$ values shown in the ^{1}H NMR spectrum of its derivative **2** (0.66 and 3.90 Hz, respectively, Table 1), since they are in agreement with dihedral angles of *ca* 75° and 60°, respectively, but not with H-10β–H-1α and H-3–H-2α dihedral angles of *ca* 140° and 0°, respectively, for which the Karplus equation in its original form [8] gave J values of 5 and 8 Hz, respectively. Furthermore, an α-configuration for the 1,2-epoxide of salvifarin (**1**) was also supported by the fact that the acetalic proton showed a strong paramagnetic shift ($\Delta\delta$ +0.50) in the ^{1}H NMR spectrum of the derivative **2**. This was due to an increase of the deshielding effect of the oxirane ring, which was more close to the H-20 proton in compound **2** than in salvifarin (**1**) (see the molecular models of **1** and **2**).

In agreement with all the above deductions, the ^{13}C NMR spectrum of salvifarin (Table 2) showed carbon resonances which were only compatible with structure **1**. In particular, the δ_{C-4} of 137.8 clearly established [9] an AB *trans*-clerodane skeleton for this new diterpenoid.

Finally, the negative Cotton effect ($\Delta\varepsilon_{265} - 5.68$) shown by the exocyclic α,β-unsaturated-γ-lactone group indicated [5, 10] that salvifarin (**1**) had the same absolute configuration as neo-clerodane [11]. The C-12 configuration of salvifarin (**1**) was not ascertained; however, on biogenetic grounds we suppose that it is *R*, as it was found in all the neo-clerodane diterpenoids isolated from *Salvia* spp. [3, 4]. A neo-clerodane derivative possessing an identical acetalic moiety to that of salvifarin (**1**) has been previously synthesized from cascarillin A [12], a diterpenoid found in cascarilla bark.

The other diterpenoid isolated from *S. farinacea*, salvifaricin, had a $C_{20}H_{20}O_5$ molecular formula and its structure (**3**) was established from its ^{1}H and ^{13}C NMR data (Tables 1 and 2), which were identical with those of

salvifarin (**1**) except for the existence in salvifaricin (**3**) of a C-1–C-2 double bond instead of the oxirane ring of compound **1**. In agreement with an $\alpha,\beta:\gamma,\delta$-diunsaturated-$\gamma'$-lactone moiety, salvifaricin showed an identical UV spectrum and H-1–H-3 and H-10 proton resonances (see Experimental and Table 1) to those of gensnerofolin B, a neo-clerodane-1,3-dien-18,19-olide isolated from *Salvia gensneraefolia* [13]. The absolute configuration of salvifaricin (**3**) was not ascertained; however, compound **3** is believed to belong to neo-clerodane series [11], like salvifarin (**1**), co-occurring in the same species. The similar variation of the [α] values of compounds **1** and **3** (see Experimental) also supported this point.

EXPERIMENTAL

Mps are uncorr. For general details on experimental procedures see refs. [2, 3]. Plant materials were collected in the Botanic Garden of Palermo, Italy, in June 1981, and voucher specimens are deposited in the Herbarium of this centre.

Isolation of the diterpenoids. Dried and finely powdered *S. farinacea* Benth. aerial parts (800 g) were extracted with Me_2CO (6 l.) as previously described for other *Salvia* spp. [1–4]. The extracts were evaporated to dryness yielding a residue (60 g), which was chromatographed over a Si gel column (800 g, deactivated with 15% H_2O) eluted with *n*-hexane and *n*-hexane–EtOAc mixtures. Elution with EtOAc–*n*-hexane (3:1) yielded salvifaricin (**3**, 102 mg, less polar diterpenoid) and salvifarin (**1**, 1040 mg).

Salvifarin (**1**). Mp 220–222° (from MeOH); $[\alpha]_D^{20}$ −4.3°, $[\alpha]_{365}^{20}$ −53.9° ($CHCl_3$; *c* 0.93); IR ν_{max}^{KBr} cm^{-1}: 3155, 3135, 1605, 1508, 885 (furan ring), 3085, 1755, 1675 (α, β-unsaturated-γ-lactone), 3050 (epoxide), 3000, 2980, 2960, 2920, 2895, 2875, 1470, 1440, 1385, 1270, 1235, 1225, 1205, 1165, 1050, 1020, 1005, 960, 840, 795, 740, 715; UV λ_{max}^{EtOH} nm (log ε): 208 (3.70) (furan ring), 236.5 (3.45) (α, β-unsaturated-γ-lactone). CD nm (Δε): 350 (0), 265 (−5.68), 254 (0), 220 (+13.85), 200 (0) (MeOH; *c* 0.212); ^1H NMR (100 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (20.15 MHz, $CDCl_3$): see Table 2; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 356 $[M]^+$ (3), 340 (1), 327 (4), 325 (2), 309 (3), 256 (12), 205 (15), 203 (13), 187 (20), 171 (20), 157 (21), 150 (100), 145 (27), 131 (35), 115 (28), 105 (24), 95 (71), 94 (69), 91 (42), 81 (60), 77 (29), 65 (20), 55 (23). (Found: C, 67.39; H, 5.89. $C_{20}H_{20}O_6$ requires: C, 67.40; H, 5.66%.)

Pyrazoline derivative **2**. A MeOH soln of **1** (50 mg) was treated with an Et_2O soln of CH_2N_2 for 2 hr at room temp. After evaporation of the solvents and crystallization from EtOAc–*n*-hexane, pure **2** (48 mg) was obtained: mp 168°; $[\alpha]_D^{20}$ −253.0° ($CHCl_3$; *c* 0.249); IR ν_{max}^{KBr} cm^{-1}: 3160, 3130, 1610, 1510, 880 (furan ring), 1773 (γ-lactone), 3040 (epoxide), 1550 (pyrazoline), 2990, 2950, 2900, 1470, 1430, 1375, 1335, 1250, 1215, 1185, 1160, 1050, 1020, 1000, 955, 900, 800, 795, 720; ^1H NMR (360 MHz, $CDCl_3$): see Table 1; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): $[M]^+$ absent, 370 $[M-N_2]^+$ (3), 354 (2), 270 (13), 203 (20), 183 (21), 171 (29), 164 (99), 149 (41), 131 (36), 115 (36), 105 (37), 95 (80), 94 (100), 91 (68), 81 (80), 77 (44), 65 (26), 55 (32), 41 (29). (Found: C, 63.66; H, 5.69; N, 6.97. $C_{21}H_{22}O_6N_2$ requires: C, 63.31; H, 5.57; N, 7.03%.)

Salvifaricin (**3**). Mp 214–215° (from MeOH); $[\alpha]_D^{20}$ −155.2°, $[\alpha]_{365}^{20}$ −1438.9° ($CHCl_3$; *c* 0.393); IR ν_{max}^{KBr} cm^{-1}: 3160, 1500, 880 (furan ring), 3040, 3020, 1743, 1675, 1585 (α, β:γ, δ-diunsaturated-γ'-lactone), 2970, 2950, 2925, 2895, 2870, 1465, 1375, 1265, 1240, 1165, 1055, 1030, 1010, 980, 940, 900, 800, 730, 700, 680; UV λ_{max}^{EtOH} nm (log ε): 207 (3.83) (furan ring), 297.5 (3.67) (α,β:γ,δ-diunsaturated-γ'-lactone); ^1H NMR (90 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (20.15 MHz, $CDCl_3$): see Table 2; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 340 $[M]^+$ (11), 282 (10), 259 (9), 243 (7), 216 (28), 200 (13), 189 (18), 173 (16), 163 (74), 135 (58), 115 (38), 105 (29), 95 (100), 94 (48), 91 (47), 81 (75), 77 (32), 69 (24), 55 (33), 41 (19). (Found: C, 70.19; H, 5.98. $C_{20}H_{20}O_5$ requires: C, 70.57; H, 5.92%.)

Acknowledgements—We thank Palermo Botanic Garden Office for the facilities given for the collection and botanical classification of the plant material, Miss M. D. Casado and Mrs. M. Plaza, Madrid, for recording the ^{13}C NMR spectra, and Mr. J. Prieto, Madrid, for elemental analyses. This work was supported in part by a grant of 'Progetto Finalizzato per la Chimica Fine e Secondaria', C. N. R. (Rome), and in part by the 'Comisión Asesora de Investigación Científica y Técnica' (grant No. 11/81), Madrid. The financial support of the Spanish Foreign Ministry for travel facilities between Italy and Spain is gratefully acknowledged.

REFERENCES

1. Savona, G. and Rodríguez, B. (1980) *An. Quím. Ser. C* **76**, 187.
2. García-Alvarez, M. C., Savona, G. and Rodríguez, B. (1981) *Phytochemistry* **20**, 481.
3. Savona, G., Bruno, M., Paternostro, M., Marco, J. L. and Rodríguez, B. (1982) *Phytochemistry* **21**, 2563.
4. Savona, G., Paternostro, M., Piozzi, F., Hanson, J. R., Hitchcock, P. B. and Thomas, S. A. (1978) *J. Chem. Soc. Perkin Trans.* 1, 643.
5. Wagner, H., Seitz, R., Lotter, H. and Herz, W. (1978) *J. Org. Chem.* **43**, 3339.
6. Alvarenga, M. A., Gottlieb, H. E., Gottlieb, O. R., Magalhaes, M. T. and Da Silva, V. O. (1978) *Phytochemistry* **17**, 1773.
7. Eguren, L., Perales, A., Fayos, J., Savona, G., Paternostro, M., Piozzi, F. and Rodríguez, B. (1981) *J. Org. Chem.* **46**, 3364.
8. Karplus, M. (1963) *J. Am. Chem. Soc.* **85**, 2870.
9. Luteijn, J. M., van Veldhuizen, A. and de Groot, A. (1982) *Org. Magn. Reson.* **19**, 95.
10. Stapel, G., Menssen, H. G. and Snatzke, G. (1980) *Planta Med.* **38**, 366.
11. Rogers, D., Unal, G. G., Williams, D. J., Ley, S. V., Sim, G. A., Joshi, B. S. and Ravindranath, K. R. (1979) *J. Chem. Soc. Chem. Commun.* 97.
12. Halsall, T. G., Oxford, A. W. and Rigby, W. (1965) *J. Chem. Soc. Chem. Commun.* 218 (and references therein).
13. Jiménez, M., Moreno, E. D. and Díaz, E. (1979) *Rev. Latinoam. Quim.* **10**, 166.