PERGAMON

Phytochemistry 53 (2000) 103–109

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Polystachynes A–E, five *cis*-neo-clerodane diterpenoids from *Salvia polystachya*☆

Emma Maldonado*, Alfredo Ortega

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, D.F., Mexico

Received 5 May 1999; accepted 9 July 1999

Abstract

From the aerial parts of *Salvia polystachya* five new neo-clerodane diterpenoids, polystachynes A–E, have been isolated. The structures were established by spectroscopic methods, including the X-ray analysis of polystachynes C and D. The known clerodanes salvifaricin, linearolactone and dehydrokerlin were also isolated. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Salvia polystachya*; Labiatae; Neo-clerodanes; Diterpenes; Polystachynes A–E; X-ray diffraction analysis

1. Introduction

As continuation of our search for new natural compounds from the genus *Salvia* (Labiatae) (Maldonado & Ortega, 1997a, 1997b; Maldonado, Ortega, Díaz & Reynolds, 1998), we have analyzed the aerial parts of *Salvia polystachya* Ort. (section *Polystachyae*, subgenus *Calosphace*), which is used in Mexican folk medicine as anti-gastralgic, anti-dysenteric, purgative and emollient (Instituto Nacional Indigenista, 1994). As a result, we have isolated eight clerodane diterpenoids whose structural elucidation is described in this paper.

2. Results and discussion

The aerial parts of *S. polystachya* gave the known neo-clerodane diterpenes, salvifarcin (**2**) (Savona, Raffa, Bruno & Rodríguez, 1983; Eguren, Fayos, Perales, Savona & Rodríguez, 1984), linearolactone (**5**) (Esquivel, Cárdenas, Ramamoorthy & Rodríguez-Hahn, 1986; Soriano-García, Esquivel, Toscano & Rodríguez-Hahn, 1987) and dehydrokerlin (**9**) (Fernández, Esquivel, Cárdenas, Sánchez, Toscano & Rodríguez-Hahn, 1991). In addition, five new neo-clerodane diterpenes designated as polystachynes A–E were isolated.

Polystachyne A (**1**) was determined as $C_{20}H_{22}O_5$ on the basis of its EI-mass spectrum. The IR spectrum exhibited a band at 1774 cm^{-1} due to a saturated γ-lactone and the characteristic absorptions at 1502 and 876 cm^{-1} due to a β-substituted furan ring. Its ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) were quite similar to those of salvifaricin (**2**) (Savona et al., 1983) except for the signals attributables to ring A, which in the case of compound **1**, indicated the presence of only one double bond in that ring. The cross-peaks in the ^{1}H–^{1}H-COSY spectrum led to the sequence ◆–CH–CH_2–CH=CH–CH–◆, establishing that polystachyne A is a dihydro derivative of salvifaricin (**2**) with either 1,2- or 2,3-double bond. The C–H correlations observed in the long-range HETCOR spectrum between C-4/H-19β, H-6β; C-9/H-20, H-10, H-17; C-5/H-1, H-4, H-6β, H-7, H-19β; C-18/H-4, H-19β and H-1/C-3, C-5, C-10 lead us to put aside the possibility of a 2,3-double bond, thus establishing the structure of polystachyne A as depicted in **1**. Its stereochemistry will be discussed afterward.

Polystachyne B (**3**) and C (**4**), showed molecular ions at *m*/*z* 358 (EIMS) in agreement with the molecu-

☆ Contribution No. 1704 of the Instituto de Química, UNAM.

* Corresponding author.

PII: S0031-9422(99)00466-5

Table 1
^{1}H-NMR spectral data of compounds **1**, **3**, **4**, **6** and **8** ($CDCl_3$, 300 MHz, TMS as internal standard)

H	1	3	4	6[a]	8
1	5.48 *br d* 9.9	3.09 *br d* 3.5, 3	3.14 *br d* 4.5	3.02 *dd* 3.8, 2.2	3.41 *dd* 3.6, 2.7
2	5.94 *ddd* 9.9, 5.4, 2.7	3.25 *dd* 5.7, 3.5	3.31 *m*[b] 4.5, 3	2.91 *ddd* 3.8, 2.4, 1.2	3.51 *dd* 3.6, 2.4
3α	2.4 *m*[b]	2.28 *ddd* 14.5, 5.7, 5.1	2.4 *m*[b]	2.1–2.3 *m*[b]	6.98 *dd* 2.4
3β	2.1 *m*[b]	1.81 *dd* 14.5, 12.9	2.01 *m*[b]	2.1–2.3 *m*[b]	–
4	2.33 *dd* 14.4, 5	2.18 *dd* 12.9, 5.1	2.4 *m*[b]	2.06–2.2 *m*[b]	–
6α	1.79 *dd* 14.2, 4.4	1.71 *br dd* 13.6, 4.2	2.16 *dd* 14.1, 3.9	1.6-1.8 *m*[b]	1.5–1.7 *m*[b]
6β	1.30[b]	1.4.7 *br d* 13.6	1.51 *br d* 14.1	2.06–2.2 *m*[b]	1.5–1.7 *m*[b]
7α	–	–	–	1.6–1.8 *m*[b]	2.27[b]
7β	4.45 *d* 4.4	4.48 *br d* 4.2	4.42 *dd* 3.9, 1.2	1.6–1.8 *m*[b]	1.5–1.7 *m*[b]
8	1.97 *q* 7.2	2.95 *q* 7	2.42 *q*[b] 7	2.25–2.4 *m*[b]	2.57 *d* 3.3
10	2.68 *br s*[b]	2.12 *br s* $w/2 = 5$	2.24 *s*	1.71 *br d* 2.2	1.86 *br s*
11α	1.88 *dd* 13.2, 7.5	1.90 *dd* 13.2, 7.5	1.89 *dd* 13, 7.6	2.17 *dd* 14.4, 7.5	2.29 *d* 16
11β	2.66 *dd*[b] 13.2, 7.5	2.79 *dd* 13.2, 7.5	2.81 *dd* 13, 7.6	1.73 *dd* 14.4, 7.5	2.90 *dd* 16, 7.5
12	5.28 *t* 7.5	5.30 *t* 7.5	5.29 *t* 7.6	5.47 *t* 7.5	5.76 *br d* 7.5
14	6.33 *dd* 1.7, 0.8	6.29 *br s*	6.32 *dd* 1.8, 0.9	6.37 *dd* 1.8, 0.9	6.41 *dd* 1.6, 1
15	7.41 *t* 1.7	7.37 *t* 1.5	7.41 *t* 1.7	7.32 *t* 1.8	7.49 *t* 1.6
16	7.37 *m*	7.37 *br s*	7.37 *m*	7.45 *m*	7.42 *m*
17	1.28 *d*[b] 7.2	1.29 *d* 7	1.36 *d* 7		–
19α	4.04 *dd* 8, 2	3.87 *dd* 7.8, 2.4	4.20 *dd* 9, 1	4.22 *d* 8.4	3.36 *dd* 9, 1
19β	4.85 *d* 8	4.83 *d* 7.8	4.53 *d* 9	3.52 *d* 8.4	4.42 *d* 9
20	5.24 *s*	5.15 *s*	5.33 *s*	1.06 *s*	1.28 *s*

[a] Determined in $(CD_3)_2CO$–C_6D_6 1:1.
[b] Superimposed signal.

lar formula $C_{20}H_{22}O_6$. Both compounds exhibited IR absorptions for a saturated γ-lactone and β-substituted furan ring (see Section 3). Their ^{1}H- and ^{13}C-NMR signals (Tables 1 and 2) were assigned by COSY, HETCOR and long-range HETCOR spectra. They were quite similar to those of polystachyne A (**1**) except for those concerning to C-1 and C-2, since in polystachynes B and C, an epoxy group is present instead of the $\Delta^{1,2}$ of **1**. This was indicated by the signals for H-1 (δ 3.09, *dd*, J = 3.5, 3 Hz in **3** and 3.31 *m* in **4**) and supported by the signals at δ 55.3 (*d*, C-1) and 50.8 (*d*, C-2) exhibited by **3** and the corresponding signals at δ 52.9 (*d*, C-1) and 48.9 (*d*, C-2) of compound **4**, observed in their respective ^{13}C-NMR spectra. These facts allowed to formulate the structures of both compounds with the planar structure **3**. Evidently, this also established a stereoisomeric relationship between them.

The stereochemistry of polystachyne A (**1**), B (**3**) and C (**4**) was established as follows. The three compounds exhibited NOEs between H-20/ H-10, H19α, H-19β, which indicated a *cis* A/B ring fusion and an α-orientation of H-10 and the acetalic proton, H-20. This was supported, in the case of polystachyne B, by the presence of a W-coupling between H-6α and H-10α.

Compounds **1**, **3** and **4** also showed NOEs between H-12/H-17; H-8/H-6β, H-17 and H-7/H-6α, H-6β, H-17. This was indicative of a β-orientation of H-7, H-8 and H-12 and let to establish that the three compounds possesses the same configuration at the chiral centers C-5, C-7–C-10, C-12 and C-20, as depicted in **1**, **3** and **4**. At this point only the configuration at C-4 for **1** and C-1, C-2 and C-4 for **3** and **4** remained unassigned.

The H-4 coupling constants of **1** (J = 14.4, 5 Hz) and **3** (J = 12.9, 5.1 Hz) are in accordance with the dihedral angles: H-4α–H-3β = 180° and H-4α–H-3α = 55°. These angles were observed only when the C-18–C-19-lactone ring is *trans*-fused, and therefore, H-4 is α-oriented. In the case of polystachyne B (**3**), this was confirmed by the NOEs cross-peaks of H-19α with H-4, H-10 and H-20. The NOE between H-4 and H-19α was not observed in compound **1**, nevertheless, the

Table 2
^{13}C-NMR spectral data of compounds **1**, **3**, **4**, **6** and **8** ($CDCl_3$, 75 MHz)

C	1	3	4	6	6[a]	8
1	128.7 *d*	55.3 *d*	52.9 *d*	51.5 *d*	52.2 *d*	53.2 *d*
2	127.4 *d*	50.8 *d*	48.9 *d*	49.9 *d*	50.5 *d*	49.0 *d*
3	20.5 *t*	19.4 *t*	22.3 *t*	23.8 *t*	24.3 *t*	130.2 *d*
4	45.3 *d*	46.2 *d*	44.6 *d*	40.1 *d*	40.0 *d*	142.5 *s*
5	42.0 *s*	43.7 *s*	40.5 *s*	38.2 *s*	38.5 *s*	42.9 *s*
6	30.3 *t*	31.0 *t*	43.7 *t*	35.9 *t*	36.3 *t*	29.3 *t*
7	86.7 *d*	88.0 *d*	86.5 *d*	21.0 *t*	21.7 *t*	17.6 *t*
8	39.9 *d*	38.7 *d*	39.8 *d*	47.8 *d*	48.9 *d*	40.7 *d*
9	58.6 *s*	59.5 *s*	58.8 *s*	35.4 *s*	36.0 *s*	36.7 *s*
10	50.3 *d*	47.9 *d*	46.5 *d*	44.7 *d*	44.6 *d*	52.8 *d*
11	38.3 *t*	38.1 *t*	38.5 *t*	34.3 *t*	34.7 *t*	37.6 *t*
12	75.8 *d*	76.1 *d*	75.2 *d*	70.8 *d*	71.3 *d*	71.7 *d*
13	128.7 *s*	129.0 *s*	128.6 *s*	125.8 *s*	127.5 *s*	126.2 *s*
14	108.3 *d*	108.3 *d*	108.2 *d*	108.2 *d*	109.7 *d*	108.5 *d*
15	143.8 *d*	143.8 *d*	143.8 *d*	144.0 *d*	144.7 *d*	144.5 *d*
16	138.5 *d*	138.5 *d*	138.5 *d*	139.3 *d*	140.6 *d*	138.6 *d*
17	14.6 *q*	14.7 *q*	14.7 *q*	172.5 *s*	174.0 *s*	168.0 *s*
18	175.3 *s*	174.1 *s*	178.3 *s*	178.3 *s*	179.4 *s*	173.2 *s*
19	79.5 *t*	80.1 *t*	80.7 *t*	74.9 *t*	80.6 *t*	75.1 *t*
20	110.1 *d*	110.4 *d*	110.2 *d*	31.6 *q*	31.8 *q*	31.5 *q*

[a] Determined in $(CD_3)_2CO$–C_6D_6 1:1.

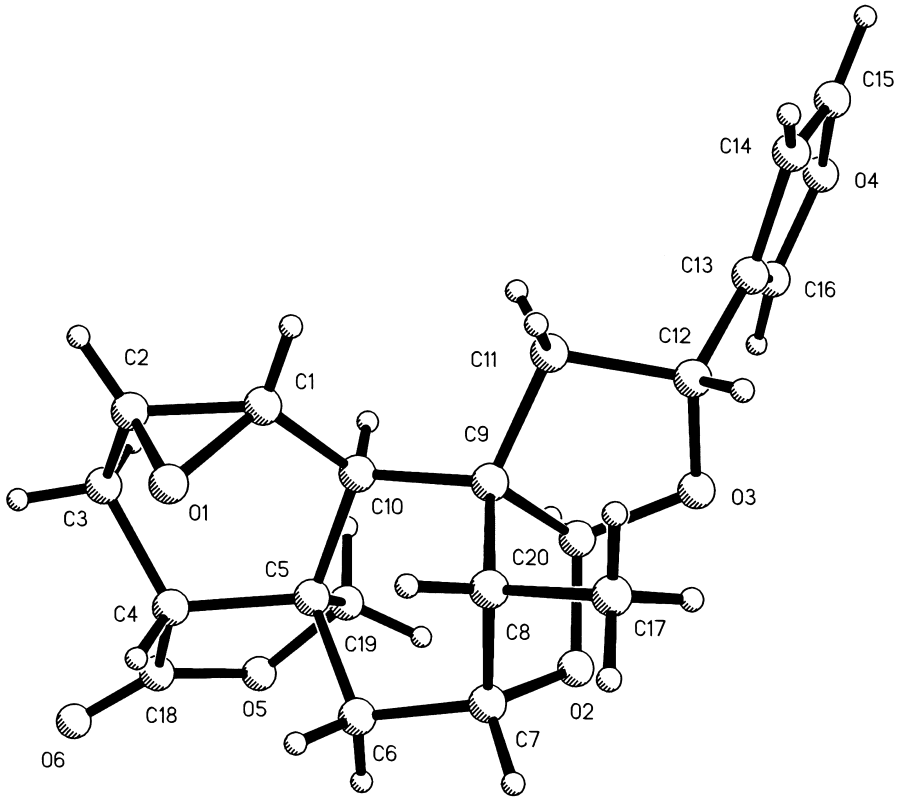


Fig. 1. Computer-generated perspective drawing of compound **4**.

above mentioned and the similar chemical shift of carbons 3–6 and 18 of **1** and **3** allowed to postulate the same configuration at C-4 in both compounds, thus establishing the structure of polystachyne A as that represented in **1**.

The assignment of the configuration at C-4 for polystachyne B (**3**) presented several difficulties: (i) the superposition of the signals for H-3β, H-4 and H-8 made useless the observed NOEs of these protons and (ii) the signals for H-2, H-3α, H-3β and H-4 were more complex than expected. Thus, H-2 appeared as a broad asymmetric signal with six lines. This signal was transformed into a *dd* signal (J = 4.5, 3 Hz) by irradiation of H-3α. In turn, the H-3α signal, which should be a *ddd*, appeared as an asymmetric signal with ten lines. The irradiation of H-2 turned it into a symmetric six line multiplet with intensities 2:1:3:3:1:1.5. The additional multiplicity of these signals was attributed to a conformational equilibrium involving C-2–C-4. This assumption was supported by the partial collapsing of the H-2–H-4 signals when the ^{1}H-NMR spectrum was determined at 48°C. Nevertheless, the J values of H-4 could not be determined.

In spite of these facts, the different chemical shift of C-3, C-5 and mainly, of C-6 and C-18 in **4**, in relation with the corresponding signals of **1** and **3**, allowed to propose that compound **4** possesses more probably a *cis*-C-18–C-19-lactone ring fusion. This arrangement could explain the difference in chemical shift of C-6 in the stereoisomers **3** (δ 31.0) and **4** (δ 43.7), because of the presence in **3** of the γ-gauche effects ($\Delta\delta = -12.7$ ppm) of C-3 and C-18 on C-6, which do not exist in **4**.

Finally, the orientation of the C-1–C-2-epoxy group in polystachyne B (**3**) was proposed to be β on the basis of the J values ($J_{1-10} = 3$ Hz; $J_{2-3_\alpha} = 5.7$ Hz; $J_{2-3_\beta} = 0$ Hz) which are in accordance with the dihedral angles H-1–H-10 = 60°; H-2–H-3α = 35°; H-2–H-3β = 90°. Further support was given by the NOE cross-peaks of H-1/H-10 and H-2/H-3α, but the conclusive evidence was the remarkable deshielding effect that the oxirane induces to H-8, which appeared at an unusual low field (δ 2.95) for this kind of protons. From the above mentioned, structure **3** was formulated for polystachyne B.

Polystachyne C (**4**) showed $J_{1-10} = 0$ Hz; $J_{2-3_\beta} = 3$ Hz (J_{2-3_α} could not be determined). These J values are in agreement with a β-oriented epoxy group if the C-18–C-19-olide is *cis*-fused to ring A. On the other hand, the signal for H-8 appeared at higher field (δ 2.42) as compared with those of **3** (δ 2.95) and salvifarin (δ 2.67) (the β-epoxy derivative of **2**, determined by X-ray analysis; Savona et al., 1983). This fact could be attributed to a longer distance between the ethereal

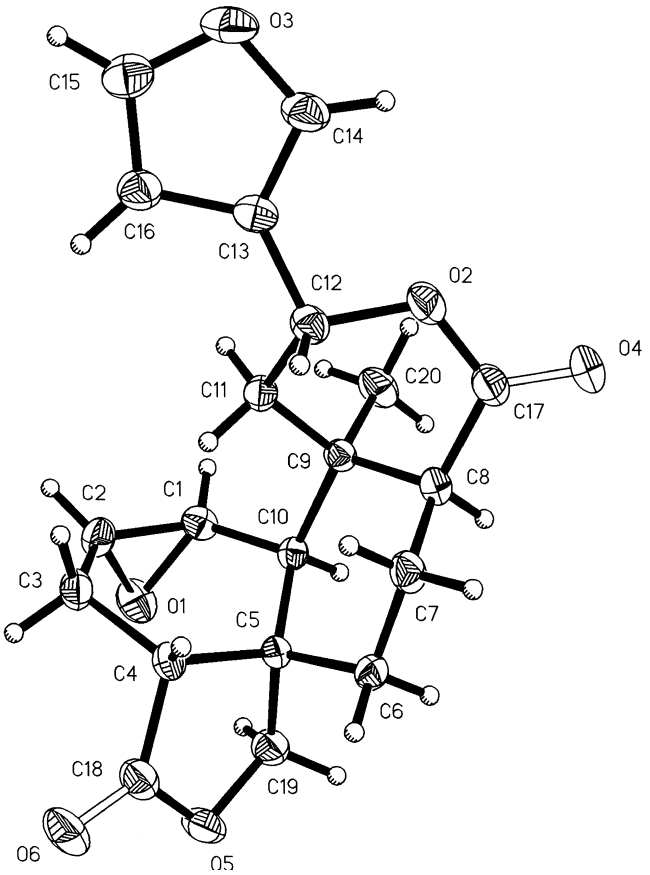


Fig. 2. Computer-generated perspective drawing of compound **6**.

oxygen and H-8 or to an α-oriented oxirane ring. In order to solve these ambiguities a single crystal of polystachyne C was submitted to X-ray crystallographic analysis (Fig. 1). The results established the orientation of the oxirane as β and confirmed the C-18–C-19-lactone fusion as *cis*, thus establishing the structure **4** for this compound.

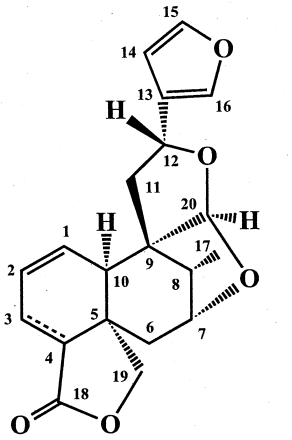
The fourth new compound, polystachyne D (**6**), $C_{20}H_{22}O_5$ (EIMS) showed IR bands for a β-substituted furan ring (1503, 875 cm^{-1}) and saturated γ- and δ-lactone functions (1767 and 1739 cm^{-1}). Its ^1H, ^{13}C, COSY, HETCOR and long-range HETCOR spectra resemble those of **3** and **4**, but instead of the signals indicating the bicyclic ketal, the corresponding signals for a δ-lactone function were present in compound **6**. This came from the presence of the ABX system (C-11-2H–C-12-H, Table 1) observed in the COSY spectrum and from the ^{13}C signals for C-12 (δ 71.3, *d*), C-8 (δ 48.9, *d*) and C-17 (δ 174.0, *s*). Therefore, structure **6** (without stereochemistry) was established for polystachyne D. A compound with the same structure has been isolated from *S. reptans* and its stereochemistry determined as **7** by X-ray analysis (Esquivel et al., 1991). Comparison of physical and spectroscopic data of **6** and **7** indicated a stereoisomeric relationship between them. The configuration of **6** could not be determined solely on the basis of the NMR spectral data, due to the overlapping of 10 proton signals (two multiplets, 5H each one) in the ^1H-spectrum. However, some relevant information was obtained from these data. First of all, the chemical shift of the C-20 methyl group at δ 31.8 and the NOEs between H-10/H-19α, H-20 indicated an A/B *cis* ring fusion as in compounds **1**–**4**. The observed NOEs between H-19α/H-10 and between H-19β/H-6β allowed to establish that in **6** the C-19 protons appeared in an opposite order to that observed in compounds **1**–**5** and **7**. This and the absence in **6** of the ^4J-coupling between H-19α and H-6β (observed in **1**–**5** and **7**) let to suspect that **6** could be the C-4 epimer of **7**. This was supported, as in the case of the C-4-epimers, **3** and **4**, by the remarkable γ-gauche effect ($\Delta\delta = -16.6$ ppm) observed for C-6 when the ^{13}C-NMR data of **6** (*cis*-lactone) and **7** (*trans*-lactone) were compared.

Due to the before mentioned overlapping of proton signals, the stereochemistry at C-1, C-2, C-8 and C-12 of compound **6** remained uncertain; therefore, the X-ray analysis of this compound was undertaken. The results confirmed the A/B ring fusion as *cis* and established that the only difference between **6** and **7** is the 18,19-lactone fusion, which in **6** is *cis*-fused to ring A (Fig. 2). The configuration at C-12 is *R* and the orientation of the C-1–C-2-epoxy group, H-8, H-10, C-18, C-19 and C-20 was determined to be α.

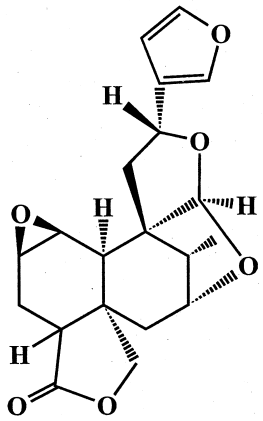
Polystachyne E (**8**), showed a molecular formula $C_{20}H_{22}O_6$ (EIMS). Its IR spectrum exhibited bands at 1766 and 1683 cm^{-1} assigned to an α,β-unsaturated-γ-lactone attached to ring A. It also showed a band at 1744 cm^{-1} attributed to a δ-lactone and the characteristics absorptions due to a β-substituted furan ring at 1500 and 876 cm^{-1}.

The homo and heteronuclear, mono and bidimensional NMR spectra indicated the presence of a C-1–C-2-epoxy group (δ 3.41 *dd*, J = 3.6, 2.7 Hz, H-1) and (δ 3.51 *dd*, J = 3.6, 2.4 Hz, H-2). The signal at δ 3.51 was coupled with a doublet at δ 5.98 (J = 2.4 Hz) which was assigned to the β-proton (H-3) of the α,β-unsaturated-γ-lactone. The remaining spectral data, which are similar to those of **7**, are in agreement with structure **8** assigned to this compound.

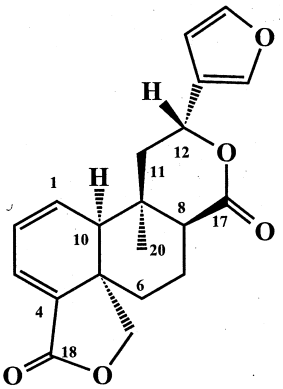
Its relative stereochemistry was based on the NOESY spectrum. An A/B *cis* ring fusion was revealed by the NOEs between H-10/H-11β, H-19α, H-20. The *R*-configuration of C-12 was substantiated by the NOEs between H-16/H-8, H-11α, H-20, while the α-orientation of the oxirane was deduced from both, the J values of H-1 (which are similar to those of compound **6**) and the NOEs between H-1/H-6β (weak), H-10 (weak) and H-11β.



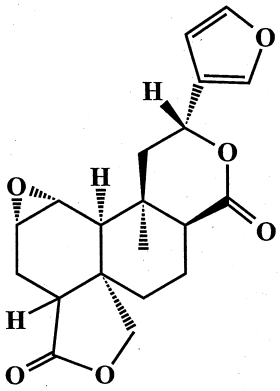
1 H-4β
2 $\Delta^{3,4}$



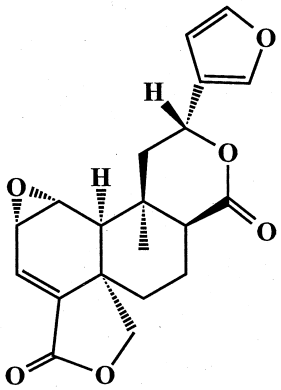
3 H-4α
4 H-4β



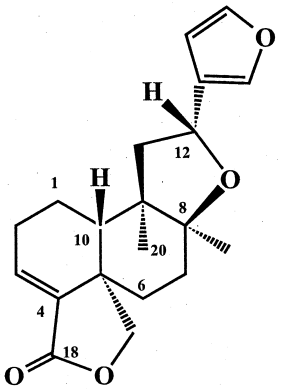
5



6 H-4β
7 H-4α



8



9

3. Experimental

3.1. Plant material

Salvia polystachya Ort. was collected in Huitzilac (Morelos State, México), in October 1992. A voucher specimen was deposited at the Herbarium of the Instituto de Biología, UNAM (MEXU-573762).

3.2. Extraction and isolation

Dried and finely powdered aerial parts of the plant (956 g) were extracted with Me_2CO and MeOH to obtain, after solvent evaporation 77 and 29.8 g of extracts, respectively. Both extracts were combined and partitioned between MeOH–H_2O (4:1) and hexane to give, after solvent evaporation 63.6 and 41 g of residues, respectively. The MeOH–H_2O residue was fractioned by CC (silica gel G; hexane with increasing amounts of EtOAc). Repeated CC (silica gel G; hexane–EtOAc, 17:3 or 4:1) of frs. eluted with hexane–EtOAc 4:1 and 7:3 afforded **1** (83.7 mg), **2** (23.9 mg) and **9** (23 mg). Frs. eluted with hexane–EtOAc 7:3 and 3:2 contained a very complex mixture, which was resolved after repeated CC separations (silica gel G; hexane–EtOAc, 4:1 or hexane–$CHCl_3$–Me_2CO 4:4:1 or hexane–*i*PrOH 9:1) and crystallizations. Compounds **3** (441.3 mg), **4** (96 mg), **5** (2.124 g), **6** (2.21 g) and **8** (61.4 mg) were obtained. The analysis of the hexane residue and of the more polar frs. of the MeOH–H_2O residue are actually in progress.

The known compounds **2**, **5** and **9** were identified by

comparison of their physical and spectrosopic data with those published in literature (Savona et al., 1983; Esquivel et al., 1986; Fernández et al., 1991).

*3.2.1. Polystachyne A (**1**)*

Mp 204–206°C; $[\alpha]_D^{20}$ 8.5° ($CHCl_3$; *c* 0.155); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1782, 1711, 1636, 1503, 873. EIMS (70 eV) *m/z* (rel. int.): 342 (M+, $C_{20}H_{22}O_5$) (10); 297 (5); 287 (3); 246 (8); 217 (3); 115 (33); 105 (25); 95 (60); 91 (100); 77 (50); 65 (45); 55 (24); 53 (28); 41 (32).

*3.2.2. Polystachyne B (**3**)*

Mp 217–219°C; $[\alpha]_D^{20}$ 0.0° ($CHCl_3$; *c* 0.159); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1782, 1503, 1148, 1047, 1020, 997, 876. EIMS (70 eV) *m/z* (rel. int.): 358 (M+, $C_{20}H_{22}O_6$) (87); 340 (3); 329 (23); 312 (7); 302 (7); 295 (8); 262 (9); 211 (10); 197 (36); 122 (68); 94 (100); 81 (38); 55 (16).

*3.2.3. Polystachyne C (**4**)*

Mp 244–247°C; $[\alpha]_D^{20}$ −15.09° ($CHCl_3$; *c* 0.159); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1774, 1502, 1192, 1047, 1026, 876. EIMS (70 eV) *m/z* (rel. int.): 358 (M+, $C_{20}H_{22}O_6$) (100); 340 (4); 329 (10); 312 (9); 295 (8); 294 (8); 236 (14); 211 (14); 197 (17); 179 (37); 161 (13); 122 (22); 94 (56); 81 (28); 55 (12).

*3.2.4. Polystachyne D (**6**)*

Mp 180–182°C; $[\alpha]_D^{20}$ −78.3° ($CHCl_3$; *c* 0.279); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1767, 1739, 1598, 1503, 875. EIMS (70 eV) *m/z* (rel. int.): 358 (M+, $C_{20}H_{22}O_6$) (11); 343 (1); 340 (1); 329 (2); 220 (4); 203 (8); 189 (4); 177 (6); 145 (10); 95 (46); 94 (100); 91 (33); 81 (18); 77 (23); 65 (15); 55 (16).

*3.2.5. Polystachyne E (**8**)*

Mp 251–253°C; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1766, 1744, 1683, 1601, 1500, 876. EIMS (70 eV) *m/z* (rel. int.): 356 (M+, $C_{20}H_{22}O_6$) (6); 340 (6); 338 (2); 327 (2); 262 (7); 203 (10); 189 (13); 115 (19); 105 (18); 95 (100); 91 (40); 77 (37); 65 (22); 55 (26).

*3.2.6. X-ray structure determination of polystachyne C (**4**)*

Crystals of **4** are orthorhombic, space group $P2_12_12_1$, $Z = 4$ with $a = 11.387$ (1), $b = 12.089$ (1), $c = 12.218$ (1) Å, $V = 1681.9$ (2) $Å^3$ and $D_{calc} = 1.415$ mg/m^3. Unit cell and intensity data were recorded on a Siemens P4/PC diffractometer with MoK_α radiation ($\lambda = 0.71073$ Å), $\mu = 0.104$ mm^{-1}. Crystal size: $0.60 \times 0.40 \times 0.36$ mm. Of the 1707 collected reflections with $2\theta \leq 50°$, 1707 were unique and were used in the structure solution and refinement. The structure was solved by direct methods (Altomare, Cascarano, Giacovazzo, Guagliardi, Burla, Polidori & Camalli, 1994) and refined by full-matrix least-square on F^2 method (Sheldrick, 1997). Hydrogen atoms were included at idealised positions with an isotropic temperature factor of 1.2 U_{eq} of the parent C-atom. Refinement converged to a *R* value of 0.043 in the final cycle. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-120563.

*3.2.7. X-ray structure determination of polystachyne D (**6**)*

Compound **6** crystallized in the monoclinic space group $P2_1$, with $a = 7.857$ (1), $b = 12.654$ (2), $c = 8.588$ (1) Å, $\beta = 102.13(1)°$, $V = 834.8$ (2) $Å^3$, $Z = 2$ and $D_{calc} = 1.426$ Mg/m^3. Unit cell and intensity data were recorded on a Siemens P4/PC diffractometer with MoK_α radiation ($\lambda = 0.71073$ Å), $\mu = 0.105$ mm^{-1}. Crystal size: $0.74 \times 0.44 \times 0.32$ mm. Of the 1666 collected reflections with $2\theta \leq 50°$, 1552 were unique and were used in the structure solution and refinement. The structure was solved by direct methods (Altomare et al., 1994) and refined by full-matrix least-square on F^2 method (Sheldrick, 1997). Hydrogen atoms were included at idealized positions with an isotropic temperature factor of 1.2 U_{eq} of the parent C-atom. Refinement converged to a *R* value of 0.037 in the final cycle. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-120564.

Acknowledgements

We are very grateful to Rubén A. Toscano for the X-ray analysis and to Rubén Gaviño for the NMR experiments. We also thank Mssrs. Luis Velasco, Javier Pérez and Rocío Patiño for technical assistance.

References

Altomare, A., Cascarano, G., Giacovazzo, C., Guagliardi, A., Burla, M. C., Polidori, G., & Camalli, M. (1994). *Journal of Applied Crystallography*, *27*, 435.

Eguren, L., Fayos, J. F., Perales, A., Savona, G., & Rodríguez, B. (1984). Salvifarin, X-ray structure determination of a *cis* neo-clerodane diterpenoid from *Salvia farinacea*. *Phytochemistry*, *23*(2), 466–467.

Esquivel, B., Cárdenas, J., Ramamoorthy, T. P., & Rodríguez-Hahn, L. (1986). Clerodane diterpenoids from *Salvia lineata*. *Phytochemistry*, *25*(10), 2381–2384.

Esquivel, B., Esquivel, O., Cárdenas, J., Sánchez, A. A., Ramamoorthy, T. P., Toscano, R. A., & Rodríguez-Hahn, L. (1991). Clerodane and secoclerodane diterpenoids from *Salvia reptans*. *Phytochemistry*, *30*(7), 2335–2338.

Fernández, M. C., Esquivel, B., Cárdenas, J., Sánchez, A. A., Toscano, R. A., & Rodríguez-Hahn, L. (1991). Clerodane and

aromatic seco-clerodane diterpenoids from *Salvia rhyacophila*. *Tetrahedron*, *47*(35), 7199–7208.

Instituto Nacional Indigenista (Argueta, V.A., Co-ordinator), (1994). *Atlas de las plantas de la medicina tradicional mexicana* (vol. I, pp. 398). México, D.F.

Maldonado, E., & Ortega, A. (1997a). Languidulane, clerodane and secoclerodane diterpenes from *Salvia tonalensis*. *Phytochemistry*, *45*(7), 1461–1464.

Maldonado, E., & Ortega, A. (1997b). Neo-clerodane diterpenes from *Salvia thymoides*. *Phytochemistry*, *46*(7), 1249–1254.

Maldonado, E., Ortega, A., Díaz, E., & Reynolds, W. F. (1998). Variable temperature NMR studies on the conformations of tonalensin in solution. *Spectrochimica Acta, Part A*, *54*(5), 659–670.

Savona, G., Raffa, D., Bruno, M., & Rodríguez, B. (1983). Salvifarin and salvifaricin, neoclerodane diterpenoids from *Salvia farinaca*. *Phytochemistry*, *22*(3), 784–786.

Sheldrick, G. M. (1997). *SHEXL-97, Program for refinement of crystal structures*. Germany: University of Göttingen.

Soriano-García, M., Esquivel, B., Toscano, R. A., & Rodríguez-Hahn, L. (1987). Structure and stereochemistry of (8*S*,12*R*)-*cis*-clerodane-1,3,13(16),14-tetraene-15,16-epoxy-12(17);18,19-diolide (linearolactone), a diterpene. *Acta Crystallographica*, *C43*, 1565–1567.