Polystachyne F, a 5,10-*seco*-Neoclerodane from *Salvia polystachya*[1)]

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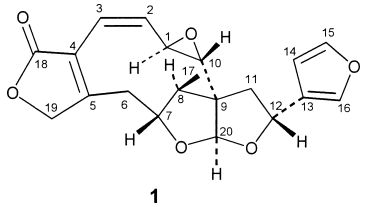
A new 5,10-*seco*-neoclerodane diterpene, polystachyne F (1), was isolated from the aerial parts of *Salvia polystachya*. Its structure was established on the basis of its spectroscopic properties and X-ray crystallographic analysis. Some correlations in the NOESY spectrum of 1 suggested the existence of a conformational equilibrium.

Key words *Salvia polystachya*; Lamiaceae; 5,10-*seco*-neoclerodane; polystachyne F

Salvia polystachya Ort. (Lamiaceae) is a small shrub native from México where its seeds, known as "chía", are used as food and beverage ingredients.[2,3)] In Mexican traditional medicine, this plant is mainly used as purgative and antigastralgic, although other uses such as antipyretic, antipaludic, antihemorragic and emollient, have also been described.[2)] In an earlier work, we isolated eight clerodane type diterpenes (salvifaricin, linearolactone, dehydrokerlin and polystachynes A—E) from this plant.[4)] Now, we described the isolation of a further clerodane derivative from this species.

Results and Discussion

Polystachyne F (**1**) was obtained as colorless plates. Its molecular formula, $C_{20}H_{20}O_6$, deduced from the quasimolecular ion peak at *m/z* 357 (ESI-MS-positive mode), together with the data of the ^{13}C-NMR DEPT spectrum, revealed its diterpenic nature. The IR spectrum exhibited absorption bands at 1752 (α,β-unsaturated-γ-lactone), 1645 (double bonds), 1502 and 874 (β-substituted furan ring) cm^{-1}. The ^{13}C-NMR spectrum confirmed the presence of the γ-lactone by the signals of a carboxylic and a oxygenated methylene carbons at δ_C 172.3 (C-18) and 72.4 (C-19). The latter correlated in the HSQC spectrum with the proton signals of an AB system (J=17.5 Hz) at δ_H 5.07 (H-19a) and 4.70 (H-19b). ^2J, ^3J H–C-couplings of these protons with two non protonated vinylic carbons at δ_C 126.3 (C-4) and 161.0 (C-5) led to propose a C-4 double bond, whose presence was supported by the allylic couplings between the CH_2-19 protons and an olefinic proton at δ_H 6.29 (1H, d, J=11 Hz, H-3), observed in the ^{1}H–^{1}H COSY spectrum. In this spectrum, the observed couplings between the H-3 signal and the signal at δ_H 5.90 (1H, br t, J=8.8 Hz, H-2), and of the latter with that at δ_H 3.42 (1H, br d, J=5.5 Hz, H-1), which in turn was coupled with the singlet signal at δ_H 3.01 (1H, H-10), allowed us to established a C-2 double bond and a 1(10) epoxy group. The presence of these functionalities was corroborated by the ^{13}C-NMR signals at δ_C 132.9 (C-2), 122.5 (C-3), 57.9 (C-1) and 58.3 (C-10).



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Clerodane diterpenes usually posses one secondary and one tertiary methyl groups at C-8 and C-9 respectively. In the ^{1}H-NMR spectrum of **1** only one methyl signal at δ_H 1.46 (3H, d, J=7.5 Hz) was observed. This signal was assigned to the secondary methyl group (CH_3-17) at C-8, since the C-8 proton δ_H 2.11 (1H, q, J=7.5 Hz, H-8) correlated, in the HMBC spectrum with C-17, C-10, and with the signals of a quaternary carbon at δ_C 56.8 (C-9), a CH_2 carbon at δ_C 36.1 (C-6), and an acetalic carbon at δ_C 108.2. The last carbon signal was attributed to C-20 by its ^2J, ^3J H–C-couplings with the signals of H-8, H-11a and with two CH protons whose chemical shifts at δ 5.16 and 4.66, indicated be geminal to oxygenated functions. The former was assigned to H-12 by its ^{1}H–^{1}H couplings with both C-11 protons, while the latter was attributed to H-7 due to its couplings with the C-6 protons. The above mentioned and the observed HMBC correlations of C-20 with H-7 and H-12 implies the presence of ethereal C-7—C-20 and C-12—C-20 linkages.

The ^{1}H- and ^{13}C-NMR spectra of **1** showed signals for a β-substituted furan at δ_C 128.2 (C-13), δ_C108.5, δ_H 6.34 (CH-14), δ_C 143.7, δ_H 7.37 (CH-15) and δ_C 138.9, δ_H 7.35 (CH-16). The C-13, C-14 and C-15 carbon signals correlated in the HMBC spectrum with the H-12 proton signal, thus establishing the position of the furan ring and the structure **1** for polystachyne F.

Analysis of both, Dreiding models and NOESY spectrum of **1** led us to establish its relative configuration as follows: NOE interactions of the equatorial CH_3-17 with H-7, H-11a and H-12 indicated a 7*R*, 8*S*, 9*R* configurations, if a 12*R* configuration, as those assigned to the neoclerodanes previously isolated from *S. polystachya*,[4)] is assumed. On the other hand, the β axial H-8 showed strong interactions with H-6, H-7 and H-10, thus establishing a β-orientation of H-10 and, as consequence, a 10*R* configuration. The NOE between H-2 and H-3 indicated a *Z* configuration of the C-2 double bond and the NOEs between H-2 and H-10 and between H-1 and H-20 established a *trans* epoxy group, and therefore *R* configurations at C-1 and C-20. These assignments were confirmed by the X-ray crystallographic analysis of **1** (Fig. 1).

Conformation A (Fig. 2) agrees with the above mentioned NOEs exhibited by compound **1**, nevertheless, the observed NOEs of H-1 with H-2 and H-6 and that of H-8 with H-19b (weak), can only be explained if compound **1** adopts conformation B (Fig. 2). The broadening of the signals for C-1, C-2 and C-6 protons, supports the presence of both conformers, but they should be in a rapid equilibrium since the ^{1}H- and

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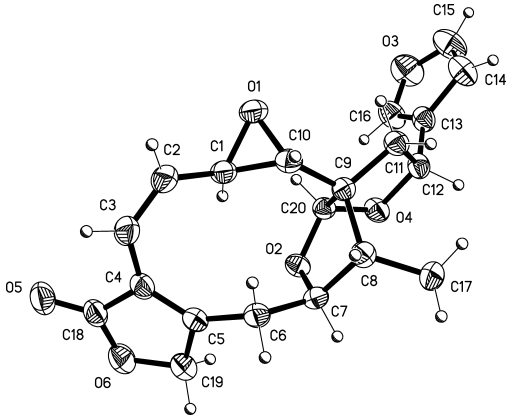

Fig. 1. ORTEP Diagram of Polystachyne F (**1**)

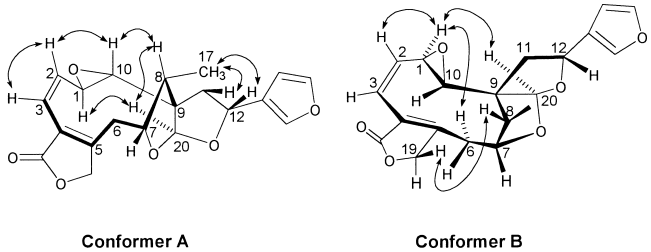

Fig. 2. NOE Interactions for Conformers A and B of Polystachyne F

^{13}C-NMR signals did not appear in duplicate. A similar conformational behavior was observed in other 5,10-*seco*-neoclerodanes like tonalensin, a quite related compound isolated from *Salvia tonalensis*,[5] and 7α-hydroxystrictic acid methyl ester isolated from *Coniza hypoleuca*.[6]

Experimental

General Experimental Procedures The melting point (uncorrected) was determined in a Fisher–Johns apparatus. Column chromatographies were performed on silica gel 60 (Merck G), while silica gel 230—400 mesh (Macherey–Nagel) was used for flash chromatography. TLC was carried out on precoated Macherey–Nagel Sil G/UV$_{254}$ plates of 0.25 thickness, and spots were visualized by spraying with 3% $CeSO_4$ in H_2SO_4 2 N followed by heating. The optical rotation was measured on a Perkin Elmer 343 polarimeter. The UV and IR spectra were recorded on a Shimadzu UV 160U and a Bruker Tensor 27 spectrometers, respectively. ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR spectra were recorded on a Varian Unity Plus 500 spectrometer with TMS as internal standard. Mass spectrum was recorded on a Bruker Daltonics Esquire 6000 spectrometer.

Plant Material Aerial parts of *Salvia polystachya* Ort. were collected, as in the previous study, in Huitzilac, State of Morelos, México, in June 2005. A voucher specimen (MEXU-573762) is deposited at the Herbarium of the Instituto de Biología, UNAM.

Extraction and Isolation The air-dried powered leaves of *S. polystachya* (1.2 kg) were packed in a glass column and extracted with Me_2CO (10 l). The extracts were combined and concentrated *in vacuo* to dryness. The dried residue (86.9 g) was chromatographed on a column of silica gel eluted with CH_2Cl_2 (fr. A, 2 l, 32.4 g; fr. B, 4 l, 14.67 g); CH_2Cl_2–EtOAc 19 : 1 (fr. C, 1.5 l, 14.2 g); CH_2Cl_2–EtOAc 7 : 3 (fr. D, 1.5 l, 21.37 g); EtOAc (fr. F, 2 l, 3.04 g). Fraction B was chromatographed over silica gel using mixtures of hexane–EtOAc–Me_2CO (80 : 10 : 4, frs. 1—36; 70 : 15 : 9, frs. 37—82; 200 ml each). Fractions 41—66 were combined and further purified by column chromatography eluted with hexane–EtOAc 7 : 3. 37 fractions of 50 ml each were obtained, from which, fractions 20—27 (210.9 mg) were combined and submitted to flash chromatography on silica gel 230—400-mesh eluted with mixtures of hexane–EtOAc (4 : 1, frs. 1—30; 4 : 2, frs. 31—85; 25 ml each). Compound **1** (32.1 mg) was purified from fractions 50—64 by crystallization from Me_2CO–hexane.

Table 1. ^{1}H- and ^{13}C-NMR Data of Polystachyne F (**1**) (500, 125 MHz in $CDCl_3$, TMS)

Position	δ_H (J in Hz)	δ_C	DEPT	HMBC (H–C) couplings
1	3.42 br d (5.5)	57.9	CH	C-2, C-10
2	5.90 br t (8.8)	132.9	CH	C-4
3	6.29 d (11)	122.5	CH	C-1, C-5
4		126.3	C	
5		161.0	C	
6,6′	2.80 br s (w/2=17)	36.1	CH_2	C-5, C-7, C-8, C-19
7	4.66 t (9)	85.0	CH	C-9, C-17, C-20
8	2.11 q (7.5)	47.9	CH	C-6, C-9, C-10, C-17, C-20
9		56.8	C	
10	3.01 s	58.3	CH	C-9, C-20
11	2.58 br dd (13.5, 8.5)	34.3	CH_2	C-8, C-9, C-13, C-20
	2.28 dd (13.5, 7.5)			C-8, C-9, C-10, C-12, C-13
12	5.16 t (7.5)	73.4	CH	C-11, C-13, C-14, C-16, C-20
13		128.2	C	
14	6.34 s	108.5	CH	C-13, C-15, C-16
15	7.37 t (2.5)	143.7	CH	C-13, C-14, C-16
16	7.35 br s	138.9	CH	C-14, C-15
17	1.46 d (7.5)	17.1	CH_3	C-7, C-8, C-9
18		172.3	C	
19	5.07 d (17.5)	72.4	CH_2	C-4, C-5
	4.70 d (17.5)			C-4, C-5
20	5.05 s	108.2	CH	C-7, C-8, C-9, C-10

Polystachyne F (**1**) was obtained as colorless plates (Me_2CO–hexane); mp 135—138 °C. ^{1}H- and ^{13}C-NMR spectroscopic data and HMBC correlations, see Table 1. IR (film) cm^{-1}: 1752, 1645, 1502, 874. UV λ_{max} (MeOH) nm (ε): 210 (14820). ESI-MS (positive mode) m/z: 379 $[M+Na]^+$, 357 $[M+H]^+$. $[\alpha]_D^{22}$ −166.8° (c=0.180, $CHCl_3$).

X-Ray Crystallographic Analysis of Polystachyne F (1) Crystals data: $C_{20}H_{20}O_6 \cdot 0.5C_3H_6O$, formula weight 385.40, crystal size 0.446×0.322×0.058 mm, monoclinic, space group C2, Z=4 with a=23.772(2) Å, b=8.406(1) Å, c=9.898(1) Å, β=104.034(2)°, V=1918.9(3) Å^3, D_{calc}=1.334 mg/m^3, $F(000)$=816 and GOF=1.039. Unit cell and intensity data were collected on a Bruker Smart Apex CCD diffractometer with Mo$K\alpha$ radiation (λ=0.71073 Å), μ=0.099 mm^{-1}. The structure was solved by direct methods and refined by full-matrix least square on F^2 method.[7] The final data for R and wR factors with $I>2\sigma(I)$ were 0.0367 and 0.0884, respectively. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC 612712).

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References and Notes

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