A *CIS*-LANGUIDULANE DITERPENOID FROM *SALVIA MEXICANA* VAR. *MAJOR* (LABIATAE)

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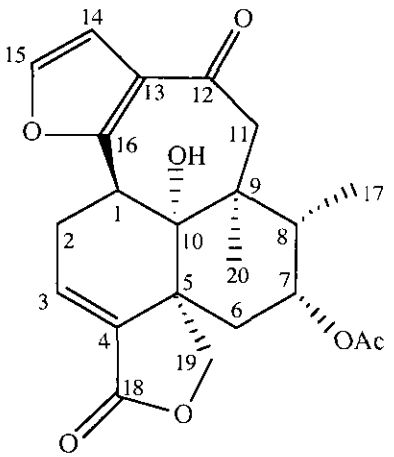
Abstract- A new *cis*-languidulane diterpenoid, named Salvimexicanolide has been isolated from the aerial parts of *Salvia mexicana* var. *major* besides the known flavonoid naringenin. The structure of the new diterpenoid was established by spectroscopic methods and confirmed by X-Ray analysis.

Salvia L. (Tribe Mentheae) is one of the most diversified and larger genera of the Labiatae. It is a tropical and subtropical genus with over 900 species.[1] Most of the 500 species of Salvia found in Mexico, Central and South America belong to the Subgenus Jungia (formerly Calosphace).[2] Several diterpenes have been isolated from this genus with abietane, *neo*-clerodane or rearranged *neo*-clerodane skeleton. Most of the diterpenoids isolated from the American *Salvia sp.* are neoclerodane or can be biogenetically derived from a clerodane precursor.[3] The languidulane skeleton, for instance, could be originated for the union of the C-16 to the C-1 of a clerodane and is somewhat distributed in the genus.[4-7] As a part of our ongoing chemical studies on Mexican *Salvia* species we report in this paper the structure of a new languidulane diterpenoid named salvimexicanolide (**1**), isolated from the aerial parts of *Salvia mexicana* var. *major* Benth. (Subgenus Jungia, Section Briquetia), a species endemic to Mexico.[8] Previous work on this plant revealed the presence of triterpenoids.[9] The structure of **1** was established from its spectroscopic data and confirmed by X-Ray analysis. Salvimexicanolide (**1**) is the first example of an A/B *cis* fused languidulane diterpenoid.

Extraction of the aerial parts of *Salvia mexicana* var. *major* afforded after extensive chromatography, naringenine[10] and a new *cis*-languidulane diterpenoid to which we have assigned structure (**1**) on the following considerations.

Compound (**1**) was assigned the molecular formula $C_{22}H_{24}O_7$ by HRMS. Its IR spectrum showed absorptions for a γ-lactone (1755 cm^{-1}), hydroxyl group (3414 cm^{-1}), ester carbonyl (1723 cm^{-1}) and an α, β-unsaturated ketone (1665 cm^{-1}). The absorptions at 1581, 926 and 860 cm^{-1} were attributed to a disusbtituted furan ring. The ^1H NMR spectra of salvimexicanolide (**1**) (Table 1) showed two one-proton doublets (J = 1.8 Hz) at δ 7.57 and 6.63 characteristics of the C-14 and C-15 protons of a languidulane diterpenoid with an α, β-substituted furan ring. The chemical shift of these protons indicated the presence of a carbonyl group at C-12 as in languiduline,[4] salvisousolide[5] and related languidulane derivatives.[6,7] The ^{13}C NMR spectrum of **1** (Table 2) showed signals at δ 125.3 *s*, 109.3 *d*, 143.5 *d* and 161.6 *s*, corresponding to the α, β-substituted



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furan ring. The signal for the C-12 carbonyl group was observed at δ 194.9 *s*. An AB system observed, in the ^{1}H NMR spectrum of **1**, at δ 4.93 and 4.5 (J = 8.1 Hz) was assigned to the C-19 methylene protons. The *pro-S* H-19 (δ 4.5) shows an additional long-range coupling of 1.8 Hz with the H-6β. This fact indicated the lack of a substituent at the C-6β position and the *axial* orientation of the C-19 methylene group.[11] Furthermore the chemical shift of the H-19 *pro-R* (δ 4.93) indicated the presence of an acetate group bound to the C-7α *axial* poisition.[12] A double triplet at δ 4.82 (J = 3.9 and 3.3 Hz) was assigned to the geminal proton of this moiety. On the other hand, the H-19 *pro-S* shows a downfield shift ($\Delta\delta \approx 0.4$) respect to the same signal described for other languidulane and *neo*-clerodane diterpenoids.[4-7] The downfield shift observed is due to the deshielding effect produced by the α oriented hydroxyl group at C-10, whose existence was confirmed by the ^{13}C NMR spectrum (δ 77.7, s) and indicated by the IR data. On the basis of the previous discussion an A/B *cis* fusion is proposed for salvimexicanolide (**1**). Inspection of a Dreiding model of **1** supports this assumption. The chemical shift observed for the C-20 methyl group (δ 20.8), in the ^{13}C NMR spectrum of **1** (Table 2), is consistent with this fact.

Table 1.^{1}H NMR Data for Salvimexicanolide (**1**)†

H	δ	J	H	δ	J
1	4.18 dd	2.1, 9.4	15	7.57 d	1.8
2 α	3.29 ddd	2.1, 3.9, 21	3H-17	0.81 d	7
2 β	2.97 ddd	3.9, 9.4, 21	19 pro *R*	4.93 d	8.1
3	6.93 t	3.9	19 pro *S*	4.5 dd	1.8, 8.1
7	4.82 dt	3.3, 3.9	3H-20	1.16 s	-
11α	2.48 d	17.4	CH_3CO	2.09 s	-
11β	3.43 d	17.4	-OH	4.63 s	-
14	6.63 d	1.8			

†Run at 300 MHz, Me_2CO-d_6, TMS, J in Hz. Assignments confirmed by COSY spectrum

Other relevant signals in the ^{1}H NMR spectrum of **1** (Table 1) are those assigned to the H-1α, H-2α and H-2β protons. The coupling constants observed for these signals support an α orientation for H-1. An AB system at δ 2.48 (J = 17.4 Hz) and 3.43 was assigned to the C-11 methylene protons.

The structure of **1** was confirmed by X-Ray diffraction analysis of a single crystal. The molecular structure is illustrated in Figure 1. This analysis confirmed the A/B *cis* fusion and the relative stereochemistry depicted in **1**. The interatomic distance between the H-19 *pro-S* and the oxygen of the hydroxyl group at C-10 is 2.22 Å, leading to a strong interaction, thus accounting for the downfield shift observed for this proton

(*vide supra*). Salvimexicanolide (**1**) is, to the best of our knowledge, the first example of an A/B *cis* fused languidulane derivative.

Languidulane diterpenoids have been previously isolated from species belonging to Section Angulatae (*S. urolepis* and *S. languidula*)[4,6] and Polystachyae (*S. sousae* and *S. tonalensis*).[5,7] From a chemotaxonomic point of view is interesting the presence of a languidulane diterpenoid in a plant belonging to Section Briquetia, which is unrelated to the Sections Angulatae and Polystachyae.[8] Although a correlation between the diterpenoid content of the *Salvia* spp. studied and the botanical section to which it belongs, has been proposed,[13] several exceptions are now known.[14,15] An analysis of recent findings[16] on the distribution of diterpenoids in *Salvia* Subgenus Jungia indicated that this proposal must be reconsidered.

Table 2.^{13}C NMR Data for Salvimexicanolide (**1**)¶

C	δ	C	δ
1	37.4 d	14	110.3 d
2	22.5 t	15	142.6 d
3	136.4 d	16	154.1 s
4	132.7 s	17	11.7 q
5	48.7 s	18	175.3 s
6	29.5 t	19	73.0 t
7	71.9 d	20	21.3 q
8	39.6 d	$O\underline{C}OCH_3$	169.9 s
9	46.5 s	$OCO\underline{C}H_3$	21.4 q
10	78.1 s		
11	52.4 t		
12	193.8 s		
13	122.4 s		

¶Run at 75 MHz, $CDCl_3$, TMS. Assignments confirmed with the aid of DEPT and HETCOR spectra.

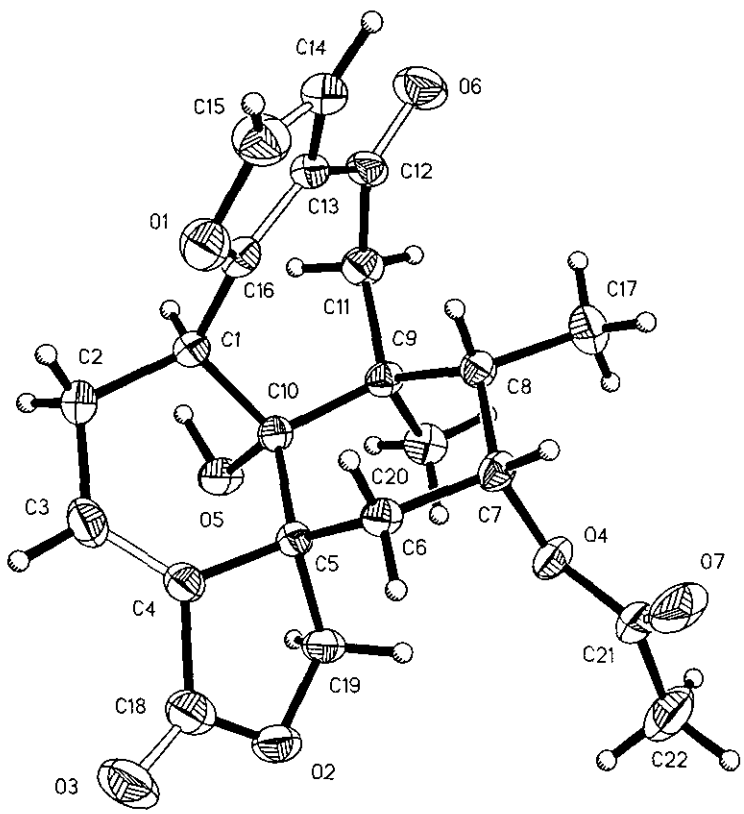

Figure 1. Computer generated perspective drawing of salvimexicanolide (**1**)

EXPERIMENTAL

Mps are uncorrected. *Salvia mexicana* var. *major* was collected in the State of Veracruz (México) in May 1996. Voucher specimen (MEXU702360) was deposited at the herbarium of the Instituto de Biología UNAM). *Extraction, Fractionation and Isolation of Salvimexicanolide from Salvia mexicana* var. *major*. Dried and powdered aerial parts of *S. mexicana* (451 g) were extracted with Me_2CO (5 L) for 5 days at rt. The solvent was removed in *vacuo* to yield 39 g of a gummy residue which was partitioned between MeOH-H_2O (4:1) and benzene-petrol ether (1:1). The aqueous methanolic fraction was concentrated in *vacuo*, water was added and the mixture was extracted with EtOAc. The organic phase was dried with anh. Na_2SO_4 and the solvent removed to yield 8.9 g of a gum. A portion (5.3 g) of this gum was subjected to vacuum chromatography over silica gel. Mixtures of petrol ether-EtOAc of increasing polarity were used as eluents. From the fractions eluted with petrol ether-EtOAc (4:6), salvimexicanolide (**1**) (12.2 mg) was isolated after crystallization with EtOAc. The mother liquors were subjected to flash chromatography (petrol ether-EtOAc, 7:3) to yield naringenin (10 mg). The physical data obtained (mp, MS, IR and ^{1}H NMR) were identical with those published in the literature.[10] Salvimexicanolide (**1**). Crystalline pale-yellow solid, mp 285-286°C; $[\alpha]_D$ 144° (c 0.1; MeOH); IR ν_{max} (KBr) cm^{-1}: 3414, 1755, 1723, 1665,1581, 926, 860; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; MS m/z (rel. int.): 400 (100), 382 (10), 368 (15), 358 (65), 340 (32), 322 (8), 295 (25), 267 (25), 235 (20), 225 (20), 189 (20), 177 (20), 163 (20), 147 (20), 135 (44), 121 (33), 109 (26), 81 (23), 43 (85). $C_{22}H_{24}O_7$ requires M^+ at m/z 400.

*X-Ray Structure Determination of Salvimexicanolide (**1**).* The pale-yellow crystal of **1**, was obtained by slow evaporation from EtOAc. A suitable crystal with dimensions 0.24 X 0.20 X 0.18 mm was selected. The data were collected on a Siemens P4/PC diffractometer. Intensities were collected at rt using CuKα radiation (λ = 1.54178 Å) and were corrected for background, Lorentz and polarization effects. The structure was solved by direct methods[17] and refined by full-matrix least-squares with anisotropic temperature factors for the non-hydrogen atoms. The hydrogen atoms were included at idealized positions except for the hydrogen atom bounded to oxygen.

ACKNOWLEDGMENT

The authors thanks to Ms. Sci. María del Rosario García-Peña for plant identification. We are also indebt Ms. Sci., I. Chávez, B. Quiroz, W. Matus, R. Patiño, L. Velasco and F. Pérez for technical assistance. This work was supported in part by DGAPA , UNAM (México, Grant PAPIIT IN216698).

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Received, 19th February, 1999