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A NEW *NEO*-CLERODANE DITERPENOID FROM *SALVIA GESNERAEFLORA* (LABIATAE).

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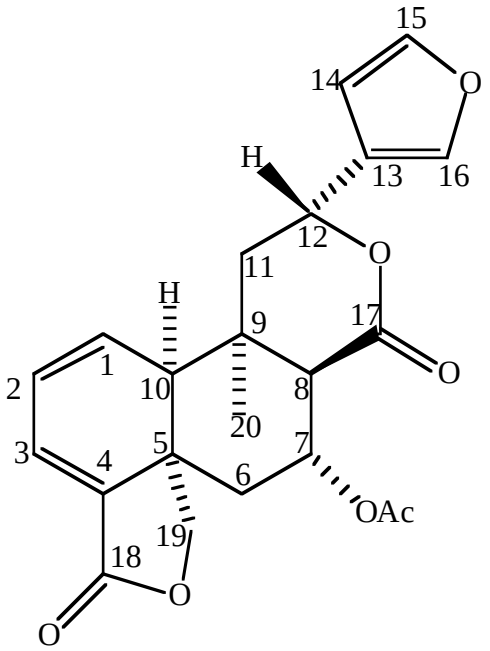
Abstract- The aerial parts of *Salvia gesneraeflora* afforded, besides salviarin and gensnerofolin B, a new neoclerodane named 7α-acetoxy-7,8α–dihydrogensnerofolin B. Its structure was elucidated by spectroscopy methods and chemical correlation with gensnerofolin B.

Plants of the Labiatae Family have attracted much attention owing to a variety of medicinal properties and biological activities attributed to them.[1,2] *Salvia* constitutes the largest genus in the Labiatae family with about 900 species in the world. This genus is well represented in México with *ca.* 300 species. *Salvia gesneraeflora* (Subgenus Jungia, Section Nobiles) is a shrub which grows abundantly nearby Mexico City and is related to *Salvia fulgens*.[3] Previous work on this species led to the isolation of gensnerofolines A and B.[4] The former was later identified as salviarin, the main constituent from *Salvia splendens*.[5,6] The ^{13}C NMR data of gensnerofolin B were not reported in the earlier paper and the stereochemisty was not ascertained although was later proposed to be related to salviarin, based on biogenetic grounds.[7] As a part our ongoing phytochemical studies on Mexican Labiatae spp,[8,9] looking for potential insect antifeedant *neo* -clerodane diterpenoids, in this paper we described the isolation and characterization of the chemical constituents of a new collection of *Salvia gesneraeflora*. Besides salviarin and gensnerofolin B (**2**) we isolated a new *neo* -clerodane derivative whose structure was established as 7α-acetoxy-7,8α-dihydrogensnerofolin B (**1**). The relative steroechemistry of **1** was established unambiguously by spectroscopic methods. Chemical correlation of **1** with **2** led us to deduce the relative stereochemistry of gensnerofolin B.

Extraction of the aerial parts of *S. gesneraeflora* afforded after extensive chromatographic purification, salviarin, gensnerofolin B (**2**) and a new neoclerodane diterpenoid to which we have assigned structure (**1**) on the following considerations. Compound (**1**) was isolated as a crystalline solid, mp 193 - 195°, and was assigned the molecular formula $C_{22}H_{22}O_7$ by FAB^+, HRMS. Its IR spectrum showed absorptions for a γ-lactone ring (1757 and 1175 cm^{-1}), δ-lactone ring and ester carbonyl (1712 cm^{-1}), double bonds (1668 cm^{-1}), a β-substituted furan ring (1580 and 875 cm^{-1}) and the absence of hydroxyl groups. The UV spectrum showed absorption in agreement with the presence of a homoannular diene system at 298 nm (log ε = 3.522).[5,10]

The relative stereochemistry proposed for **1** in this paper is supported by the following data. The ^{1}HNMR spectra of **1** (Table 1) showed, in addition to the characteristic signals for a β - substituted furan ring and the protons of a dienic moiety (H-1, H-2, and H-3),[5,10] an AB system at δ 4.86 and 4.15 (J = 9.0 Hz) which was assigned to the C-19 methylene protons. The *pro-S* H-19 (δ 4.15) shows an additional long-range coupling of 1.5 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 4.86) indicated the presence of an acetate group bound to the C-7α *axial* poisition.[11] A double triplet at δ 5.68 (J = 5.7 and 3.0 Hz) was assigned to the geminal proton of this moiety (H-7) and a singlet at δ 2.10 to the corresponding methyl group. The ^{13}C NMR spectra of **1** (Table 2) showed the signals for the acetate group at δ_C 169.5 s and 21.4 q. A doublet at δ 2.83 was attributed to H-8. The J value (3.0 Hz) indicated a β-orientation for the C-17 carbonyl group present in **1** , as in salviarin,[6] 1(10)-dehydrosalviarin[5] and linearolactone.[5,10]



1

Table 1. ^{1}H NMR Data for compounds (**1**) and (**2**)†

	1		**2**	
H	δ	J	δ	J
1	6.11 dd	9.5, 2.1	6.04 dd	9.5, 2.5
2	6.43 ddd	9.5, 5, 3	6.43 ddd	9.5, 5.0, 3.5
3	7.00 d	5.0	7.00 d	5.0
6			α 2.39 dd	18.0, 3.5
			β 2.35 ddd	18.0, 3.5, 1
7	5.68 dt	5.7, 3	6.85 t	3.5
8	2.83 d	3.0		
10	2.70 br t	2.1	2.70 dd	3.5, 2.5
11α	1.95 dd	15.3, 12.3	2.11 dd	15.0, 12.0
11β	2.53 dd	15.3, 4.0	2.54 dd	15.0, 2.5
12	5.35 dd	12.3, 4.0	5.13 dd	12.0, 2.5
14	6.47 dd	2.0, 0.5	6.49 dd	2.0, 1.0
15	7.46 t	2.0	7.46 t	2.0
16	7.53 m		7.54 m	
19 pro *R*	4.86 d	9.0	4.46 d	8.5
19 pro *S*	4.15 dd	9.0, 1.5	4.24 dd	8.5, 1.0
3H-20	1.35 s		1.28 s	
C$\underline{H}_3$CO	2.10 s			

†Run at 300 MHz, $CDCl_3$, TMS, J in Hz. Assignments confirmed by COSY spectrum.

The ^{1}HNMR spectra of **1** also exhibited an ABX system. The X part ascribed to H-12, was located at δ 5.35 (1H, *dd* , J=12.3 and 4.0 Hz). The *J* values of this signal suggested [5,10] an *axial* orientation for H-12. The AB part was localized a δ 1.95 (*dd*, J = 15.3, 12.3, H-11 α-axial) and δ 2.53 (*dd* , J=15.3, 4.0 Hz, H-11 *β* -equatorial). A three-protons singlet at δ 1.35 in the ^{1}H NMR spectra of **1** (Table1) was assigned to the C-20 methyl group. This fact together with the chemical shift observed for C-20 at δ 29.1 (q), in the ^{13}C NMR spectra of **1** (Table 2) indicated an A/B *cis* fusion, as in linearolactone,[5] a *cis neo*-clerodane diterpenoid previously isolated form *Salvia lineata* (Subgenus Jungia, Section Fulgentes), whose strucutre was confirmed by X-Ray analysis.[10] NOESY spectrum confirms the relative stereochemistry depicted in **1**. The signal ascribed to H-10 showed strong NOE cross peak with Me-20, which in his turn correlate with H-19 *pro S*. These facts indicate that these groups are in the same plane of the molecule.

Table 2.^{13}C NMR Data for Compounds **1** and **2**¶

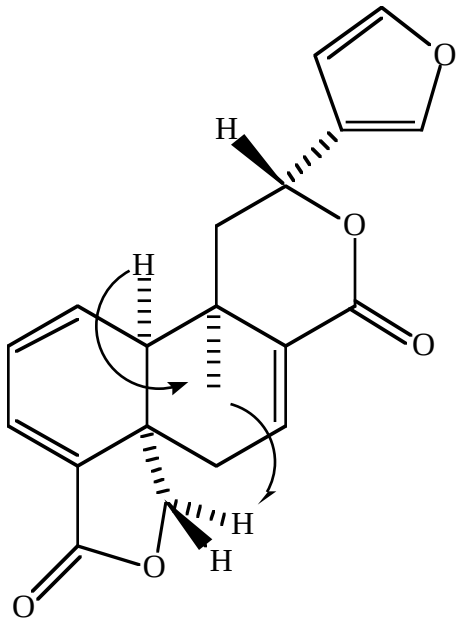
C	**1**	**2**	C	**1**	**2**
1	132.2 d	133.7 d	12	71.1 d	72.1 d
2	127.4 d	127.1 d	13	135.1 s	134.3 s
3	128.6 d	128.4 d	14	108.4 d	108.4 d
4	132.5 s	133.0 s	15	143.9 d	143.9 d
5	38.8 s	38.5 s	16	139.9 d	139.9 d
6	31.2 t	32.4 t	17	169.9 s	167.9 s
7	64.7 d	138.8 d	18	168.2 s	168.1 s
8	44.1 d	123.2 s	19	77.8 t	79.0 t
9	35.4 s	34.3 s	20	29.1 q	33.1 q
10	50.6 d	48.6 d	$O\underline{C}OCH_3$	169.5 s	
11	42.0 t	40.8 t	$OCO\underline{C}H_3$	21.4 q	

¶Run at 125 MHz. $CDCl_3$, TMS. Assignments confirmed with the aid of HMBC and HMQC spectra.

Compound (**2**) was isolated as a crystalline solid, mp 206-208^{o} and its spectral data IR, ^{1}HNMR (Table 1) and MS are identical to those described for gensnerofolin B, previously isolated from the same source.[4] Nevertheless, the relative stereochemistry of **2** was not assigned in the previous work. The previously not described ^{13}C NMR dat of **2** is included in Table 2 for comparison. The chemical shift observed for C-20 (δ 33.1, q) indicated an A/B *cis* fusion as in **1**. This fact was confirmed by NOE difference spectrscopy, since irradiation of the C-20 methyl protons produce the increment of the signals ascribed to H-10 and the H-19 pro *S* (see Table 1 and structure **2**). Ont he previous discusion the A/B ring fusion, as *trans*, assigned to gensnerofolin B, based on biogenetic grounds must be amended.[7]

On the other hand it is interesting to note that all the neo-clerodane diterpenoids with and α,β-γ,δ-diunsaturated γ-lactone function in ring A so far isolated from *Salvia* spp possesses an A/B *cis* ring fusion.[5,7,10]

Treatment of **1** with $KHCO_3$ in aq. MeOH under mild conditions (see experimental) afforded **2** in an straightforward manner. The β-elimination of the acetoxy group could occur *via* an E1cB mechanism after the epimerization at C-8, rather than a *syn* elimination process.[12] The epimerization reaction proposed has been observed in related clerodanic dilactones, for example in salviarin, under mild alkaline conditions.[13] On the previous discusion compound (**1**) must be named 7α-acetoxy-7,8α-dihydrogensnerofolin B.



2, arrows indicate NOE interactions.

EXPERIMENTAL

Mps are uncorrected. *S. gensneraeflora* was collected in Ajusco Mountain (México, D.F.) in December 1999. Voucher specimens were deposited at the herbarium of the Instituto de Biología UNAM.

Extraction, fractionation and isolation of salviarin, gensnerofolin B, and 7α-acetoxy-7,8α-dihydrogensnerofolin B from S. gensneraeflora. Dried and powdered aerial parts of *S. gensneraeflora* (4500 g) were extracted x 4 with Me_2CO (10 L) for 8 days at rt. The gummy extract (150 g), obtained after evapn. of the solvent under reduced pressure, was partitioned between petrol - C_6H_6 (1:1) and MeOH-H_2O (4:1) The polar portion (32.5 g) 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 (4:1), 2000 mg (0.044 % dry wt) of salviarin were isolated. Some fractions eluted with petrol ether - EtOAc (3:2) (2.44 g) were rechromatographed over silica gel using mixtures of petrol ether - EtOAc as eluents. Elution with petrol ether - EtOAc (3:2) yielded 30 mg (6.7×10^{-4} %dry wt) of gensnerofolin B (**2**). The fractions eluted with petrol ether - EtOAc (1:1)(1.53 g) were subjected to flash chromatography purification using $CHCl_3$ - Me_2CO (9:1) as eluent to afford 650 mg (0.014 % dry wt) of 7α-acetoxy-7,8α- dihydrogensnerofolin B (**1**).

*Compound (**1**).* Crystalline solid, mp 193 - 195^o ; $[\alpha]^{20}_D$ - 94.8^o (c 2.3 x 10^{-3}, EtOH); IR ν_{max} ($CHCl_3$) cm $^{-1}$: 1775, 1712, 1668; UV λ_{max} (EtOH) nm (log ε): 209 (4.014), 298 (3.522); ^{1}HNMR see Table 1; ^{13}C NMR see Table 2; FAB$^+$MS m / z (rel. int.): 399 ($M^+ + 1$)(5), 398 (M^+)(20), 356 (30), 338 (35), 323 (33), 300 (7), 282 (5), 244 (17), 230 (6), 204 (82), 189 (38), 160 (40), 135 (78), 121 (50), 81 (85), 77 (27), 43 (100). $C_{22}H_{22}O_7$ requires M^+ at m / z 398.1444, found HRMS 398.1448.

*Treatment of **1** with with $KHCO_3$.* Compound (**1**) (57 mg) in MeOH (30 mL) was treated with $KHCO_3$ (30 mg in 1 mL H_2O) for 5 h, at rt. After usual work-up and flash chromatography purification, 36 mg of **2** were obtained, identical in all aspects (mp, IR, ^{1}H NMR) to gensnerofolin B (**2**).

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REFERENCES

1. B. Esquivel, A.A. Sánchez and E. Aranda, In Phytochemicals and Phytopharmaceuticals, ed. by F. Shahidi and Ch. Ho, AOCS Press, Champaign Illinois, 2000, pp. 371-385.
2. M. Nieto, E.E. García, O.S. Giordano and C.E. Tonn, *Phytochemistry*, 2000, **53**, 911.
3. C. Epling, *Rep. Spec. Nov. Regni Veg. Beih*., 1939, **110**, 1.
4. M. Jiménez, E.D. Moreno and E. Díaz. *Rev. Latinoamer. Quím.*, 1979, **10**, 166 (*Chem. Abstr*., 1980, **93**, 8324z).
5. B. Esquivel, J. Cárdenas, T.P. Ramamorthy and L. Rodríguez-Hahn *Phytochemistry* 1986, **25**, 2381.
6. G. Savona, M.P. Paternostro, F. Piozzi , J.R. Hanson, P.B. Hitchcock, S.A. Thomas, *J.Chem. Soc. Perkin Trans. 1*, 1978, 643.
7. L. Rodríguez-Hahn, B. Esquivel, and J. Cárdenas, *Progress in the Chemistry of Natural Products*, 1994, **63**, 107.
8. B. Esquivel, R.M. Domínguez, M.A. Martínez and G. Espinosa-Pérez, *Heterocycles*, 1997**, 45**, 2247.
9. B. Esquivel, N. Ramírez-Dávalos and G. Espinoza-Pérez, *Heterocycles*, 1999, **51**, 1647.
10. M. Soriano-García, B. Esquivel, R.A. Toscano and L. Rodriguez-Hahn, *Acta Cryst.*, 1987, **C43**, 1565.
11. B. Esquivel, L. M. Hernández, J. Cárdenas, T. P. Ramamoorthy, and L. Rodríguez-Hahn, *Phytochemistry,* 1989, **28**, 561.
12. J. March, Advanced Organic Chemistry. Reactions, Mechanism and Strucutres, 4th. ed. John Wiley and Sons, New York, NY, 1992, pp. 991-995.
13. M.C. Fernández , B. Esquivel, J. Cárdenas, A.A. Sánchez, R. A. Toscano and L. Rodríguez-Hahn, *Tetrahedron*,1991, **47**, 7199.