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STRUCTURE OF SALVIGENOLIDE, A NOVEL DITERPENOID WITH A REARRANGED *NEO*-CLERODANE SKELETON FROM *SALVIA FULGENS*

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Abstract - From the aerial parts of *Salvia fulgens* Cav (Labiatae) a new diterpenoid with a rearranged *neo*-clerodane skeleton was isolated This novel compound named salvigenolide, showed a six - seven A/B ring system with a *trans* fussion A probable biogenetic route is proposed Its structure and relative stereochemistry as in 1, were established by spectroscopic means and X-ray diffraction analysis

In recent years, plants of Labiatae family have been the subject of chemical and biological studies [1] A variety of bi and tricyclic diterpenoids which show antifeedant, antitumor or antifungal properties, have been isolated from some plants of this family [2] In Mexico, Labiatae family is well represented, mainly by genus *Salvia*, which is one of the largest in our country with over 257 species [3] Some of these plants are used as folk medicine,[4] hallucinogens[5] or culinary herbs [2] In a previous paper, we described the structure of melisodoric acid from *Salvia melissodora* Lag [6] Recently *Salvia keerlii* Benth , a closely related species, was studied and two new *neo*-clerodane diterpenoids were isolated [7]

In this paper we describe the structure and stereochemistry of salvigenolide (1), a new diterpenoid with a novel rearranged *neo*-clerodane skeleton, isolated from the aerial parts of *Salvia fulgens* Cav (*Salvia*, section Fulgentes, Epling) a shrub which grows in the valley of Mexico

Salvigenolide (1) was isolated as a crystalline product, mp 218-220°C, and showed molecular formula $C_{22}H_{22}O_7$ by mass spectrometry Its IR spectrum exhibited the characteristic absorption for a furan ring (3140, 1501, 871 cm^{-1}), two γ-lactone functions (1771 and 1761 cm^{-1}), an ester carbonyl (1743 cm^{-1}) and the absence of hydroxyl groups

The ^1H NMR spectrum (Table I) of salvigenolide (1), showed signals for a β-substituted furan ring and an α,β-unsaturated 18 19 - olide, functions very common in clerodane type diterpenoids [8,9] A singlet at δ 2 05 (3H) was ascribed to an acetate group whose geminal proton was observed as a triplet at δ 5 3 This multiplicity, and the fact that none of the C-19 protons (see Table I) showed a longe-range coupling indicated[11] that the acetate group must be bound to C-6 and β axial

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TABLE I ^{1}H NMR Data for Salvigenolide and Derivatives †

Compound	H 3	H-6	H 7	H-8	H 10	H-14	H-12	H-15 16	H 19	H-20	CH_3COOR
1	7 05dd (8 4)	5 3t (3)	β2 45dt (14,3) α1 35m	3 8bd (12)	3 45dd (14 4)	6 25t (2)	6 0bd (1)	7 45d 2H(2)	α3 70d (10) β3 95 (10)	1 6bs 3H	2 05s 3H
1*	6 8dd (8 4)	5 27t (3)	β2 25dt (14 3) α1 35m	3 2bd (12)	2 75dd (14 4)	5 95t (2)	5 45bs	7 15bs 2H	α3 15d (10) β3 57d (10)	1 0bs 3H	1 6s 3H
2		5 05dd (3 4)		3 6bd (10)	3 1m	6 25t (2)	6 0bs	7 45d (2)	α3 85d (10) β4 1d (10)	1 6bs 3H	2 15s 3H
3	7 05dd (8 3)	5 2t (4)		2 95 (12 4)		6 4t (2)	5 45s	7 5m	α3 6d (8) β3 9d (8)	1 35s 3H	2 0s 3H
4	7t (4)	4 6dd (12 4)	β2 4m α3 1dd (16,4)			6 15dd (2,1)	5 75d (2)	7 4t (2) 7 55brs	3 8 bs 2H	1 20d 3H(7)	

‖ Run at 80 MHz using $CDCl_3$ as solvent and TMS as internal standard Coupling constant in Hz are in parenthesis Chemical shifts are in δ values
* Run in C_6D_6 solution

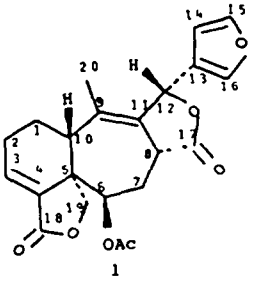
A broad singlet at δ 6 0 was attributed to an allylic proton bound to a carbon bearing oxygen These requirements are satisfied by H-12 if we assume that it is the geminal proton of a γ-lactone function (ν max 1760 cm^{-1}) The fragments at m/e 81 and 95 in ratios 25 and 49% in the mass spectra of 1 (see experimental) support[12] the existence of the lactone group with the alcoholic oxygen at C-12 The absence of a secondary methyl group doublet, frequently observed in the ^{1}H NMR spectra of clerodane type diterpenoids,[1] suggested that C-17 was responsible for the carbonyl group of the γ-lactone function A broad singlet (3H) observed at δ 1 6 in the ^{1}H NMR spectrum of 1 (Table I) was ascribed to the C-20 vinylic methyl group The absence of vinylic protons coupled to it, showed that it is bound to a fully substituted double bond Two sp^2 singlets at δ 133 5 and 133 2 observed in the ^{13}C NMR spectrum of 1 (Table II) were assigned to this double bond

The ^{13}C NMR spectra of clerodane type diterpenoids described in the literature,[7-11] show two sp^3 singlets due to C-5 and C-9, salvigenolide showed only one at δ 48 3 which was assigned to C-5 This fact and the analysis of the data presented, suggested that salvigenolide (1) possesses a rearranged clerodane skeleton

TABLE II ^{13}C NMR Data for Compounds 1 2 and 4 ‡

	1‡	1*	2§	4*
C 1	24 94(t)	25 1 t*	23 t	23 2 t
C-2	25 48(t)	25 5 t**	24 93 t	26 1 t
C 3	140 8 (d)	141 89 d	21 04 t	142 8 d
C 4	133 20(s)	133 64 s	44 98 d	133 8 s
C-5	48 33(s)	48 75 s	46 08 s	49 62 s
C 6	71 42(d)	71 83 d	75 5 d	75 9 d
C 7	30 02(t)	30 39 t	30 69 t	38 97 t
C-8	39 32(d)	39 6 d	37 83 d	121 16 s
C 9	133 5 (s)**	134 6 s**	135 22 s**	23 3 d
C 10	39 32(d)	39 4 d	38 31 d	38 30 d
C-11	133 2 (s)**	133 8 s**	133 87 s**	169 3 s
C-12	75 11(d)	75 21 d	75 03 d	82 75 d
C 13	124 15(s)	124 9 s	124 10 s	121 08 s
C-14	108 44(d)	109 16 d	108 54 d	108 63 d
C-15	144 76(d)	145 13 d	144 69 d	145 04 d
C-16	140 94(d)	140 7 d	141 10 d	137 46 d
C 17	176 69(s)	177 0 s	176 8 s	173 29 s
C-18	168 44(s)	169 s	177 8 s	168 18 s
C-19	68 99(t)	69 23 t	69 09 t	66 8 t
C-20	15 7 (q)	15 40 q	16 67 q	14 28 q
C-21	169 (s)	169 3 s	169 4 s	
C-22	20 8(q)	20 58 q	20 7 q	

† Recorded at 20 MHz Chemical shifts in δ values from TMS SFORD multiplicity in parenthesis
* Run in Py-d_5 as solvent
‡ Run in $CDCl_3$ as solvent
** Values in any vertical column may be interchanged



1

X-ray diffraction analysis of a single crystal of salvigenolide revealed the correct structure and relative stereochemistry as shown in 1

The molecular structure with numbering scheme is illustrated in Fig 1 The salvigenolide molecule comprises an unusual system of six-seven-five *trans*-fused rings with *trans* angles of -19 0 (5), 84 6 (4)° and 62 6 (6), -1 8 (5)°, respectively The cyclohexene ring takes a distorted sofa conformation and the C(2)-C(3)-C(4)-C(5) part is nearly planar with a torsion angle of 3 5 (7)° The cycloheptene ring adopts a conformation of the chair type with an approximate mirror plane through the C-6 atom and their torsion angles in this ring are close to those of the cycloheptene ring in the triterpene 3β-methoxy-21-keto-Δ^{13}-serratene[13] and deacetyl dihydro gaillardin *p*-bromobenzoate [14] Both γ-lactone rings have the envelope conformation with flaps at C(5) and C(17), respectively The furan ring is planar, within the limit of experimental error The γ-lactone ring containing the O (5) atom and the acetate group at C(6) are oriented to minimize transannular repulsions between their oxygen atoms The angle between normals to the planes of the ring and the group is 102 5(5)° The acetate group at C(6) lies almost perpendicular to the cycloheptene ring (torsion angle, C(21)-O(1)-C(6)-C(5) = 107 4 (5)°) serving to bring this group well clear off the ring system The molecules are held in the crystal by van der Waals forces

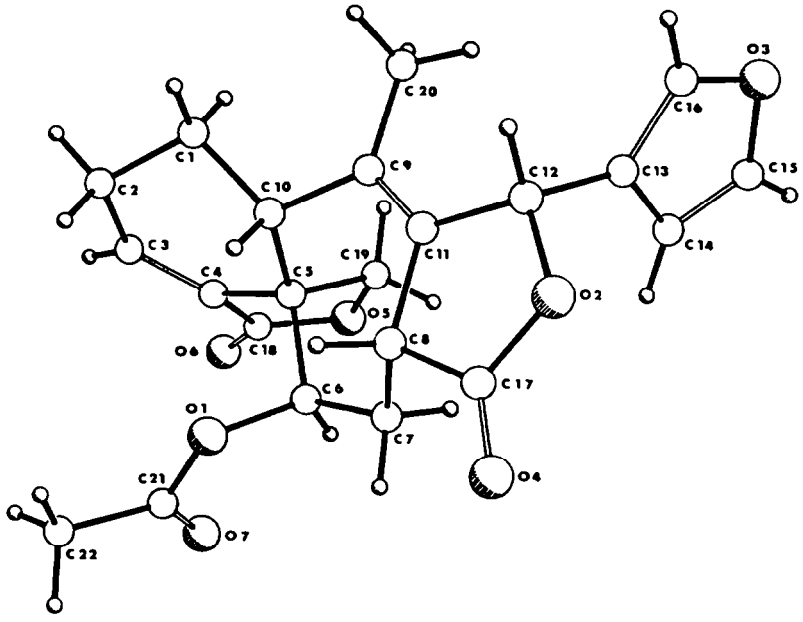
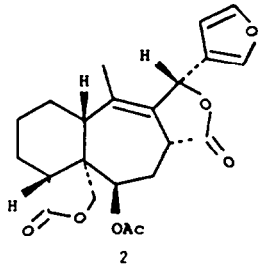


Fig 1 A molecule of salvigenolide showing the atom labelling

Catalytic hydrogenation of salvigenolide 1, gave the dihydroderivative 2 The configuration shown for C-4, was deduced by comparison of the ^{13}C NMR spectrum of 2 (Table II) with literature data for similar structures [10]



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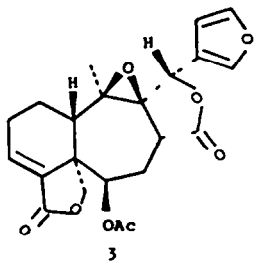
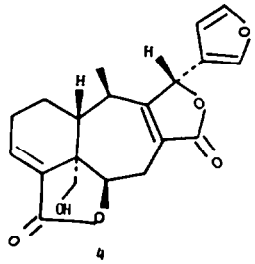


3

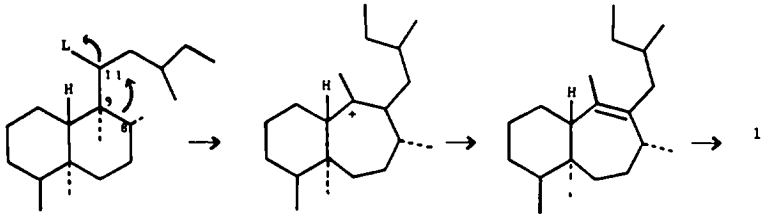


4

Treatment of 1 with MCPBA produced the epoxy-derivative 3 Its ^{1}H NMR spectrum (Table I) exhibited a sharp singlet (3H) at δ 1 35, showing that epoxydation had occurred on the 9,11 double bond The upfield shifts observed for H-12 and H-8 suggested that epoxydation had taken place from the less hindered β face of the molecule

Saponification of 1 yielded the hydroxyderivative 4 Its ^{1}H NMR spectrum (Table I) revealed that the 9,11 double bond of 1 has been moved to 8,11, showing the C-20 methyl as a doublet at δ 1 20 The multiplicity and chemical shifts exhibited by H-3 and H-6 indicated that a relactonization has taken place giving rise to an α,β-unsaturated 18 6β olide and an hydroxymethylene group at C-5, as has been found in plaunol B [17] The relative stereochemistry proposed for 4, was deduced from a study of the changes produced in its ^{1}H NMR spectrum on addition of Eu(fod)$_3$ (experimental).

The structure 1 can be derived biogenetically by the migration of the C8-C9 bond of a normal clerodane skeleton to C-11 with a concomitant loss of an adequate leaving group (L) as outlined in scheme 1 To our knowledge, salvigenolide is the first natural product[15] described with an A/B six-seven ring system derived from a *neo*-clerodane[16] skeleton



Scheme 1

EXPERIMENTAL SECTION

Melting points were determined in a Fisher Jones apparatus and are uncorrected Column chromatography was carried out by using Merck silica Gel 60 (0 063-0 2 mm) UV and IR spectra were determined on Nicolet FT 5X and Perkin Elmer 552 spectrometers, respectively Optical rotations were measured with a Perkin Elmer 241 polarimeter with a 1 dm cell ^{1}H and ^{13}C NMR spectra were determined at 80 and 20 MHz respectively in $CDCl_3$, C_6D_6 or pyridine-d_5 solutions with Me_4Si as internal standard Mass spectra were obtained at 70 eV on a Hewlett-Packard 5985-B spectrometer Plant material was collected in November 1983, 7 5 Km west of Huitzilac, state of Morelos, México Voucher specimen (MEXU-379095) was deposited at the Herbarium of the Instituto de Biología, UNAM

Isolation of Salvigenolide (1) Dried and powdered aerial parts (2300 g) of *Salvia fulgens* Cav were extracted with acetone (20 1) at room temperature for one week Evaporation of the solvent yielded a gum (50 g) which was subjected to dry-column chromatography over silica gel (1250 g) deactivated with 5% water Elution with petroleum ether-EtOAc (1 3) gave 1 (500 mg 0 021% dry weight) mp 218-220°C from MeOH, $|\alpha|_D^{20}$ -191 6 (c 0 21, MeOH), IR (Nujol) 3140, 1771, 1761, 1743, 1665, 1501, 887, 871 cm^{-1}, UV (MeOH) λ_{max} 208 nm (log ε 4 5), for ^{1}H and ^{13}C NMR see table I and II respectively, MS (direct inlet) m/z (rel intensity) 398 (M^+, 4 3), 357 (15), 356 (44 9), 338 (20), 95 (49 5), 91 (29), 81 (25), 43 (100 base peak) $C_{22}H_{22}O_7$ requires M^+ at m/z 398

Catalytic hydrogenation of 1 Salvigenolide (1) (100 mg) in methanol (5 ml) was hydrogenated using Pd/C (10%, 25mg) as catalyst After usual work up, product 2 (80 mg) was obtained Mp 110-112°C from acetone-*i*-propylether, $|\alpha|_D^{20}$ -106 (c 0 25, MeOH), UV (MeOH) λ_{max} 206 nm (log ε 4 26), IR (Nujol) 1760, 1740, 1590, 1500, 900, 870 cm^{-1}, for ^{1}H and ^{13}C NMR see tables I and II respectively, MS (direct inlet) m/z (rel intensity) 400 (M^+,2 5), 340 (46), 95 (35), 91 (30), 81 (20), 79 (21), 77 (20), 43 (base peak) $C_{22}H_{24}O_7$ requires M^+ at m/z 400

Epoxidation of 1 A solution of 1 (100 mg) in CH_2Cl_2 (5 ml) was treated with 90 mg of MCPBA The mixture was stirred during 72 hr at room temperature The solution was washed with 10% $NaHCO_3$ aq soln and water, dried and the solvent removed under vacuum The solid product obtained was recrystallized from EtOAc to yield 3 (60 mg) mp 222°C, $|\alpha|_D^{20}$ -132 (c 0 21, $CHCl_3$), UV (MeOH) λ_{max} 207 nm (log ε 4 5), IR ($CHCl_3$) 2948, 1778, 1678, 1600, 1502, 896, 876, 860 cm^{-1}, ^{1}H NMR see Table I, MS (direct inlet) m/z (rel intensity) 372 (1 8), 356 (1), 354 (2), 219 (5), 192 (8), 179 (6), 120 (5), 95 (10), 91 (15), 81 (5), 79 (7), 77 (10), 43 (100 base peak) $C_{22}H_{22}O_7$ requires M^+ at m/z 414 (not observed)

Saponification of 1 A solution of 1 (100 mg) in methanol (10 ml) was treated with 50 mg of solid $KHCO_3$ in an argon atm The mixture was stirred during 12 hr at room temperature The solution was neutralized with the stoichiometric amount of acetic acid (0 360 ml of a 10% aq soln) The solvent was removed at reduced pressure (2 mm Hg), and the residue extracted with EtOAc, washed with water, dried and the solvent removed under vacuum to yield 4 (60 mg) as a crystalline solid

mp 248-250°C dec from EtOAc, $|\alpha|_D^{20}$ -206 (c 0 21, MeOH), UV (MeOH) λ_{max} 210 nm (log ε 4 66), IR (Nujol) 3464, 1752, 1679, 1659, 1502, 874, 811, 781 cm^{-1}, ^1H and ^{13}C NMR see Tables I and II respectively Addition of 4 2 mg of $Eu(fod)_3$ produced downfield shifts of the signals ascribed to H-6 (Δδ=2 85), 2H-19 (Δδ=2 75), H-7α (Δδ=1 5), H-3 (Δδ=1 4), 3H-20 (Δδ=0 5), and H-12 (Δδ=0 7) MS (direct inlet) m/z (rel intensity) 356 (M^+, 13 4), 327 (14), 326 (80), 95 (80), 91 (70), 81 (60), 79 (60), 77 (100 base peak) $C_{20}H_{20}O_6$ requires M^+ at m/z 356

X-ray structure determination of Salvigenolide (1) $C_{22}H_{22}O_7$, space group $P2_12_12_1$ with Z=4 and a=8 625(2), b=12 453(2), c=18 052(2)Å, V=1938 9(4)$Å^3$ and the calculated density is 1 364 $g\,cm^{-3}$ Intensities of 1500 independent reflections were collected on a Nicolet R3 diffractometer using MoK_α radiation (λ=0 7107 Å and $2\theta_{max}$ = 45°) of which 1225 were considered to be observed $|I>2\ 5\sigma(I)|$ The structure was solved by direct methods using SHELXTL programs [18] The trial structure was refined by least-squares In the final refinement, anisotropic thermal factors were used for the non-hydrogen atoms and for the hydrogen atoms riding on the bonded carbon atoms with a fixed isotropic temperature factor U=0 06 Å The final R value is 0 050 The final difference map has no peaks greater than -0 2 $eÅ^{-3}$ and the isotropic extinction parameter is X=0 0029 [19]

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