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LASIANTHIN, A *NEO*-CLERODANE DITERPENOID FROM *SALVIA LASIANTHA*

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Key Word Index—*Salvia lasiantha*; Labiatae; *neo*-clerodane diterpenoid; lasianthin.

Abstract—From the aerial part of *Salvia lasiantha* a *neo*-clerodane diterpenoid, lasianthin, was isolated. The structure of this compound was established by spectroscopic means and X-ray diffraction analysis.

INTRODUCTION

Continuing our systematic phytochemical studies of Mexican *Salvia* [1–5], we studied *Salvia lasiantha* (sect. *Mitratae*, Epling [6]) which is a variable subshrub fairly common in southern states of Mexico and Guatemala.

From the aerial parts of a population of *S. lasiantha* collected in Oaxaca (México), β-sitosterol, oleanolic acid and a new *neo*-clerodane diterpenoid, lasianthin (**1**), were isolated.

RESULTS AND DISCUSSION

Lasianthin (**1**) showed a $C_{22}H_{30}O_5$ molecular formula. Its IR spectrum showed a weak band at 1784 and a strong one at 1750 cm^{-1} due to the Fermi resonance associated with a β-substituted butenolide function [7]. An absorption at 1735 cm^{-1} was assigned to an ester function. A strong band at 1658 cm^{-1} was attributed to an α,β-unsaturated ketone and absorptions at 1617, 891 and 855 cm^{-1} to trisubstituted double bonds.

The ^{1}H NMR spectrum of lasianthin (**1**) (Table 1) confirmed the presence of a β-substituted butenolide function. The ester function was shown to be an acetate group (δ2.1, 3H *s*) which must be attached to C-7 and axially oriented since its geminal proton appeared as a broad double doublet at δ5.21 with coupling constants (J = 3.6 and 6 Hz) due to equatorial–equatorial and equatorial–axial interactions. The α,β-unsaturated ketone function must be at C-2 since the ^{1}H NMR spectrum of **1** showed a broad singlet at δ5.74 and a vinylic methyl group at δ1.9 (3H, *d*, J = 1 Hz), which were assigned to H-3 and to the methyl group bound to C-4. Two double doublets found at δ2.55 (1H, J = 14, 19 Hz) and 1.54 (1H, J = 19,

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Table 1. ^{1}H NMR data of compounds **1**, **2**, **3a** and **3b***

H	**1** (300 MHz)	**2** (80 MHz)	**3a** (80 MHz)	**3b** (80 MHz)
H-1ax	2.55 *dd* (14, 19)			
H-1eq	1.54 *dd* (19, 3.3)			
H-2	—	—	4.17 *m*	5.15 *m*
H-3	5.74 *s* (*br*)		5.17 *m*	5.10 *s* (*br*)
H-7	5.21 *br dd* (3.6, 6)	5.1 *dd* (4, 9)	5.17 *m*	5.09 *m*
H-10	1.93 *dd* (3.3, 14)			
H-14	5.83 *t* (1.8)		5.85 *t* (2)	5.85 *t* (2)
2H-16	4.72 *d* (1.8)	4.4 *dd* (7, 10) 3.85 *dd* (7, 10)	4.72 *d* (2)	4.72 *d* (2)
Me-17	0.96 *d* (7)	0.92 *d* (7)	0.92 *d* (7)	0.90 *d* (7)
Me-18	1.9 *d* (1)	0.85 *d* (6)	1.65 *d* (1)	1.62 *s* (*br*)
Me-19	1.32 *s*	1.15 *s*	1.27 *s*	1.25 *s*
Me-20	1.14 *s*	1.00 *s*	1.07 *s*	1.05 *s*
OAc	2.1 *s*	2.07 *s*	2.07 *s*	2.07 *s* 2.05 *s*

*Coupling constants in Hz are in parentheses. Chemical shifts are in δ values.

3.3 Hz) were attributed to axial H-1 and equatorial H-1 protons. The coupling constants shown by these protons (J = 3.3 and 14 Hz) can be explained if we consider the equatorial–axial and axial–axial interactions with the axially oriented H-10 which was responsible for a double doublet (J = 3.3 and 14 Hz) found at δ1.93. In the methyl region two singlets at δ1.32 and 1.14 and a doublet (J = 7 Hz) at δ0.96 were assigned to the Me-19, Me-20 and Me-17 groups, respectively.

The relative chemical shifts shown by the tertiary and secondary methyl groups in the ^{1}H NMR spectrum of lasianthin (**1**) suggested an A/B *cis* stereochemistry [8], as has been found in clerodane diterpenoids isolated from *Solidago* species [9] (Compositae). These *cis*-clerodane diterpenoids usually have an ester function at the C-6 position which does not exert any influence on the chemical shifts found for the C-8 and C-9 methyl groups [8].

The ^{1}H NMR spectroscopic data and the ^{13}C NMR spectrum of **1** (Table 2) were insufficient to establish without any doubt the stereochemistry of the A/B ring junction. An X-ray diffraction analysis of the single crystal of lasianthin was performed in order to clarify this point.

The molecular structure together with the numbering scheme is illustrated in Fig. 1. The cyclohexane ring exhibits a chair conformation. The methyl groups at C(5) and C(9) and the acetate group at C(7) occupy axial sites whereas the methyl group at C(8) and the side chain at C(9) are equatorial. The average distance and angle for the chain structure is 1.539(7) Å and 112.6(4)°, respectively, and are in agreement with the values obtained for cyclohexane [10]. The angle between normals to the cyclohexane ring and the side chain at C(9) is 90.9(6)°. The cyclohexane ring is *trans*-fused to the cyclohexanone ring which exhibits a 1,2-diplanar (puckered sofa) conformation. The five-membered ring is planar but with some minor deviations from 0° torsion angles, maximum 0.7(6)° for C(14)–C(13)–C(16)–O(3). The C=O bonds agree well with the accepted value of 1.215(5) Å [11]. The arrangement of molecules in the crystal appears to be determined by van der Waals interactions.

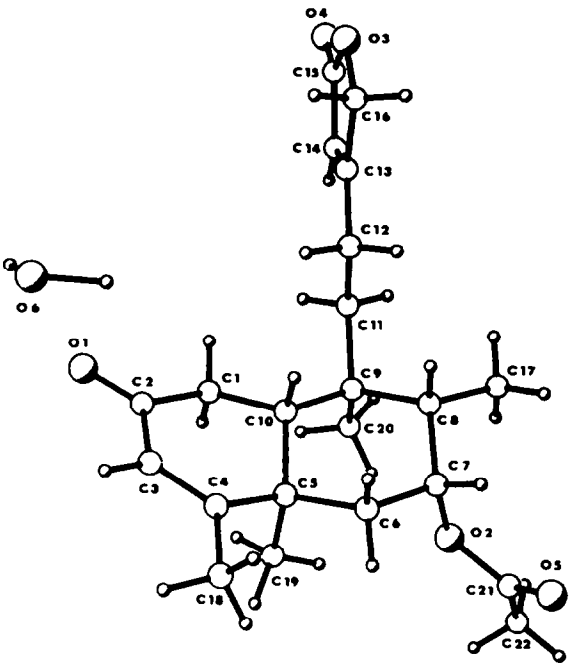


Fig. 1. Molecular structure of lasianthin.

Table 2. ^{13}C NMR data for compounds **1** and **3b** (20 MHz, $CDCl_3$, TMS as internal standard)

C	1	3b
1	35.64 *t*	24.52 *t*
2	198.91 *s*	74.34 *d*
3	125.25 *d*	119.87 *d*
4	169.99 *s*	149.37 *s*
5	38.62* *s*	38.14* *s*
6	34.78† *t*	36.45† *t*
7	74.10 *d*	71.76 *d*
8	37.91 *d*	38.14 *d*
9	38.43* *s*	38.07* *s*
10	45.72 *d*	45.17 *d*
11	35.74† *t*	39.40† *t*
12	22.12 *t*	24.40 *t*
13	173.67 *s*	173.75 *s*
14	115.32 *d*	115.42 *d*
15	171.73 *s*	170.74 *s*
16	73.05 *t*	72.90 *t*
17	12.00 *q*	11.92 *q*
18	19.91 *q*	19.36 *q*
19	19.01 *q*	17.49 *q*
20	18.09 *q*	17.70 *q*
OCOMe	170.32 *s*	170.20 *s*
		170.10 *s*
OCOMe	19.19 *q*	19.22 *q*
		19.36 *q*

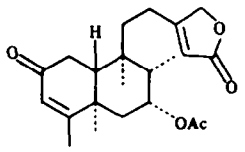
*† Values in any vertical column may be interchanged.

As shown by the X-ray diffraction analysis, lasianthin has A/B-*trans* stereochemistry. Therefore, it belongs to the *trans neo*-clerodane (*ent*-clerodane) type of diterpenoids recently isolated from many *Salvia* species of the *Calosphace* subgenus [1–5, 12]. The fact that the Me-17 doublet (δ0.96) in the ^{1}H NMR spectrum of **1** is observed at higher magnetic field than the Me-20 singlet (δ1.14) can be explained by the magnetic deshielding exerted by the axial C-7 acetate group on the C-9 and the C-5 methyl groups.

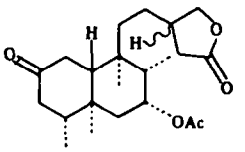
Catalytic hydrogenation of **1** gave the tetrahydroderivative **2**, which did not show Fermi resonance and the 1658 cm^{-1} band ascribed to the α,β-unsaturated ketone (see Experimental).

Sodium borohydride reduction of lasianthin (**1**), in the presence of $CeCl_3$ [13], gave preferentially the hydroxyderivative **3a**. The α-equatorial orientation assigned to the hydroxy group was based on steric considerations and the coupling constant (2 Hz) shown by the vinylic H-3 when the signal for Me-18 was irradiated in the ^{1}H NMR spectrum of the acetyl derivative **3b** (Table 1). H-2 could be detected on addition of Eu(fod)$_3$ and appeared as a broad double triplet ($W_{1/2}$ = 18 Hz).

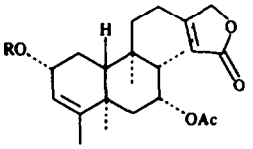
The ^{13}C NMR spectra of **1** and **3b** (Table 2) are in agreement with the structure and relative stereochemistry proposed for these compounds. Assignments were based on data described for similar structures [14, 15].



1



2



3a R = H
3b R = Ac

EXPERIMENTAL

Mps: uncorr; MS: direct inlet 70 eV; ^{1}H NMR and ^{13}C NMR: 80 and 20 MHz, respectively, $CDCl_3$, TMS as internal standard, unless otherwise stated. Assignments of ^{13}C NMR chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Plant material was collected at 3 km NE of Tamazulapan, Oaxaca (México) and a voucher specimen (MEXU 379092) is deposited at the Herbarium of the Instituto de Biología, UNAM.

Isolation of lasianthin. Dried and powdered aerial parts of *S. lasiantha* (800 g) were extracted with Me_2CO at room temp. for 8 days. The solvent was removed at red. press. and the gummy residue obtained (55 g) was chromatographed over silica gel (1.5 kg deactivated with 10% H_2O), using as eluents hexane–EtOAc mixtures of increasing polarity. Elution with hexane–EtOAc (9:1) gave β-sitosterol, mp 133–136, identified by comparison with an authentic sample. The fractions eluted with hexane–EtOAc (3:1) gave a triterpenoid acid (3 g) identified as oleanolic acid by comparison of its methyl ester with an authentic sample (mp, mmp, and IR and ^{1}H NMR spectra).

Elution with hexane–EtOAc (1:1) yielded lasianthin (**1**, 2 g), mp 174–176° from Me_2CO–hexane, $[\alpha]_D^{20}$ −93.46 ($CHCl_3$, *c* 0.49), UV λ_{max}^{MeOH} nm (ε): 208 (18 500), 232 (13 000); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1784, 1750, 1735 (shoulder), 1658, 1617, 891, 855. ^{1}H NMR (300 MHz): Table 1; ^{13}C NMR: Table 2; MS *m/z* (rel. int.): 374 $[M]^+$ (32), 346 (1), 332 (5), 317 (8), 314 (10), 299 (10), 272 (12), 203 (20), 189 (12), 175 (20), 161 (48), 109 (37), 83 (55), 43 (100%). $C_{22}H_{30}O_5$ requires: $[M]^+$ at *m/z* 374.

Catalytic hydrogenation of lasianthin. Lasianthin (100 mg) in EtOAc (10 ml) was hydrogenated using Pd-C (10%, 20 mg) as catalyst. After the usual work-up, the crystalline product obtained (85 mg) showed mp 153–155°, $[\alpha]_D^{20}$ −4.7 ($CHCl_3$, *c* 0.17), UV end absorp. (ε = 600); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780, 1735, 1720; ^{1}H NMR, Table 1; MS *m/z* (rel. int.): 378 $[M]^+$ (1.1), 335 (2.8), 318 (4), 205 (22), 147 (18), 114 (5), 85 (15), 69 (32), 43 (100%) ($C_{22}H_{34}O_5$ requires $[M]^+$ at *m/z* 378).

$NaBH_4$ *reduction of* **1** *in the presence of* $CeCl_3$. Lasianthin (400 mg) was dissolved in methanolic $CeCl_3 \cdot 6H_2O$ (2.5 ml, 0.4 M) and $NaBH_4$ (60 mg) was slowly added with stirring at 0°. After 10 min at room temp. the mixture was extracted with EtOAc. The EtOAc phase was then washed with H_2O, dried and the solvent removed under vacuum. The solid product (**3a**) obtained (310 mg) was crystallized from Me_2CO–hexane. It showed mp 72–75°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1783, 1750, 1640, 855. ^{1}H NMR: Table 1. MS *m/z* (rel. int.): 358 $[M-18]^+$ (1.4), 343 (2), 316 (8), 187 (10), 119 (29), 111 (10), 105 (32), 91 (41), 43 (100%) ($C_{22}H_{32}O_5$ requires $[M]^+$ at *m/z* 376).

Acetylation of **3a** (300 mg) with Ac_2O (1 ml) in py (1 ml) at room temp. for 18 hr gave **3b**, mp 206–208°, $[\alpha]_D^{20}$ −11.17 ($CHCl_3$, *c* 0.17), UV λ_{max}^{MeOH} nm (ε): 208 (12 000); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780, 1741, 1638, 756. ^{1}H NMR: Table 1; ^{13}C NMR: Table 2. MS *m/z* (rel. int.): 418 $[M]^+$ (2), 375 (11), 356 (5), 298 (4), 186 (8), 123 (57), 119 (19), 98 (83), 43 (100%) ($C_{24}H_{34}O_6$ requires $[M]^+$ at *m/z* 418).

X-Ray structure determination of lasianthine. Crystals of lasianthine were prepared by slow evaporation of a MeOH soln. Initial photographic studies showed 2/m Laue symmetry. The crystals are monoclinic, space group C2. Unit cell dimensions were obtained by a least-squares fit to the angular settings of 25 centred reflections with $5.2° < 2\theta < 15.1°$ on a Nicolet R3m diffractometer equipped with a graphite monochromator crystal. Crystal data for lasianthine: $C_{22}H_{30}O_5$ 1/2 H_2O, M_r = 383.5, a = 15.074(4), b = 9.125(3), c = 15.176(5) Å, β = 91.37(2)°, V = 2087(1) Å^{-3}, d_{calc} = 1.22 g/cm^3, $F(000)$ = 828, Z = 4 and μ(Mo K_α) = 0.81 cm^{-1}. The crystal chosen for intensity measurement had the dimensions 0.31 × 0.36 × 0.44 mm, and was mounted on a glass fibre. Intensity measurements were made with Mo K_α (λ = 0.7107 Å) radiation utilizing the ω-scan technique, the rate of scanning being varied from 4.0 to 29.3 deg/min^{-1}. Two reflections ($\bar{1}\,\bar{1}\,0$; 0 2 0) monitored at intervals of 50 measurements. All reflections in two octants of reciprocal space were measured with an index range of $h \pm 16$, $k\,0 \to 7$, $l\,0 \to 16$ according to $3° < 2\theta < 45°$. The total number of data collected was 1473, of which 1267 reflections had $I > 2\sigma(I)$ and these formed the basis of the structural solution and refinement; these reflections were corrected for Lorentz and polarization effects; no absorption correction was applied. The crystal structure was solved by direct methods using the SHELXTL program package [16]. The trial structure was refined by least-squares procedures with anisotropic temperature factors for the non-H atoms and with a fixed isotropic temp. factor, U = 0.06 Å^2, for the H atoms bonded to C atoms; the H atoms bonded to 0 atom were found in a difference Fourier map. The function minimized was $\Sigma\omega(|Fo|-|Fc|)^2$ with a weighting scheme $\omega = |\sigma^2(Fo) + 0.0015 (Fo)^2|^{-1}$, where σ is the standard deviation of the observed amplitudes. The final discrepancy indexes are R = 0.049 and ωR = 0.054 for the 1267 observed reflections.* The final difference map has no peaks greater than ± 0.18e/Å^3; the isotropic extinction parameter is X = 0.0009 and the atomic scattering factor for the O, C and H atoms were from *International Tables for X-Ray Crystallography* [17].

All computations were performed in the laboratory on a Nova 4S computer and plot was drawn on a Tektronix plotter.

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*A list of the observed and calculated structure factors, anisotropic thermal parameters bond distances and angles and H-atom coordinates are deposited at the Cambridge Crystallographic Data Centre, U.K.

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