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CLERODANE-TYPE DITERPENOIDS FROM *SALVIA SOUSAE*

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Key Word Index—*Salvia sousae*; Labiatae; new clerodane derivatives; diterpenoids; 1,2-dihydro-6α,7α-epoxylinearifoline; 8β,17-dihydro-7-*epi*-2α-hydroxylanguiduline.

Abstract—The aerial parts of *Salvia sousae* afforded, in addition of two flavones of known structure, two new clerodane derivatives related to linearifoline and languiduline. The structures were elucidated by spectroscopic means.

INTRODUCTION

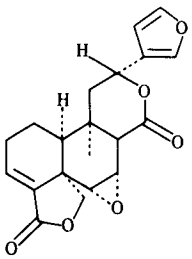
Continuing our studies on Mexican *Salvia* spp., from which diterpenoids, flavonoids, sterols and triterpenoids have been isolated [1–8], we describe in this paper the chemical components of *Salvia sousae* Ramamoorthy. This is a perennial shrub endemic to the semiarid region of Puebla (México) and classified in Section *Polystachyae* of *Salvia* Subgenus *Calosphace*.

In addition to oleanolic acid and two flavones of known structure, two new clerodane diterpenoids **1** and **2** were isolated. The chemotaxonomic situation of the genus is briefly discussed.

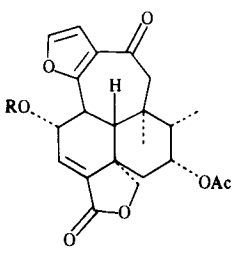
RESULTS AND DISCUSSION

Extraction of the aerial parts of *S. sousae* afforded, after extensive chromatography, oleanolic acid, the flavones eupatorine (6,7,4′-trimethoxy-5,5′-dihydroxyflavone) [9], cirsiliol (6,7-dimethoxy-5,3′,4′-trihydroxyflavone) [10] and two diterpenoids related to linearifoline and languiduline to which we assigned structures **1** and **2** on the following considerations.

Compound **1** was isolated as a crystalline solid, mp 235–238°, the mass spectrum is consistent with the molecular formula $C_{20}H_{20}O_6$. Its IR spectrum exhibited the characteristic absorptions for a furan ring (1500, 870 cm^{-1}), α,β-unsaturated-γ-lactone (1785 cm^{-1}), δ-lactone (1720 cm^{-1}), double bonds (1650 cm^{-1}) and the absence of hydroxyl groups. The UV spectrum [λ_{max}^{MeOH} nm (ε): 209 (10300)] supports these assignments.



1



2 R = H
3 R = Ac

The ^{1}H NMR spectrum of **1** showed a triplet at δ6.65 (J = 4 Hz) which was ascribed to the olefinic β-proton of an α,β-unsaturated γ-lactone group, coupled to a methylene moiety and therefore it was assigned to H-3 (see Table 1). An AB system at δ4.2 and 3.95 (J = 8 Hz) was attributed to the C-19 methylene group. The pro-*S*-diastereotopic proton of this group is also *W*-coupled with the C-6β proton indicating an axial orientation for C-19 and the absence of a C-6β substituent. This behaviour has been described in both *cis* [11, 12] and *trans* [13, 14] *neo*-clerodan-18:19 olides. Other relevant signals in the ^{1}H NMR spectrum of **1** are those due to an ABX system at δ5.15 (X parts J_{AX} = 12 Hz, J_{BX} = 3 Hz), 2.6 (B part J_{AB} = 16 Hz) and 2.15 (A part), ascribed to the axial H-12 and the C-11 methylene moiety. Comparison of the ^{13}C NMR spectrum of **1** (Table 2) with that of salviarin [15], 1(10)-dehydrosalviarin, 1α,10α-epoxysalviarin and linearifoline [5] suggested a half chair conformation of the δ-lactone ring in **1** and therefore a 12*R*-configuration.

The ^{1}H NMR spectrum of **1** also exhibited the characteristic signals for a β-substituted furan ring (see Table 1) and a three protons singlet at δ1.25 ascribed to the α-axial methyl group attached to C-9. H-8 was reponsible for a doublet (J = 1.5 Hz) at δ2.15. The value of the coupling constant indicated an equatorial orientation for this proton, and therefore, the C-8 substituent (C-17) must be β-axially oriented as in salviarin [15], 1(10)-dehydrosalviarin, 1α,10α-epoxysalviarin and linearifoline [5]. Two one-proton multiplets at δ3.3 and 3.15 were assigned to the oxirane protons H-6 and H-7. When the ^{1}H NMR spectrum of **1** was recorded in C_6D_6 as solvent, a better resolution of these signals was obtained. In this solvent H-6 and H-7 were observed at δ2.45 (*dd*, J = 3.5 and 2 Hz) and 2.35 (*dd*, J = 3.5 and 1.5 Hz). H-8 was observed at δ0.85 as a doublet (J = 1.5 Hz). These assignments were confirmed by double resonance experiments. Irradiation at δ0.85 removed a 1.5 Hz coupling in the signal ascribed to H-7 (now as a doublet J = 3.5 Hz). Irradiation at δ2.4 (centre of the oxirane proton signals) transformed the

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Table 1. ^{1}H NMR (80 MHz) data of compounds **1–3**

H	1	1*	2	3
1			3.1 *t* (8)	3.25 *t* (9)
2			4.7 *dd* (8, 1)	5.8 *dd* (9, 1)
3	6.65 *t* (4)	6.4 *t* (4)	6.65 *d* (1)	6.6 *d* (1)
6	3.3 *m*	2.45 *dd* (3.5, 2)		
7	3.15 *m*	2.35 *dd* (3.5, 1.5)	5.25 *dt* (4, 2)	5.3 *dt* (4, 2)
8	2.25 *d* (1.5)	0.85 *d* (1.5)		
10				2.35 *d* (9)
12	5.15 *dd* (12, 4)	5.05 *dd* (10, 4)		
11-A	2.15 *dd* (12, 16)	1.1 *dd* (16, 10)	2.5 *d* (16)	2.55 *d* (16)
11-B	2.6 *dd* (16, 4)	1.95 *dd* (16, 4)	3.05 *d* (16)	3.05 *d* (16)
14	6.4 *br s*	6.15 *br s*	6.75 *d* (2)	6.7 *d* (2)
15	7.4 *m*	7.1 *m*	7.4 *d* (2)	7.25 *d* (2)
16	7.4 *m*	7.2 *m*		
19 pro-*S*	3.95 *dd* (8, 2)	3.05 *dd* (8, 2)	4.05 *dd* (8, 1)	4.1 *dd* (8, 1)
19 pro-*R*	4.2 *d* (8)	3.4 *d* (8)	4.9 *d* (8)	4.95 *d* (8)
17			1.1 *d* (7)	1.1 *d* (7)
20	1.25 *s*	0.55 *s*	0.9 *s*	0.9 *s*
OCOMe			2.15 *s*	2.1 *s* 2.15 *s*

Coupling constants in Hz are in parentheses. Chemical shifts are in δ values. Run using $CDCl_3$ are solvent and TMS as internal reference.

*Run in benzene-d_6 as solvent.

Table 2. ^{13}C NMR data for compound **1** (20 MHz, $CDCl_3$–DMSO-d_6 1:1, TMS as int. standard)

C	δ	C	δ
1	18.9 (*t*)	11	35.7 (*t*)
2	29.1 (*t*)	12	71.4 (*d*)
3	135.7 (*d*)	13	123.8 (*s*)
4	133.2 (*s*)	14	108.7 (*d*)
5	41.8 (*s*)	15	143.6 (*d*)
6	54.5 (*d*)	16	139.9 (*d*)
7	50.8 (*d*)	17	174.4 (*s*)
8	45.5 (*d*)	18	168.2 (*s*)
9	40.4 (*s*)	19	79.5 (*t*)
10	47.1 (*d*)	20	33.2 (*q*)

SFORD multiplicities are in parenthesis.

signal ascribed to H-8 into a singlet and at the same time the *W*-type coupling was removed in the singal attributed to the pro-*S* H-19. The coupling constant between H-8 and H-7 (1.5 Hz) indicated a β-orientation for H-7, in total agreement with the dihedral angle predicted for Tori's equation [16] (calculated 57°; observed 65°, Dreiding models). These results led us to locate the oxirane ring in the C-6α-C-7α positions. The presence of signals at δ54.5 (*d*, C-6), 50.8 (*d*, C-7) and 45.5 (*d*, C-8) in the ^{13}C NMR spectrum of **1** are in agreement with the previous assignments.

Another relevant signal in the ^{13}C NMR spectrum of **1** is that due to C-20 at δ33.2 (*q*). The chemical shift observed for this carbon could be due to the lack of the γ-gauche shielding effect exerted over it when C-17 is α-equatorially oriented. A similar chemical shift had been observed for C-20 in linearifoline, a closely related *cis*-clerodane isolated from *Salvia Lineata* Benth. [5]. The relative stereochemistry of linearifoline was recently confirmed by X-ray crystallography [16a]. On the basis of this comparison, we propose an A/B *cis*-fusion for **1**, which must be named, therefore, 1,2-dihydro-6α,7α-epoxylinearifoline.

The mass spectrum of the second diterpenoid isolated from *S. sousae*, was consistent with the molecular formula $C_{22}H_{24}O_7$. Structure **2** was proposed for it, based on spectroscopic data. Its IR spectrum showed the characteristic absorptions for hydroxyl groups (3590 cm^{-1}), α,β-unsaturated-γ-lactone (1770 cm^{-1}), ester function (1740 cm^{-1}) and α,β-unsaturated ketone (1670 cm^{-1}).

The ^{1}H NMR spectrum of **2** (Table 1) showed in the methyl region, a singlet at δ0.9 and a doublet at 1.1 (J = 7 Hz) assigned to the Me-20 and Me-17 groups respectively. A singlet at δ2.15 was attributed to an acetate methyl group whose geminal proton (H-7) was responsible for a double triplet (J = 4 and 2 Hz) at 5.25. The coupling constants observed for this proton indicated an α-axial orientation for the acetate group. These data, and the comparison with related compounds [2, 6], led us to locate the acetate group at C-7. Therefore the ring B of **2** is identical to that present in kerlinolide, a *trans-neo*-clerodane diterpenoid isolated from *Salvia keerlii* Benth. [2]. These conclusions are supported by the chemical shift of the pro-*R* H-19 at δ4.9 (*d*, J = 8 Hz), since the axial acetate group at C-7 produces a strong deshielding effect on this proton. The pro-*S* H-19 was observed at δ4.05 as a double doublet (J = 8 and 1 Hz). The long-range coupling suggested an α-axial orientation for C-19 and the absence of substituents at the C-6β position [11, 13, 14].

Other relevant signals in the ^{1}H NMR spectrum of **2** are those due to a β-proton of an α,β-unsaturated-γ-lactone at δ6.55 (*d*, J = 1 Hz), ascribed to H-3. The multiplicity and coupling constant of this signal indicated the presence of an α-oriented substituent at C-2. A double doublet at δ4.7 (J = 8 and 1 Hz) was ascribed to the geminal proton of an hydroxyl group, since it was shifted downfield upon acetylation. The chemical shift of this proton revealed its allylic nature and led us to locate it at the C-2 position with a β-axial orientation.

The main feature of **2** is the presence of a seven-membered ring, with an α,β-unsaturated ketone function, due to the linkage of C-1 with C-16 of a clerodane skeleton. This fact was deduced from the careful analysis of the ^{1}H NMR spectrum of its acetyl derivative **3**, which shows an AB system at δ2.55 and 3.05 (J = 16 Hz) assigned to the C-11 methylene moiety. The presence of a carbonyl group at C-12 conjugated with the furan ring

was evidenced by the UV spectrum [λ_{max}^{MeOH} nm (ε): 205 (19000), 253 (5700)] and by the presence in the ^{1}H NMR spectrum of **3** (Table 1), of two one-proton doublets (J = 2 Hz) at δ6.7 and 7.25 ascribed to the furan protons H-14 and H-15, respectively. H-1 was responsible for a triplet at δ3.25 (J = 9 Hz) and H-10 was observed as a doublet at 2.35 (J = 9 Hz). Irradiation at δ3.25 transformed the signal ascribed to H-10 into a singlet and at the same time removed a 9 Hz coupling in the signal ascribed to H-2 (now as a doublet, J = 1 Hz). These results indicate the axial nature of H-1, H-2 and H-10. Since C-19 is α-axially oriented (*vide supra*), the A/B ring fusion must be *trans*. Compound **2**, is closely related to languiduline, a *neo*-clerodane recently isolated from *Salvia languidula*, whose structure was confirmed by X-ray crystallography (Jorge Cárdenas personal communication) and must be named 2α-hydroxy-7-*epi*-8β,17-dihydrolanguiduline.

The chemical profile found for *Salvia sousae*, is very similar to that of most of the *Salvia* spp. from Subgenus *Calosphace* studied up to now. Recently the phytochemical study of several spp. of this Subgenus [1–7, 12, 15, 17–20] led to the isolation of a number of diterpenes, mainly *neo*-clerodanes, in more than the 80% of the species studied. Some abietane-type diterpenoids have been isolated from spp. belonging to Section *Erythrostachys* [21] and *Tomentellae* [8, 22]. On the other hand, almost 100% of the European and Asiatic *Salvia* spp., belonging to Subgenus *Salvia* and *Sclarea* studied up to now, afforded abietane-type diterpenoids [23–25]. On the basis of these data, the American *Salvia* Subgenus *Calosphace* seems to be chemically different to the European and Asiatic Subgenera. These findings could be of chemotaxonomic, phytogeographic and phyllogenetic [26] importance and further studies on selected species are in progress to support these observations.

EXPERIMENTAL

Mps: uncorr. MS were obtained at 70 eV by direct inlet. ^{1}H and ^{13}C NMR were performed at 80 and 20 MHz, respectively, using TMS as int. standard. Plant material was collected in September 1984 at Acatepec, Puebla (México) and a voucher specimen (MEXU 404002) was deposited in the Herbarium of Instituto de Biología, UNAM.

Isolation of the constituents from S. sousae. Dried aerial parts of *S. sousae* (2695 g) were extracted with Me_2CO (18 l) at room temp. for 5 days. The gummy extract (160 g), obtained after evapn of the solvent at red. pres., was chromatographed over silica gel (1.5 kg), deactivated with 10% H_2O). Mixtures of petrol–EtOAc of increasing polarity were used as eluents. Fractions of 500 ml were collected. From the fractions eluted with petrol–EtOAc (8:2), 51 g (1.89%, dry wt) of oleanolic acid were isolated. The identity of this compound was confirmed by comparison of its methyl ester derivative with an authentic sample. Elution with petrol–EtOAc (4:6) yielded 523 mg (0.0194% dry wt) of 6,7,4′-trimethoxy-5,5′-dihydroxyflavone which was identified by comparison with literature data [9]. From the fractions eluted with petrol–EtOAc (8:2) two components were isolated after extensive chromatographic purification. The most polar was the 6,7-dimethoxy-5,3′4′-trihydroxyflavone, a compound previously reported in the literature [10].

The less polar compound was a novel diterpenoid named 1,2-dihydro-6α,7α-epoxylinearifoline (**1**), Mp 235–238° from Me_2CO–hexane; $[\alpha]_D^{20} = -36.2°$ (MeOH; c 0.2); UV λ_{max}^{MeOH} nm (ε): 209 (10360); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1785, 1720, 1650, 1500, 870; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; MS m/z (rel. int.): 357 (3.3), 356 (9), 323 (2.8), 244 (5.8), 234 (4), 189 (5), 176 (20), 115 (30), 95 (90), 94 (60), 91 (70), 77 (78), 65 (60), 39 (100). $C_{20}H_{20}O_6$ requires M^+ at m/z 356.

The mother liquors of **1** were rechromatographed over silica (200 g) using a mixture of Me_2CO–CH_2Cl_2 (1:9) as eluent, to yield a second diterpenoid compound named 2α-hydroxy-7-*epi*-8β,17-dihydrolanguiduline (**2**) as an unstable oil: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 1770, 1740, 1670, 875; ^{1}H NMR see Table 1; MS m/z (rel. int.): 400 (7.4), 313 (7.4), 264 (7.5), 239 (10), 179 (30), 162 (60), 161 (100), 149 (50), 109 (40), 105 (40), 83 (40), 82 (40), 81 (45), 80 (30), 79 (30), 71 (60), 69 (64), 57 (78), 55 (79). $C_{22}H_{24}O_7$ requires M^+ at m/z 400.

Acetylation of compound **2**. Compound **2** (100 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) at room temp. for 0.5 hr. After usual work-up product **3** was isolated as a crystalline solid: mp 300° from EtOAc–hexane; $[\alpha]_D^{20} = -179.7°$ (MeOH; c0.22); UV λ_{max}^{MeOH} nm (ε): 205 (19000), 253 (5700); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775, 1740, 1660, 870; ^{1}H NMR see Table 1; MS m/z (rel. int.): 442 (10), 400 (60), 382 (8), 340 (9), 311 (2), 285 (2), 189 (1), 179 (7), 161 (100), 159 (5), 95 (3), 91 (1), 81 (3), 43 (78). $C_{24}H_{26}O_8$ requires M^+ at m/z 442.

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