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CLERODANE DITERPENOIDS FROM *SALVIA UROLEPIS*

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Key Word Index—*Salvia urolepis*; Labiatae; clerodane derivatives; diterpenoids.

Abstract—From the aerial parts of *Salvia urolepis*, three new neoclerodane diterpenoids were isolated. Their structures were determined by spectroscopic means. The known languidulane diterpenoid 2α-hydroxy-7-*epi*-8β,17-dihydrolanguiduline was also obtained.

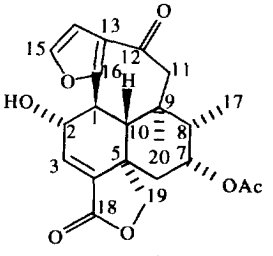
INTRODUCTION

In a continuation of our systematic studies of Mexican *Salvia* species we have analysed the diterpenoid content of *Salvia urolepis*, Fern. This species has been classified [1] in section Angulatae, subsection Glumacea (*Salvia*, subgenus Calosphace), which also includes *S. languidula* [2]. A phytochemical study of *S. languidula* led to the isolation of several diterpenoids with rearranged clerodane skeletons named languidulane [2] and salvilanguidulane [3]. Diterpenoids with rearranged skeletons of clerodanic origin were also isolated from *S. tiliaefolia* [4] and *S. rhyacophila* [5], two *Salvia* species included [1] in the section Angulatae, subsection Tiliaefolia. In this paper, we describe the isolation and structure determination of the diterpenoids found in *S. urolepis*.

RESULTS AND DISCUSSION

The aerial parts of *S. urolepis* afforded a mixture of oleanolic and ursolic acids, eupatorine [6] (6,7,4′-trimethoxy-5,5′-dihydroxy flavone); 5,6,3′-trihydroxy-7,4′-dimethoxy flavone [7], the languidulane diterpene 2α-hydroxy-7-*epi*-8β,17-dihydrolanguiduline (**1**) previously isolated [8] from *S. soussae* (*Salvia*, sect. Polystachyae) and named salvisousolide, and three new neoclerodane diterpenoids whose structures (**2**–**4**) were determined by spectroscopic means.

The languidulane **1** was the most abundant diterpenoid. It was obtained as an amorphous powder and identified by comparison of its IR and ^{1}H NMR spectra with those of the previously obtained diterpenoid [8]. The use of high-field 2D NMR experiments allowed the unambiguous assignment of all the proton resonances (Table 1). The COSY experiments showed a *W* coupling between H-10 and the Me-20 protons which is only possible in a *trans* A/B ring fusion of the decalin with the Me-20 α-axial. The ^{13}C NMR spectrum of **1**, not previously described, was consistent with the languidulane



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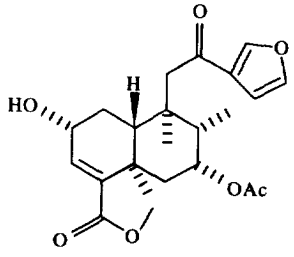

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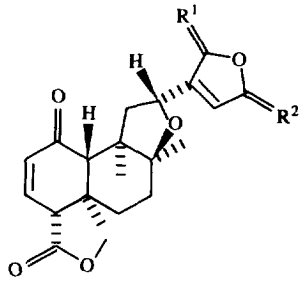

	R¹	R²
3a, b	H,OH	O
4a, b	O	H,OH
5	O	O

structure shown. The carbon resonances (Table 2) of the protonated carbon **5** were assigned by DEPT and HETCOR experiments. The three sp^3 doublets of carbons not bound to oxygen, were unambiguously assigned to C-1, C-8 and C-10. The quaternary and carbonyl carbon signals were established by comparison with the spectra of similar structures [2, 9].

The neoclerodane diterpene **2** was obtained as an unstable oil which showed in its IR spectrum bands due to a hydroxy group (3603 cm^{-1}), an α,β-unsaturated γ-lactone (1772 cm^{-1}), an ester function (1738 cm^{-1}), an α,β-unsaturated ketone (1673 cm^{-1}) and a furan ring (1607, 1560 and 872 cm^{-1}). The mass spectrum was consistent with the molecular formula $C_{22}H_{26}O_7$ (see experimental). A strong peak at *m/z* 95 (93%) suggested that the α,β-unsaturated ketone group was at C-12 [10]. The

Table 1. ^{1}H NMR spectral data for **1**–**4** (200 MHz, $CDCl_3$, TMS)

H	1*	2	3a	3b	4a	4b
1	3.11 *t* (9.3)		—	—	—	—
2	4.72 *dd* (9.3, 1.5)	4.52 *br dd* (8, 4)	6.21 *dd* (9.9, 2.7)	6.22 *dd* (10, 2.4)	6.21 *dd* (10.2, 3.0)	6.21 *dd* (10.2, 3)
3	6.68 *d* (1.5)	6.6 *br s* $W_{1/2}=4$	6.60 *dd* (9.9, 3.3)	6.59 *dd* (10, 3.3)	6.57 *dd* (10.2, 3.3)	6.57 *dd* (10.2, 3.3)
4			3.25 *dd* (3.3, 2.7)	3.23 *dd* (3.3, 2.4)	3.23 *dd* (3.3, 3.0)	3.26 *dd* (3.3, 3.0)
6α	2.38 *dd* (15, 2.2)					
6β	1.55 *ddd* (15, 4.0, 2.0)					
7	5.3 *dt* (4, 2.2)	5.26 *dt* (4, 2)				
8	1.68 *dq* (7, 4.0)					
10	2.28 *d* (9.3)		2.85 *s*	2.83 *s*	2.83 *s*	2.84 *s*
11A	3.04 *d* (15.1)	2.98 *d* (16)	2.92 *br t* (8.1)	2.97 *br t* (8.1)	3.01 *m*	3.0 *m*
11B	2.54 *d* (15.1)	2.72 *d* (16)				
12			4.89 *ddd* (9.6, 8.1, 1.8)	4.80 *ddd* (9.6, 8.1, 1.8)	4.76 *br t* (7.8)	4.77 *br t* (7.8)
14	6.79 *d* (2)	6.75 *d* (1.6)	6.04 (1)	5.99 *d* (1)	7.05 *br s*	7.05 *br s*
15	7.47 *d* (2)	7.47 *t* (1.6)	—	—	6.13 *br s*	6.13 *br s*
16		8.1 *br s*	5.97 *s*	6.13 *s*		
H_3-17	1.04 *d* (7)	0.94 *d* (7.2)	1.12 *s*	1.12 *s*	1.15 *s*	1.12 *s*
19 *pro*-R	4.96 *d* (8.3)	4.82 *d* (8)	4.37 *d* (9)	4.37 *d* (9)	4.37 *d* (9)	4.38 *d* (9)
19 *pro*-S	4.11 *dd* (8.3, 2.0)	4.0 *dd* (8, 2)	4.06 *d* (9)	4.06 *d* (9)	4.06 *d* (9)	4.07 *d* (9)
H_3-20	0.90 *s*	0.91 *s*	1.08 *s*	1.07 *s*	1.06 *s*	1.05 *s*
$MeCO_2$	2.13 *s*	2.1 *s*	—	—	—	—

* Run at 300 MHz.
J in Hertz in parentheses.

^{1}H NMR spectrum (Table 1) showed the typical resonances of a β-substituted furan ring conjugated to the ketone group (δ8.1, *br s*, H-16). The signal at δ193.65 in the ^{13}C NMR spectrum of **2** (Table 2) was attributed to this carbon. An AB system at δ2.98 and 2.72 (two doublets, $J=16$ Hz) was ascribed to the C-11 methylene. The rest of the ^{1}H NMR spectrum of **2** was very similar to that of **1** (Table 1). These data suggested the same substitution pattern in the decalin ring system of both diterpenoids, with the exception of the H-2 resonance observed as a doublet (J = 8 and 4 Hz) at δ4.52 in **2**. The multiplicity and coupling constants of this signal confirmed that **2** had no substituent at C-1. The carbon resonances and multiplicities (Table 2) were established by comparison with those found for the spectra of similar structures [9] and DEPT experiments. A triplet at δ30.25 assigned to C-1 confirmed the absence of a substituent at this carbon. The neoclerodane **2** can be considered as a biogenetic precursor of the languidulane **1** [11].

The hydroxy lactone **3** was obtained as an oily C-16 epimeric mixture (**3a** and **b**) whose mass spectrum was in accordance with the molecular formula $C_{20}H_{22}O_7$, although the parent peak was not observed. The IR spectrum contained bands due to a hydroxy group (3582 and 3360 cm^{-1}), a γ-lactone (1774 cm^{-1}), an α,β-unsaturated ketone (1681 cm^{-1}), an ether function (1026 cm^{-1}) and double bonds (1632 and 830 cm^{-1}). The ^{1}H NMR spectrum showed duplicate signals for most protons (Table 1) which suggested that the sample was a mixture of C-16 epimers. The vinylic protons, H-14, of the hydroxy lactone moiety, were observed as doublets ($J=1$ Hz) at δ5.99 and 6.04. Two singlets δ6.13 and 5.97 were ascribed to the protons geminal to the hydroxyls of this function. For simplicity, we will analyse the ^{1}H NMR of the

Table 2. ^{13}C NMR spectral data for compounds **1** and **2** (50 MHz, $CDCl_3$, TMS)

C	1	2
1	45.1 *d*	30.3 *t*
2	75.1 *d*	69.5 d
3	139.5 *d*	140.1 *d*
4	143.3 *s*	137.3 *s*
5	46.3 *s*	44.5 *s*
6	36.9 *t*	37.5 *t*
7	71.6 *d*	73.2 *d*
8	44.6 *d*	39.6 *d*
9	37.3 *s*	39.9 *s*
10	51.8 *d*	42.3 *d*
11	56.5 *t*	45.9 *t*
12	192.4 *s*	193.7 *s*
13	124.5 *s*	128.9 *s*
14	109.3 *d*	108.5 *d*
15	143.3 *d*	144.6 *d*
16	157.4 *s*	147.0 *d*
17	12.1 *q*	11.9 *q*
18	167.4 *s*	168.7 *s*
19	70.7 *t*	72.1 *t*
20	13.9 *q*	19.2 *q*
21	169.8 *s*	169.8 *s*
22	21.1 *q*	21.2 *q*

hydroxy lactone **3b** in which two double doublets at δ6.22 (J = 10 and 2.4 Hz) and 6.59 (J = 10 and 3.3 Hz) were assigned to the vinyl protons H-2 and H-3, respectively. They were shown to be coupled to H-4 which appeared at δ3.23 (*dd*, J = 3.3 and 2.4 Hz). The chemical shifts and multiplicities of H-2 and H-3 suggested that the ketone group must be located at C-1. An AB system observed at δ4.37 and 4.06 (*d*, J = 9 Hz) was attributed to the C-19 methylene. A complex signal at δ4.80 was ascribed to H-12, geminal to an ethereal oxygen. Two methyl singlets at δ1.07 and 1.12 were assigned to Me-20 and Me-17, respectively. Therefore, the ethereal function observed in the IR (1026 cm^{-1}) must be bound to C-8 and C-12 as found in kerlin (from *S. keerlii*) [12] and a related neoclerodane diterpenoid isolated from *S. rhyacophila* [5]. A singlet at δ2.83 was assigned to H-10. COSY experiments proved that it was *W* coupled to the Me-20 protons, thus showing a *trans* steroidal decalin ring system in **3** [9].

The mass spectrum of the C-15 epimeric mixture of the hydroxy lactones **4a**, **b** was determined by FAB-mass spectrometry and indicated the molecular formula $C_{20}H_{22}O_7$. It showed the same main bands in the IR spectrum as **3a**, **b**. The ^{1}H NMR was very similar to that of **3a**, **b** from which it differed in the chemical shifts and multiplicities of the hydroxy lactone protons H-14 (δ7.05, *br s*) and H-15 (δ6.13, *br s*) as expected for an α substituted hydroxylated butenolide. The H-12 resonance was observed as a broad triplet (J = 7.8 Hz) at δ4.76.

Attempted oxidation of the hydroxy lactones **3a**, **b** and **4a**, **b** to the anhydride **5**, were unsuccessful.

The presence of the languidulane diterpenoid **1** in *S. urolepis* has chemotaxonomic interest. It supports the botanical classification [1] of this species in sect. Angulatae, subsect Glumacea in which *S. languidula* [2] is also included. It is interesting to note that *S. soussae*, from which salvisousolide (**1**) was first isolated, has been included in sect. Polystachyae, a section botanically related to sect Angulatae, subsect. Glumacea.

EXPERIMENTAL

Mps.: uncorr; MS: 70 eV, direct inlet; ^{1}H NMR: 200 and 300 MHz, $CDCl_3$, TMS as int. standard; ^{13}C NMR: 50 MHz. Plant material was collected in Zimapán, State of Hidalgo (México) and a voucher specimen (TPR4852) is deposited in the Herbarium of Instituto de Biología UNAM.

Isolation of the constituents. Dried aerial parts of *Salvia urolepis* (3 kg) were extracted twice (2 × 20 l) with Me_2CO at room temp. for five days. The solvent was removed under red. pres. to yield 116.1 g of a gummy residue. This extract (78 g) was subjected to dry CC over silica gel (1 kg, 35–70 mesh, deactivated with 10% H_2O). Mixtures of petrol–EtOAc and EtOAc–MeOH of increasing polarity were used as eluents.

Elution with petrol–EtOAc (4:1) afforded β-sitosterol and a mixture of oleanolic and ursolic acids, which were identified by comparison with authentic samples. Elution with petrol–EtOAc (7:3) gave the flavone eupatorine (40 mg). The 5,6,3′-trihydroxy-7,4′-dimethoxy flavone (20 mg) was obtained from the fractions eluted with petrol–EtOAc (1:1). Both flavonoids were identified by comparison with literature data.

The fractions eluted with petrol–EtOAc (1:4) were subjected to extensive chromatographic purifications to afford 100 mg of **1**, 40 mg of **2**, 50 mg of the epimeric mixture **3a** and **b** and 15 mg of the mixture of hydroxylactones **4a** and **b**.

Salvisousolide (**1**). Amorphous powder, mp 116–117°; $[\alpha]_D$ −136.5 ($CHCl_3$; *c* 0.2); UV λ_{max}^{MeOH} nm (log ε): 202 (4.5), 265 (3.4); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 1770, 1740, 1660, 1590, 920, 870; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; MS *m/z* (rel. int.): 401 (5), 400 (10), 179 (20), 163 (5), 162 (40), 161 (100), 159 (10), 115 (20), 95 (10), 91 (10), 79 (5), 43 (60). $C_{22}H_{24}O_7$ requires $[M]^+$ at *m/z* 400.

2α-Hydroxy-7α-acetoxy-12-oxo-15 : 16-epoxy-neoclerodan-3,13(16),14-trien-18:19-olide (**2**). Oily compound; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3603, 1772, 1738, 1673, 1607, 1560, 872; ^{1}H NMR: Table 1, ^{13}C NMR: Table 2; MS *m/z* (rel. int.): 384 (0.2), 342 (0.2), 233 (10), 215 10), 171 (15), 159 (15), 149 (20), 145 (20), 110 (22), 95 (92.7), 43 (100). $C_{22}H_{26}O_7$ requires $[M]^+$ at *m/z* 402, not observed.

Mixture of hydroxylactones **3a** *and* **b**. Oily mixture; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3582, 3360, 1774, 1681, 1632, 1026, 830; ^{1}H NMR: Table 1; MS *m/z* (rel. int.): 359 (4.9), 341 (1.8), 231 (2.5), 115 (20), 91 (50), 76 (20), 55 (40). $C_{20}H_{22}O_7$ requires $[M]^+$ at *m/z* 374, not observed.

Mixture of hydroxylactones **4a** *and* **b**. Oily mixture; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1771, 1678, 1630, 1020, 832, 753; ^{1}H NMR: Table 1; FABMS *m/z* (rel. int.): 375.2 (10),

277.2 (8), 231.2 (6), 185.2 (85), 93.1 (100), 75.1 (25), 57.0 (18). $C_{20}H_{22}O_7$ requires $[M]^+$ at m/z 374.

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