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NEO-CLERODANE AND LANGUIDULANE DITERPENOIDS FROM *SALVIA SOUSAE* AND *S. KARWINSKII*

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Key Word Index—*Salvia sousae*; *Salvia karwinskii*; Labiatae; neo-clerodane and languidulane diterpenes; salviarin; 1(10)-dehydrosalviarin; 10β-hydroxysalviarin; isosalvisousolide.

Abstract—The new languidulane diterpenoid isosalvisousolide has been isolated from the aerial parts of *Salvia sousae*, besides the known neoclerodane diterpenoids salviarin, 1(10)-dehydrosalviarin and 10β-hydroxysalviarin. The flavones jaceosidin, cirsiliol and cirsilineol and oleanolic acid were also obtained from the same source. From *S. karwinskii*, 1(10)-dehydrosalviarin was the sole neoclerodane isolated. The structure of these compounds were established by spectroscopic means.

INTRODUCTION

In a continuation of our systematic studies on Mexican *Salvia* species [1–3], we describe in this paper the diterpenoid content of *Salvia sousae* Ramamoorthy and *S. karwinskii* Benth. In a previous work on *S. sousae* [4] besides oleanolic acid and some flavonoids, a *cis*-neoclerodane diterpenoid (1,2-dihydro-6α,7α-epoxylinearolactone) and a languidulane diterpenoid named salvisousolide (**1**) were isolated. As part of our ongoing search for diterpenoids from plants of the Labiatae, with potential antifeedant activity [5, 6], we have analysed a new population of *S. sousae*.

RESULTS AND DISCUSSION

The aerial parts of *Salvia sousae* afforded, after extensive chromatographic purification, the flavones jaceosidin, cirsilineol and cirsiliol, oleanolic acid and the previously known neo-clerodane diterpenoids salviarin [7], 10β-hydroxysalviarin and 1(10)-dehydrosalviarin [8]. The neo-clerodane diterpenoid (**2**) previously known from *Salvia urolepis* [2] was also isolated from this source besides a new languidulane-type diterpenoid named isosalvisousolide (**3**).

Compound **3** ($C_{22}H_{24}O_7$ MS) was isolated as an amorphous solid which showed in its IR spectrum bands due to a hydroxy group (3467 cm^{-1}), α,β-unsaturated γ-lactone (1764 cm^{-1}), ester function (1736 cm^{-1}) and α,β-unsaturated ketone (1664 cm^{-1}). The absorptions at 1588, 918 and 870 cm^{-1} were attributed to a disubstituted furan ring. The ^{1}H NMR spectrum of **3** (Table 1) showed two one-proton doublets ($J = 2$ Hz) at δ 7.33 and 6.66 characteristics of the C-14 and C-15 protons of a languidulane diterpenoid with an α,β substituted furan ring. The chemical shift of these protons indicated the presence of a ketone at C-12, as in languiduline [9] and salvisousolide [2, 4]. The ^{13}C NMR spectrum of **3** (Table 2) showed signals at δ 123.3 *s*, 108.9 *d*, 142.3 *d* and 159.1 *s* corresponding to the α,β substituted furan ring. The signal for the C-12 carbonyl group was observed at δ 193.3.

An AB system observed at δ 4.96 and 4.12 ($J = 9$ Hz) was assigned to the C-19 methylene protons. The *pro-S* H-19 (δ 4.12) is devoid of the long-range coupling commonly found in neo-clerodane diterpenoids. This fact suggests the presence of a substituent at the C-6β position [10], furthermore, the chemical shift of H-19 *pro-R* indicated the presence of an acetate group bound to the C-7α axial position [11]. A double doublet at δ 5.15 ($J = 4.5$, 3 Hz) was assigned to the geminal proton of this moiety and a broad doublet at δ 4.02 ($J = 3$) was ascribed to the geminal proton of a hydroxy group which must be located therefore at the C-6β position. The orientation for this hydroxy group was confirmed with the aid of the coupling constant observed for H-6 and H-7.

The coupling constants observed for H-10 (δ 3.02, *d*, $J = 10.5$ Hz) and H-1 (δ 3.07 *dt* $J = 10.5$, 2.5) revealed a *trans*-axial relationship between them. An AB system at δ 2.96 and 2.63 ($J = 14.5$) was ascribed

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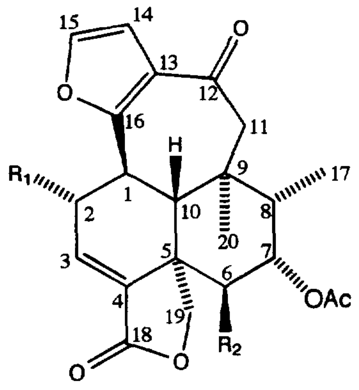

1 $R_1 = OH, R_2 = H$
3 $R_1 = H, R_2 = OH$

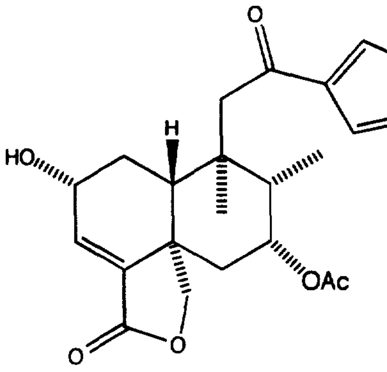

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Table 1. ^{1}H NMR data for isosalvisousolide (**3**)*

H	δ (ppm)	J (Hz)
1	3.07 *dt*	10.5, 2.5
2α	3.28 *ddd*	18, 8, 2.5
2β	2.56 *ddd*	18, 10.5, 2.5
3	7.06 *dd*	8, 2.5
6	4.02 *d*	3
7	5.15 *dd*	4.5, 3
8	2.04 *dq*	7, 4.5
10	3.02 *d*	10.5
11A	2.96 *d*	14.5
11B	2.63 *d*	14.5
14	6.66 *d*	2
15	7.33 *d*	2
3H-17	1.05 *d*	7
H-19 *pro R*	4.96 *d*	9
H-19 *pro S*	4.12 *d*	9
3H-20	0.90 *s*	–
CH_3CO-	2.14 *s*	—

* Run at 500 MHz. CDCl$_3$, TMS.

to the C-11 methylene protons and two one-proton signals at δ 3.28 (*ddd*, $J = 18, 8, 2.5$) and 2.56 (*ddd*, $J = 18, 10.5, 2.5$) were assigned to H-2α and H-2β, respectively. A double doublet at δ 7.06 was ascribed to H-3. The ^{13}C NMR spectrum of **3** (Table 2) was in total agreement with the structure and stereochemistry proposed for it. The chemical shifts observed for the C-20 and C-17 carbon atoms (Table 2) indicated an A/B *trans* fusion [2, 12]. These assignments were corroborated by the HMBC and HMQC spectra.

Compound **3** could be considered as an isomer of salvisousolide (**1**), a languidulane-type diterpenoid isolated previously from the same species and recently from *S. urolepis* [2, 4]. Other languidulane-type diterpenoids have been isolated previously from *Salvia languidula* [9].

From the aerial parts of *Salvia karwinskii* only small amounts of 1(10)-dehydrosalviarin were isolated. The presence of this diterpenoid in *S. karwinskii* has chemotaxonomic interest since *Salvia lineata*, from which 1(10)-dehydrosalviarin was first isolated [8] is included in Section *Fulgentes* [13], a section botanically related to Sect. *Holwaya*. The presence of 1(10)-dehydrosalviarin and salviarin in these plants is important, since these compounds possesses a significant antifeedant activity against *Spodoptera littoralis* [6]. These plants constitute new natural sources of these compounds. The evaluation of 10β-hyd-

Table 2. ^{13}C NMR data for isosalvisousolide (**3**)*

C	δ
1	37.8 *d*
2	34.5 *t*
3	138.4 *d*
4	135.8 *s*
5	52.3 *s*
6	67.8 *d*
7	75.9 *d*
8	38.2 *d*
9	39.3 *s*
10	47.2 *d*
11	56.7 *t*
12	193.3 *s*
13	123.3 *s*
14	108.9 *d*
15	142.3 *d*
16	159.1 *s*
17	11.1 *q*
18	167.7 *s*
19	69.2 *t*
20	14.1 *q*
21	169.6 *s*
22	21.0 *q*

* Run at 125 MHz. CDCl$_3$. TMS. Assignments confirmed with the aid of HMBC and HMQC spectra.

roxisalviarin and isosalviosusolide as insect antifeedants is in progress.

EXPERIMENTAL

Mps.: uncorr.; EIMS were obtained at 70 eV by direct inlet; UV MeOH; ^{1}H and ^{13}C NMR spectra were obtained at 500 and 125 MHz respectively, $CDCl_3$, TMS as int. standard. *Salvia sousae* was collected in Coxcatlan (state of Puebla, México) in August 1994 and *S. karwinskii* was collected in July 1994 in Zoquitlán (state of Puebla, México). Voucher specimen (BER500 and BER501, respectively) were deposited at the herbarium of the Instituto de Biología UNAM.

Extraction, fractionation and isolation of the diterpenoids from Salvia sousae. Dried and powdered aerial parts of *S. sousae* (980 g) were extracted with Me_2CO for 4 days at room temp. The solvent was removed *in vacuo* to yield 81.1 g of a gummy residue which was subjected to partition between MeOH–H_2O (4:1) and benzene–petrol (1:1). The aqueous methanolic fraction was concentrated *in vacuo*, water was added and the mixture was extracted with CH_2Cl_2. The organic phase was dried with anh. Na_2SO_4 and the solvent removed to yield 40.2 g of a gum, which was subjected to vacuum chomatography over silica gel. Mixtures of petrol–EtOAc and EtOAc–MeOH of increasing polarity were used as eluents. From the frs eluted with petrol–EtOAc (4:1) oleanolic acid (20 mg) was isolated. Elution with petrol–EtOAc (3:2) afforded 10β-hydroxysalviarin (2352 mg), cirsineol (670 mg) and jaceosidin (490 mg). Cirsiliol (264 mg) was isolated from the frs eluted with petrol–EtOAc (3:7). Some frs eluted with petrol–EtOAc (3:2) were combined and subjected to flash chromatography ($CHCl_3$–Me_2CO 4:1) to yield salviarin (25 mg), 1(10)-dehydrosalviarin (16 mg) compound **2** (154 mg) and isosalvisousolide **3** (16 mg).

Isosalvisousolide (**3**). Amorphous solid, mp 260–262°; $[\alpha]_D = -129$ (MeOH; *c* 0.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3476, 1764, 1736, 1664, 1588, 918, 870; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; MS *m/z* (rel.int.): 402 (3), 401 (15), 400 (45), 368 (15), 358 (15), 340 (20), 310 (20), 229 (25), 185 (10), 163 (25), 162 (85), 161 (199), 129 (15), 97 (15), 69 (25), 57 (30), 43 (45). $C_{22}H_{24}O_7$ requires $[M]^+$ at *m/z* 400.

Isolation of 1(10)-*dehydrosalviarin from* Salvia karwinskii. Dried and powdered aerial parts of *S. karwinskii* (80 g) were extracted with Me_2CO for 2 days at room temp. The gummy residue obtained after evaporation of the solvent was partitioned as described above. Chromatographic purification of the polar phase (8 g) afforded a mixture of oleanolic and ursolic acids and 10 mg of 1(10)-dehydrosalviarin. The identity of these compounds was confirmed by comparison with authentic samples.

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