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CLERODANE DITERPENOIDS FROM *SALVIA MELISSODORA*

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Key Word Index—*Salvia melissodora*, Labiatae, *neo*-clerodanes, diterpenoids.

Abstract—From two populations of *Salvia melissodora* Lag., four *neo*-clerodane diterpenoids were isolated. The structures of these compounds were established by spectroscopic and chemical means. The triterpenoids, oleanolic acid and ursolic acid, together with sitosterol, were also found in this species.

INTRODUCTION

Continuing with the chemotaxonomic study of Mexican Salvias [1], in this paper we report the *neo*-clerodane diterpenoids of two populations of *Salvia melissodora* Lag., (*Salvia*, section *Scorodonia* Epling) [2]. Some years ago [3], we described the structural elucidation of melisodoric acid (**1**), a *neo*-clerodane diterpenoid, whose relative stereochemistry was established recently [4]. Compound **1** was isolated from a population of *S. melissodora* collected in the valley of Mexico, and showed antifeedant activity against some species of *Heliotis* and *Euxoa* [Javier Taboada from Instituto de Química, personal communication]. We have been trying to find an adequate natural source of **1**, and have studied therefore, different populations of *S. melissodora*.

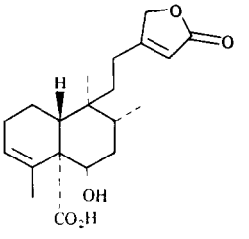
From two populations of this species, in addition to sitosterol, oleanolic and ursolic acids, we isolated four *neo*-clerodane-type diterpenoids **2**, **3a**, **4** and **5a**. Compounds **2**, **3a** and **4** have been previously described in the literature. Compound **5a**, is an isomer of **2** and constitutes a new natural product.

RESULTS AND DISCUSSION

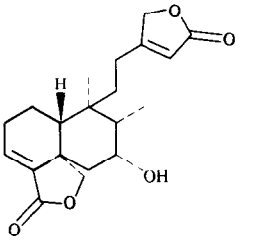
From the acetone extract of a population of *S. melissodora* collected in the State of Mexico, in addition to oleanolic acid and ursolic acid, the *neo*-clerodane diterpenoids **2** and **3a** were isolated. Compound **2** was previously isolated from *Baccharis trimera* [5] and recently from *Salvia semiatratha* Zucc. [6] and *S. microphylla* Kunt [7]. The identity of **2** was established by comparison with an authentic sample and the literature data.

Spectroscopic evidence led us to identify compound **3a** as portulide C, a *neo*-clerodane diterpenoid recently isolated from *Portulaca* cv Jewel [8]. Acetylation of **3a** in mild conditions, gave the diacetate derivative **3b**. Oxidation of **3a** with MnO_2 in CH_2Cl_2 yielded a product identical to 1-deoxybacrispine (**6**), previously isolated from *Baccharis crispa* [9]. The same product **6** was obtained by treatment of **2** with Dibal, followed by treatment with aqueous sulphuric acid.

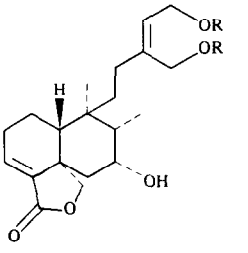
A second population of *S. melissodora*, collected in the State of Hidalgo was studied. In addition to oleanolic



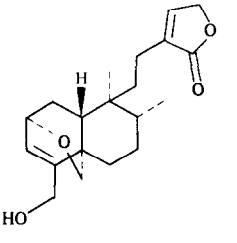
1



2



3a R = H
3b R = Ac



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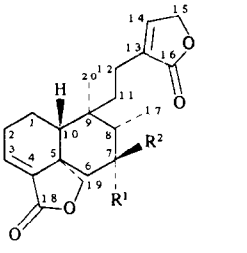
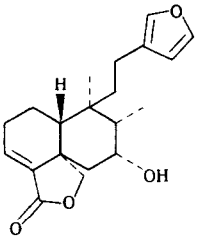


5a R^1 = OH, R^2 = H
5b R^1 = R^2 = =O



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acid and sistosterol, compounds **4** and **5a** were isolated. Spectroscopic data of **4** are identical with those described for brevifloralactone, a diterpenoid recently isolated from *Salvia breviflora* [10], a species also classified in *Salvia*, Section *Scorodonia* (Epling) [2]. The structure of **4** was supported by X-ray work.

Compound **5a** was isolated as a crystalline solid, mp 178–180° $[\alpha]_D^{20} = -154°$ ($CHCl_3$; *c* 0.16), and the mass spectrum was consistent with a $C_{20}H_{26}O_5$ molecular formula. Its IR spectrum exhibited absorptions for α,β unsaturated γ-lactone groups (1757 cm^{-1}), hydroxyl groups (3626 cm^{-1}) and double bonds (1660 cm^{-1}).

The ^1H NMR spectrum of **5a**, was very similar to that reported [5] for **2**, except for the signals due to the butenolide moiety. A broad singlet at δ 7.1 was assigned to H-14 and a broad doublet at δ 4.75 was attributed to the C-15 methylene group. These data indicate the presence of an α-substituted butenolide ring in **5a**. Hence compound **5a** is an isomer of **2**. The ^{13}C NMR spectrum of **5a** (Table 1) is also very similar to that reported [5] for **2** and supports the structure proposed for it. A singlet observed at δ 134.2 was assigned to C-13; the C-14, C-15 and C-16 are responsible for signals at δ 144.3 (*d*), 70.4 (*t*) and 174.4(*s*), respectively. A triplet observed at δ 19.2 was attributed to C-12. These chemical shifts are very different to those reported for a β-substituted butenolide ring [5, 6, 11].

Treatment of **5a** with Jones reagent yielded **5b**. In the ^1H NMR spectrum of **5b**, the signal due to the C-20 methyl group was observed at δ 0.65. The upfied shift of this signal with respect to **5a** ($\Delta\delta = 0.2$) is due to the lack of the 1,3-diaxial interactions of the C-20 methyl with the α-axial hydroxyl group at C-7 upon oxidation. Similar behaviour has been observed in the oxidation product of **2** [5].

Although melisodoric acid **1** was not isolated from these populations, the presence of *neo*-clerodane type diterpenoids is very interesting, since this type of compound frequently exhibits antifeedant properties [12]. The chemical compostion found for *S. melissodora* is in agreement with the chemical profile previously observed for several members of *Salvia* subgenus *Calosphace* [13, 14].

Table 1. ^{13}C NMR data for compound **5a***

C	δ	C	δ
1	19.2 *t*	11	36.3 *t*
2	27.7 *t*	12	19.2 *t*
3	135.2 *d*	13	134.2 *s*
4	139.2 *s*	14	144.3 *d*
5	45.0 *s*	15	70.4 *t*
6	40.7 *t*	16	174.4 *s*
7	72.3 *d*	17	11.9 *q*
8	40.7 *d*	18	170.2 *s*
9	38.6 *s*	19	72.8 *t*
10	48.4 *d*	20	19.1 *q*

*Recorded at 20 MH_2 in $CDCl_3$ with TMS as internal reference.

SFORD multiplicity in parenthesis.

EXPERIMENTAL

Mps: uncorr. MS were obtained at 70 eV by direct inlet. ^1H and ^{13}C NMR spectra were performed at 80 and 20 MHz respectively, using TMS as int. standard, coupling constants are in Hz. Plant materials were recollected in October 1985 in the State of Hidalgo (México) and in June 1985 in the State of México (México). Voucher specimens were deposited at the Herbarium of the Instituto de Biología, UNAM.

Isolation of the constituents from S. melissodora *from the State of México* (*Voucher MEXU BER* 21). Dried and powdered aerial parts of *S. melissodora* (2.7 kg) were extracted with EtOAc at room temp. for 4 days. The solvent was removed under red. pres. to yield 110 g of gummy residue. This extract (85 g) was subjected to dry CC over silica gel (1 kg deactivated with 10% H_2O). Mixtures of petrol–EtOAc and EtOAc–MeOH of increasing polarity were used as eluents. From the fractions eluted with petrol–EtOAc (1:1) 11.6 g of a mixture of oleanolic acid and ursolic acid was isolated.

Elution with petrol–EtOAc (1:4) yielded 6 g (0.22% dry weight) of **2**. The identity of this compound was confirmed by comparison with an authentic sample and the literature data [5]. The fractions obtained by elution with EtOAc–MeOH (3:1) (150 mg) were rechromatographed over silica gel (200 g) using a mixture of C_6H_6–Me_2CO (3:1) as eluent, to yield 85 mg (3.14×10^{-3}% dry weight) of **3a** as a crystalline solid: mp 185–186°. Physical data found for **3a** are identical to those reported for portulide C (lit. mp 191–192°) [8].

Acetylation of **3a**. Compound **3a** (15 mg) in pyridine (0.5 ml) was treated with Ac_2O (1 ml) at room temp. for 12 hr. After usual work-up, the crystalline product **3b** (8 mg) was obtained: mp 240–242° (EtOAc–*n*-hexane); IR, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1750, 1725, 1650, 1040; ^1H NMR ($CDCl_3$): δ 6.66 (*dd*, $J = 7$ and 2Hz, 1H, H-3), 4.05 (*m*, 1H, H-7), 5.5 (*t*, $J = 7$Hz, 1H, H-14), 4.64 (*d*, $J = 3$Hz, 2H, 2H-15), 4.65 (*s*, 2H, 2H-16), 1.03 (*d*, $J = 7$Hz, 3H, 3H-17), 3.86 (*dd*, $J = 7$ and 2Hz, 1H, H-19 pro-*S*), 5.25 (*d*, $J = 7$Hz, 1H, H-19 pro-*R*), 0.86 (*s*, 3H, 3H-20), 2.05 (*s*, 6H, –OAc); EM *m/z* (rel. int.): 434 (0.4), 43 (100), 374 (47), 416 (26). $C_{24}H_{34}O_7$ requires M^+ at *m/z* 434.

Oxidation of **3a** *with* MnO_2. Compound **3a** (35 mg) in $CHCl_3$ (10 ml) was treated with MnO_2 (250 mg) at room temp. for 5 hr. The reaction mixture was filtered and the solvent removed at red. pres. to yield 28 mg of a crystalline solid mp 198–200° (CH_2Cl_2–*n*-hexane), $[\alpha]_D^{20} = -50°$ (MeOH; *c* 0.7). Spectral data are coincident with the values reported for 1-deoxybacrispine **6** (lit. mp 198–202°, $[\alpha]_D^{20} = -50.8$ (MeOH; *c* 0.72) [9].

Conversion of compound **2** to **6**. Compound **2** (250 mg) in dry THF (2.5 ml) was treated with DIBAL (2.2 equiv.) with stirring at −35° in Ar atm. for 3 hr. After the reaction mixture reached room temp., H_2SO_4 (10 ml, 10% aq.) was added and the mixture extracted with EtOAc. The organic layer was washed with $NaHCO_3$ (10% aq.) and brine, dried over Na_2SO_4, and the solvent removed at red. pres. The crude product was purified by CC over silica gel eluted with petrol–EtOAc (7:3), to yield **6**.

Isolation of the constituents of S. melissodora *from the State of Hidalgo* (*Voucher MEXU TPR* 4749). Dried aerial parts (1290 g) of this population were extracted with Me_2CO (20 l) for 1 week at room temp. The gummy extract (204 g), obtained after evapn of the solvent at red. pres. was chromatographed over 1500 g of silica gel (deactivated with 10% H_2O). Mixtures of petrol–EtOAc of increasing polarity were used as eluents. From the fractions eluted with petrol–EtOAc (19:1) 200 mg of sitosterol were isolated. The identity of this compound was confirmed by comparison with an authentic sample.

From the first fractions eluted with petrol–EtOAc (4:1) we isolated 67 g of oleanolic acid, which was identified by compari-

son of its methyl ester derivative with an authentic sample. From the last fractions obtained with the same polarity, 176.4 mg (0.0136% dry weight) of **4** were obtained, which was identified as brevifloralactone by comparison with literature data [10].

Extensive purifications, by CC, of the fractions eluted with petrol–EtOAc (3:2) led us to isolate 622 mg (0.048% dry wt) of **5a** as a crystalline product: mp 178–180°; $[\alpha]_D^{20} = -154°$ ($CHCl_3$; *c* 0.165); UV λ_{max}^{MeOH} nm (ε): 205 (20370); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3626, 1757, 1660, 1450, 1347, 1077, 1038; ^1H NMR ($CDCl_3$): δ 6.7 (*dd*, *J* = 6 and 4Hz, 1H, H-3), 4.1 (*br dd*, *J* = 6 and 4Hz, 1H, H-7) 7.1 (*br s*, 1H, H-14), 4.75 (*br d*, *J* = 2Hz, 2H, 2H-15), 1.05 (*d*, *J* = 7Hz, 3H, 3H-17), 3.85 (*dd*, *J* = 8 and 2Hz, 1H, H-19 pro-*S*), 5.25 (*d*, *J* = 8Hz, 1H, H-19 pro-*R*), 0.85 (*s*, 3H, 3H-20); ^{13}C NMR see Table 1; MS *m/z* (rel. int.): 346 (1.1), 328 (3.7), 316 (20), 310 (10), 298 (20), 273 (10), 255 (10), 217 (25), 205 (100), 159 (35), 105 (20) 93 (20), 91 (50), 79 (30), 77 (30), 41 (30). $C_{20}H_{26}O_5$ requires M^+ at *m/z* 346.

Oxidation of compound **5a**. Compound **5a** (100 mg) in Me_2CO (10 ml) was oxidized with Jones reagent at 0°. After usual work-up, compound **5b** (77 mg) was obtained as a crystalline solid: mp 116–118° (EtOAc–*n*-hexane); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1710, 1660; ^1H NMR ($CDCl_3$): δ 6.85 (*dd*, *J* = 6 and 4Hz, 1H, H-3), 7.15 (*br s*, 1H, H-15), 4.8 (*br d*, *J* = 1Hz, 2H, 2H-15), 1.0 (*d*, *J* = 6Hz, 3H, 3H-17), 3.85 (*dd*, *J* = 8 and 1Hz, 1H, H-19 pro-*S*), 4.0 (*d*, *J* = 8Hz, 1H, H-19 pro-*R*), 0.65 (*s*, 3H, 3H-20); MS *m/z* (rel. int.): 345 (1), 344 (4.2), 233 (80), 203 (50), 175 (60), 167 (30), 91 (100), 79 (40), 77 (60), 41 (70). $C_{20}H_{24}O_5$ requires M^+ at *m/z* 344.

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