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DITERPENES FROM *SALVIA BREVIFLORA**

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Key Word Index—*Salvia breviflora*; Labiatae; neoclerodanes; brevifloralactone; X-ray structure.

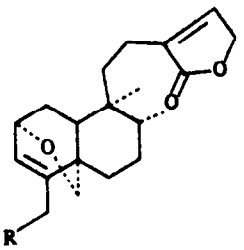
Abstract—In addition to compounds which are ubiquitous in *Salvia* species, like ursolic and oleanolic acids, we have isolated two heterobicyclic diterpenes, brevifloralactone and brevifloralactone acetate.

INTRODUCTION

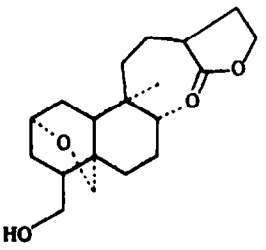
The genus *Salvia* has been found to be a rich source of terpenes, diterpenes, triterpenes and steroids [1]. A large number of clerodane diterpenes have also been isolated from plants of this genus. Some of these later compounds show antifeedant or antifungal properties [2–4]. We describe here the phytochemical investigation of *Salvia breviflora*, a species found in the state of Guerrero, México, as part of a screening of *Salvia* species growing in our country.

RESULTS AND DISCUSSION

The plant was extracted in the usual way [5] and upon column chromatography yielded ursolic and oleanolic acids, which were identified by comparison with standards. An intermediate fraction had two compounds which could be correlated with each other. The most polar one **1a** was a new diterpene for which we propose the name brevifloralactone. The spectroscopic and chemical evidence for this compound was in agreement with a neoclerodane structure. It was a solid, mp 126°, which could be crystallized only after rechromatography and slow evaporation of the solvent. The molecular formula was $C_{20}H_{28}O_4$, as determined by elemental analysis and the molecular ion at m/z 322 in the mass spectrum. The presence of ions at m/z 173 $[M-159]^+$ ($C_{13}H_{17}$) and 111 $[M-221]^+$ ($C_6H_7O_2$) were indicative of a clerodane type skeleton with a butenolide function in the side chain. Infrared spectral information showed the presence of an alcohol function (IR bands at 3609 and 1070 cm^{-1}). The ultraviolet spectrum was also in good agreement with the presence of a butenolide function (UV maxima at 208 nm in MeOH and 201 nm in hexane). The IR and UV data for this function may be compared with that of nidorelalactone [6]. The ^{13}C NMR spectrum was in agreement with structure **1a**, showing signals for the twenty carbon atoms.



1a R = OH
1b R = OAc



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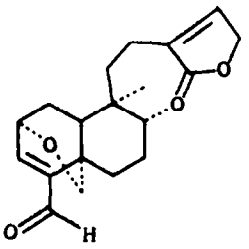

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Table 1. ^{1}H NMR data of compounds **1a**, **1b** and **3**
(80 MHz, $CDCl_3$, TMS as int. stand.)

	1a	1b	3
H-2	4.45 *m*	4.45 *m*	3.85 *m*
H-3	6.25 *dt*	6.25 *dt*	6.30 *d*
H-14	7.06 *m*	7.06 *m*	7.06 *m*
H-15	4.74 *m*	4.74 *m*	4.74 *m*
H-17	0.86 *d*	0.86 *d*	0.86 *d*
H-18	4.24 *d*	4.65 *d*	9.72 *d*
H-19	4.10 *d*	4.10 *d*	4.12 *d*
H-19′	2.83 *dd*	2.83 *dd*	2.87 *dd*
H-20	0.99 *s*	0.99 *s*	0.78 *s*
OAc		2.01 *s*	

J (Hz) H-2 $W_{h/2}$ = 11.5, H-3 = 5.5, 1.6, H-14 $W_{h/2}$ = 4, H-15 $W_{h/2}$ = 3, H-17 = 6, H-18 = 1.6, H-19 = 8.2, H-19′ = 8.2, 1.1.

The signal at δ174.1 corresponded to the carbonyl carbon in the butenolide function. Four signals at δ151.9 (*s*), 143.8 (*d*), 134.8 (*s*) and 125.0 (*d*) were assigned to two trisubstituted double bonds. The signals at δ70.2 (*t*), 67.8 (*t*), 67.0 (*d*) and 60.6 (*t*) revealed carbon atoms bearing singly bonded oxygen atoms. The first of them corresponded to the γ-carbon (C-15) of the butenolide function, the next one to the primary alcohol at C-18. The secondary carbon atom C-2 at the bridgehead in ring A and the heterobicyclic bridge methylene at C-19, explain the following two signals thus accounting for all of the oxygen substituted carbon atoms in the molecule. The ^{1}H NMR spectrum (Table 1) was examined in more detail using the technique of double resonance and it was also in agreement with structure **1a**. The vinyl proton at C-14 gave a multiplet at δ7.06 (one proton). At 6.25 there was a doublet of triplets, with coupling constants of 5.5 and 1.6 Hz (one proton), assigned to H-3. At 4.74 appeared the AB part of an ABX system assigned to the methylene group at C-15, coupled with the C-14 vinyl proton. The C-2 proton at the bridgehead gave a multiplet a 4.45 coupled to the vinyl proton at C-3 and also to the non-equivalent protons of the methylene group at C-1. There was another AB system at 4.24 which showed a small coupling constant (1.6 Hz) with the vinyl proton at C-3. This system was assigned to the methylene protons connected to the hydroxyl group. A widely separated AB system gave doublets at 4.10 (J = 8.2 Hz) and 2.83 (J = 8.2 Hz, broad doublet), the latter one showed a *W*-interaction with one of the protons at C-6 in ring B. The protons from two methyl groups appeared as a singlet at 0.99 and as a doublet at 0.86 (three protons each). The ^{1}H NMR spectrum of brevifloralactone acetate (**1b**) showed a similar pattern, except for the presence of a single signal at 2.09 for the methyl protons of the acetate group and a larger downfield displacement of the AB signal for the protons at C-18 which appeared at 4.65 in this compound.

The heterobicyclic ring in **1a** was opened by treating it with trifluoroacetic acid in chloroform. This experiment was carried out in a NMR probe containing a deuterochloroform solution of **1a**. After 72 hr there was NMR spectral evidence of an equilibrium reaction in which the heterobicyclic bridge was opened and a new double bond appeared in ring A. The signal for the methyl at δ0.99 was shifted to 0.77, while the butenolide function was unaffected.

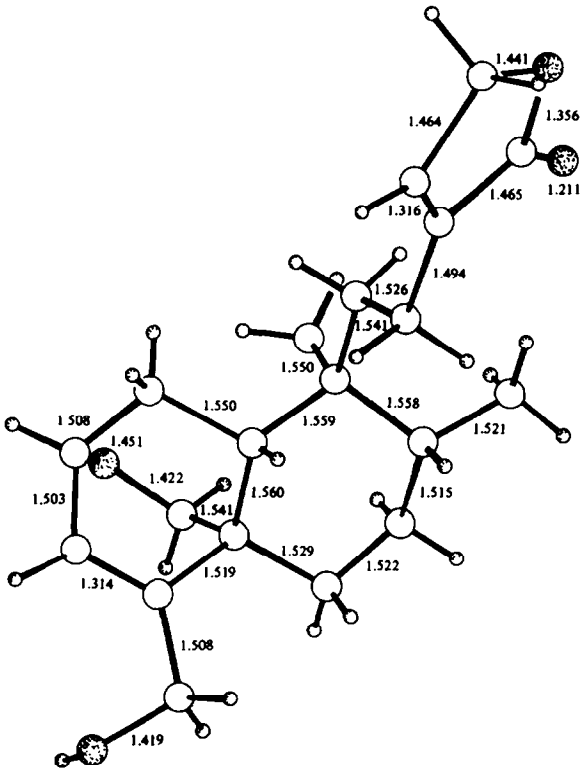

Fig. 1.

Compound **1a** was acetylated to give a monoacetate, mp 112° (identical with **1b**). It could also be hydrogenated to a tetrahydroderivative **2**, which had both double bonds saturated but retained the heterobicyclic structure in ring A. Upon oxidation, **1a** yielded among not less than eight compounds (as evidenced by HPLC) the expected aldehyde **3**. Some of the by-products showed spectroscopic evidence of extensive alteration of ring A. The NMR spectra of **1b** and **3** are reported in Table 1. All the above information is in agreement with structure **1a** and this was confirmed by X-ray crystallography except for the absolute configuration (Fig. 1). Circular dichroism showed two main positive maxima at 193 and 208 nm for compound **1a** and we believe that the depicted stereochemistry is the correct one, however this point must await further confirmation.

EXPERIMENTAL

Finely powdered dry *Salvia breviflora* Moc and Sesse (2 kg, specimen stored at Instituto de Biología, Herbario Nacional Méx. 4307072), collected in the state of Guerrero, México (aerial parts) was extracted with EtOAc. The resulting extract was separated by CC and further purified by prep. TLC developed with EtOAc–hexane (3:7). The following compounds were separated, listed in order of elution with *n*-hexane–EtOAc: ursolic and oleanolic acids, brevifloralactone acetate (**1b**) (35 mg/kg dry wt) and brevifloralactone **1a** (542 mg/kg dry wt). Ursolic and oleanolic acids were identified by comparison of their IR and ^{1}H NMR spectra and other physical properties with authentic specimens.

Brevifloralactone (**1a**) Mp 126° (uncorr.). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3609–3007, 2960, 2867, 1753, 1645, 1460, 1387, 1349, 1210, 1133, 1070, 1052, 939, 829, 750. ^{13}C NMR: δ 37.07 (*t*, C-1), 67.03 (*d*, C-2), 127.96 (*d*, C-3), 134.81 (*s*, C-4), 39.23 (*s*, C-5), 19.20 (*t*, C-6), 29.28 (*t*, C-7), 36.76 (*d*, C-8), 39.11 (*s*, C-9), 38.62 (*d*, C-10), 27.00 (*t*, C-11), 27.30 (*t*, C-12), 151.89 (*s*, C-13), 143.78 (*d*, C-14), 70.21 (*t*, C-15), 174.34 (*s*, C-16), 15.67 (*q*, C-17), 67.76 (*t*, C-18), 60.59 (*t*, C-19), 16.36 (*q*, C-20). ^{1}H MNR ($CDCl_3$): see Table 1. MS *m/z* (rel. int.): 332 $[M]^+$ (3), 315 (4), 301 (3), 302 (4), 173 (100), 111 (12), 97 (10), 91 (59). [Found C, 72.26; H, 8.21, $C_{20}H_{28}O_4$ requires C, 72.27; H, 8.44%.] CD nm (AcCN): 370 (0), 250 (+0.11), 242.9 (+0.15), 209.8 (+4.38), 208.0 (+4.13), 197 (+6.15), 193.2 (+6.41), 189.5 (5.47), 180.0 (0). $[\alpha]_D^{20}$ = +0.232 (*c* 2.3, CH_3CN).

3,4-13,14-*Tetrahydrobrevifloralactone* (**2**). Compound **1a** (88 mg) was hydrogenated in absolute EtOH (25 ml) over 10% Pd–C (10 mg). Filtration and evaporation of solvent left a residue which was purified by CC in silica gel to yield 84.5 mg (96%) of the tetrahydro derivative mp 65°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3609, 2960, 2867, 1763, 1460, 1387, 1349, 1210, 1133, 1070, 1052, 939, 829, 750. MS *m/z* (rel. int): 336 $[M]^+$ (14), 334 (1), 222 (39), 220 (3), 190 (4), 186 (49), 91 (90), 84 (30), 113 (17).

Brevifloralactone acetate (**1b**) Mp 112°. Ac_2O–pyridine treatment of **1a** at room temp. for 16 hr, yielded an acetate identical with natural product **1b**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3036, 3007, 2960, 2867, 1749, 1645, 1460, 1387, 1238, 1210, 1133, 1070, 1052, 939, 750. MS *m/z* 374 $[M]^+$ (10), 275 (5).

X-Ray analysis. X-Ray determination of **1a** was done on a sample crystallized from EtOAc, space group $P_{2_12_12_1}$, $Z = 4$ with a = 6.868(5) Å, b = 15.652(8) Å and c = 16.454(8) Å, with calculated density 1.246 g cm^{-3}, using Mo Kα radiation from 1366 reflexions; no crystal decomposition was detected during the data collection process, the structure was solved by a Shelxtl system [7] and is shown in Fig. 1. Full crystallographic information is deposited at Cambridge Crystallographic Data Centre. Final R is 0.435 and hydrogenation positions determined from bond lengths.

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