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LANGUIDULANE, CLERODANE AND SECOCLERODANE DITERPENES FROM *SALVIA TONALENSIS**

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

Abstract—In addition to several known compounds, the new languidulane diterpene, tonalenin (8β-hydroxy-7-*epi*-8,17-dihydrolanguiduline), was isolated from the aerial parts of *Salvia tonalensis*. The structures were elucidated by spectroscopic means. © 1997 Elsevier Science Ltd. All rights reserved

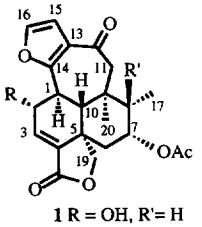
INTRODUCTION

In our search for new natural substances of the Mexican flora, several *Salvia* species have been investigated. This has resulted in the isolation of abietane, clerodane and secoclerodane diterpenoids [1–4]. Now, as a continuation of these studies, we describe the structure elucidation of the compounds isolated from *Salvia tonalensis* Brand (sect. Polystackyae, subgenus Calosphace [5]).

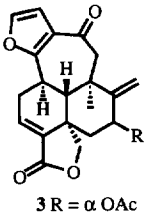
RESULTS AND DISCUSSION

The acetone extract of the aerial parts of *S. tonalensis* afforded, after extensive column chromatography, the mixture of ursolic and oleanolic acids, sitosterol, 5,3′,4′-trihydroxy-6,7-dimethoxyflavone [6, 7], the clerodane diterpene salvifaricin [8, 9] and the 5,10-secoclerodane diterpene tonalensin [10, 11].

In addition to the above mentioned compounds, two languidulane [12] diterpenoids: 2α-hydroxy-7-*epi*-8β,17-dihydrolanguiduline (**1**), previously isolated from *S. sousae* [13] and *S. urolepis* [14], and the new compound tonalenin (**2**) were also isolated.



1 R = OH, R'= H
2 R =H, R'= OH



3 R = α OAc
4 R = β OAc

The mass spectrum of tonalenin (**2**) was consistent with a molecular formula $C_{22}H_{24}O_7$. Its IR spectrum showed absorptions for hydroxyl (3490 cm^{-1}), α,β-unsaturated-γ-lactone (1770 cm^{-1}), saturated ester (1744 cm^{-1}), α,β-unsaturated ketone (1666 cm^{-1}) and disubstituted furan ring (1594, 1527 cm^{-1}). The ^1H NMR spectrum of **2** (Table 1) was very similar to that of compound **1** [13, 14] suggesting a similar skeleton for both substances. Nevertheless, the spectrum of **2** showed the H-3 signal as a double doublet and the H-1 signal as a triple doublet, indicating the absence of substituents at the C-2 position.

The ^1H NMR signals for the Me-17 and H-7 were different in both spectra. In compound **2** Me-17 was a singlet at δ 1.35 and H-7 a triplet at δ 5.09 while in **1** they were a doublet and a double of triplets, respectively. These facts, together with the singlet signal at δ 76.9 in the ^{13}C NMR spectrum of **2**, allowed location of the hydroxy group of tonalenin at C-8. The deshielding effect produced by this group on H-10 ($\Delta\delta = 0.78$), H-6β ($\Delta\delta = 0.54$) and H-11β ($\Delta\delta = 0.53$) when compared with **1** [14], indicated a 1,3-diaxial relationship between them and therefore a β-orientation of the C-8 hydroxy group. This was corroborated by the ^1H NMR spectrum of **2**, recorded after addition of TAI (trichloro acetyl isocyanate), which showed the signals for H-7, H-10 and H-11β shifted to lowfield.

The stereochemistry at C-1 and C-7 was deduced from the observed J values ($J_{1,10} = J_{1,2\beta} = 10.5$ Hz; $J_{6\alpha,7} = 2.7$ Hz; $J_{6\beta,7} = 3.6$ Hz) which indicated an α-axial orientation of H-1 and a β-equatorial disposition of H-7. The A/B *trans*-ring fusion was evidenced by the *W*-couplings of the Me-20 protons with H-10 and

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Table 1. ^{1}H NMR spectra data of compounds **2** and **3** ($CDCl_3$, 300 MHz, TMS as int. standard)

H	2	2*	2+TAI	3†
1	3.04‡	2.61 *td*	3.03 *td*	2.94 *ddd*
		10.5, 2.7	10, 2.4	11.5, 11, 3.6
2α	3.29 *dd*	2.89 *ddd*	3.3‡	3.26 *ddd*
	18, 7.8, 2.7	18, 7.8, 2.7		17.7, 7.8, 3.6
2β	2.58 *ddd*	2.07 *ddd*	2.69 *ddd*	2.33 *ddd*
	18, 9, 2.1	18, 10.5, 2.1	17, 10, 2.4	17.7, 11, 2.4
3	6.88 *dd*	6.54 *dd*	6.89 *dd*	6.85 *dd*
	7.8, 2.1	7.8, 2.1	7.5, 2.4	7.8, 2.4
6α	2.13 *d*	1.97 *dd*	2.33 *dd*	1.94 *dd*
	2.7	14.8, 2.4	15, 2.1	13.5, 7.4
6β	2.09 *dd*	1.8‡	1.82‡	2.3‡
	3.6, 1.8			
7	5.09 *dd*	4.80 *dd*	6.01 *dd*	5.71 *br ddt*
	3.6, 2.7	3.7, 2.4	3.7, 2.1	9, 7.4, 2
10	3.06‡	2.71 *d*	3.35 *d*	2.64 *br d*
		10.5	10	11.5
11α	2.86 *d*	2.79 *d*	2.83 *d*	2.64 *d*
	14	14.2	14	17.4
11β	3.07 *br d*	2.66 *br d*	3.28 *br d*	3.19 *br d*
	14	14.2	14	17.4
14	6.65 *d*	6.62 *d*	6.63 *d*	6.77 *d*
	2.2	1.8	2	2
15	7.33 *d*	7.02 *d*	7.33 *d*	7.34 *d*
	2.2	1.8	2	2
17	1.35 *s*	0.98 *s*	1.79 *s*	5.26 *d*
				2.2
17′				5.17 *d*
				1.8
$19_{pro\text{-}R}$	4.95 *d*	4.69 *d*	4.87 *d*	4.35 *d*
	8.2	8.1	8.2	8.5
$19_{pro\text{-}S}$	4.08 *dd*	3.61 *dd*	4.09 *dd*	4.03 *dd*
	8.2, 1.8	8.1, 2	8.2, 2.1	8.5, 2.3
20	1.00 *br s*	0.74 *s*	1.09 *s*	1.27 *br s*
OAc	2.14 *s*	1.75 *s*	2.13 *s*	2.16 *s*

* Determined in $CDCl_3$–benzene-d_6.
† Determined at 200 MHz.
‡ Overlapped signal.

H-11β [15, 16]. The above data, together with ^{13}C, APT, COSY and HETCOR NMR experiments, allowed formulation of tonalenin as **2**.

Dehydration of **2** afforded 7-*epi*-languiduline (**3**). The most significant difference in the ^{1}H NMR spectra of **3** and its C-7 epimer **4** [16] was the multiplicity of the H-7 signal, which is a doublet in **4** (J = 8 Hz) while in **3** is a broad *ddt* (J = 9, 7.4 and 2 Hz). Preparation of this derivative confirmed the proposed structure **2** for tonalenin.

EXPERIMENTAL

Plant material. Salvia tonalensis Brand was collected near Arriaga, Chiapas State, Mexico. A voucher specimen was deposited at the Herbarium of the Instituto de Biología, UNAM (MEXU-573763).

Extraction and isolation. Dried and ground aerial parts of the plant (501 g) were extracted with Me_2CO to obtain, after solvent evapn, 38.3 g of residue. Partition of this residue between MeOH–H_2O (4:1) and hexane–benzene (7:3) gave, after solvent evapn, a less polar (9.4 g) and a polar (27 g) fr. The less polar fr. was taken in Me_2CO, decolorized with activated charcoal and concd. From this fr. the mixt. of ursolic and oleanolic acid pptd as an amorphous solid (2.96 g). The mother liquors of these acids were submitted to CC (silica gel, hexane–EtOAc gradient elution). Frs. eluted with hexane–EtOAc (4:1 and 7:3) gave 963 mg of the above mentioned acids and a mixt. of compounds (constituted mainly by salvifaricin, tonalensisn and **3** (TLC)), which was combined with the polar fr and submitted to CC (silica gel, hexane–EtOAc gradient elution). Exhaustive CC of the frs eluted with hexane–EtOAc (4:1, 7:3 and 3:2) gave sitosterol (159 mg), tonalensin (880 mg) and salvifaricin (518 mg). Frs eluted with hexane–EtOAc (1:1 and 2:3) afforded, after repeated CC, 5,3′,4′-trihydroxy-6,7-dimethoxyflavone (10 mg), 2α-hydroxy-7-*epi*-8β-17-dihydrolanguiduline (**1**, 2.3045 g) and

Table 2. ^{13}C NMR spectral data of compounds **2** and **3** ($CDCl_3$, TMS as int. standard)

C	2*	3†
1	38.1 *d*	35.9 *d*
2	35.2 *t*	32.3 *t*
3	135.2 *d*	132.5 *d*
4	138.7 *s*	135.8 *s*
5	47.3 *s*	46.7 *s*
6	33.2 *t*	40.4 *t*
7	76.4 *d*	67.7 *d*
8	76.9 *s*	152.5 *s*
9	42.6 *s*	39.5 *s*
10	49.4 *d*	48.9 *d*
11	53.1 *t*	59.5 *t*
12	196.0 *s*	192.8 *s*
13	123.7 *s*	123.1 *s*
14	109.2 *d*	110.2 *d*
15	142.7 *d*	142.1 *d*
16	159.6 *s*	158.5 *s*
17	21.8 *q*	107.6 *t*
18	168.5 *s*	168.2 *s*
19	71.8 *t*	73.1 *t*
20	15.9 *q*	18.8 *q*
MeCO	169.8 *s*	169.7 *s*
MeCO	21.9 *q*	20.9 *q*

* Determined at 75 MHz.
† Determined at 50 MHz.

tonalenin (**2**, 1.33 g). The known compounds were identified by comparison of its physical and spectroscopic data with those published in the literature. Some previously unreported spectral data for 5,3′,4′-trihydroxy-6,7-dimethoxyflavone and tonalensin are given below.

5,3′,4′-*Trihydroxy*-6,7-*dimethoxyflavone*. Mp 285–287°; IR ν_{max}^{KBr} cm^{-1}: 3400, 1648, 1592, 1560, 1455, 1360, 1278, 1200, 1115, 1010, 835. EI-MS *m/z* (rel. int.): 330 $[M]^+$ (100); 315 (81); 301 (20); 299 (13); 287 (22); 284 (18); 181 $[A_1-Me]^+$ (13); 153 $[181-CO]^+$ (27); 135$[B_1]^+$ (9); 69 (53); 57 (15); 43 (12). ^1H NMR (200 MHz, $CDCl_3$–DMSO-d_6): δ 6.54 (1H, *s*, H-3); 6.58 (1H, *s*, H-8); 7.41 (1H, *d*, J = 2.1 Hz, H-2′); 6.97 (1H, *d*, J = 8.4 Hz, H-5′); 7.35 (1H, *dd*, J = 8.4, 2.1 Hz, H-6′); 3.98 (3H, *s*, OMe); 3.90 (3H, *s*, OMe); 12.88 (1H, *s*, C-5-OH). ^{13}C NMR (50 MHz, $CDCl_3$–DMSO-d_6): δ 164.2 *s* (C-2); 102.6 *d* (C-3); 181.9 *s* (C-4); 152.6 *s* (C-5); 131.7 *s* (C-6); 158.2 *s* (C-7); 90.5 *d* (C-8); 152.2 *s* (C-9); 105.2 *s* (C-10); 121.67 *s* (C-1′); 113.1 *d* (C-2′); 145.4 *s* (C-3′); 149.3 *s* (C-4′); 115.6 *d* (C-5′); 118.5 *d* (C-6′); 59.9 *s* (OMe) and 55.9 *s* (OMe).

Tonalensin. Crystals from EtOAc–hexane, mp 191–193°; UV λ_{max}^{MeOH} nm (log ε): 213 (30924), 272 (3238); $[\alpha]_D$ −33.19 ($CHCl_3$, *c*, 0.241). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750, 1634, 1600, 1503, 1466, 1350, 1313, 1147, 1022, 946, 874. EIMS *m/z* (rel. int.): 340 $[M]^+$ (1); 322 (1); 294 (4); 279 (6); 265 (6); 217 (24); 201 (20); 185 (21); 171 (28); 141 (49); 128 (60); 115 (68); 105 (41); 94 (74); 91 (100); 81 (75); 77 (71); 65 (43); 53 (29); 39 (24).

Tonalenin (**2**). Crystals from Me_2CO–hexane, mp 152–154°; UV λ_{max}^{MeOH} nm (log ε): 207 (10493), 249 (3681); $[\alpha]_D$ −137.35 ($CHCl_3$, *c*, 0.166). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490, 1770, 1745, 1712, 1666, 1593, 1527, 1435, 1365, 1131, 1060, 1041, 1011, 975, 933, 878. FABMS *m/z* (rel. int.): 401 $[M+1]^+$ (50); 341 (50); 323 (15); 225 (22); 99 (77); 91 (33); 83 (51); 55 (49); 43 (100).

7-epi-*Languiduline* (**3**). $SOCl_2$ (4 drops) was added to a soln of **2** (73.4 mg) in pyridine (2 ml) at 0°. After 5 min the reaction mixt. was poured into ice and worked up as usual. The obtained residue was purified by CC (silica gel, hexane–EtOAc, 7:3) to obtain 49.8 mg of **5** as colorless crystals, mp 243–245°; UV λ_{max}^{MeOH} nm (log ε): 203 (24885), 216 sh (17834), 262 (5241). $[\alpha]_D$ −281 ($CHCl_3$, *c*, 0.153). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1779, 1748, 1654, 1584, 1526, 1414, 1376, 1136, 1070, 1042, 1024, 980, 916. EIMS *m/z* (rel. int.): 382 $[M]^+$ (47); 367 (21); 339 (100); 325 (12); 322 (15); 297 (19); 280 (28); 265 (18); 249 (27); 221 (23); 217 (22); 199 (27); 165 (29); 121 (25); 115 (43); 91 (25); 77 (27); 43 (100).

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REFERENCES

1. Maldonado, E., Flores, M. de los A., Salazar, B. and Ortega, A., *Phytochemistry*, 1994, **37**, 1480.
2. Ortega, A., Cárdenas, J., Toscano, R. A., Maldonado, E., Aumelas, A., Van Calsteren, M. R. and Jankowski, C., *Phytochemistry*, 1991, **30**, 3357.
3. Ortega, A. and Maldonado, E., *Phytochemistry*, 1994, **35**, 1063.
4. Maldonado, E., Cárdenas, J., Bojórquez, H., Escamilla, E. and Ortega, A., *Phytochemistry*, 1996, **42**, 1105.
5. Epling, C., *Rep. Spec. Nov. Regni. Veg. Beih*, 1939, **110**, 1.
6. Brieskorn, C. H. and Biechele, W., *Archives of Pharmacology*, 1971, **304**, 557.
7. Morita, N., Shimizu, M. and Arizawa, M., *Phytochemistry*, 1973, **12**, 421.
8. Eguren, L., Fayos, J., Perales, A., Savona, G. and Rodríguez, B., *Phytochemistry*, 1984, **23**, 466.
9. Rodríguez, B., Pascual, C. and Savona, G., *Phytochemistry*, 1984, **23**, 1193.
10. Toscano, R. A., Maldonado, E. and Ortega, A., *Journal of Chemical Crystallography*, 1996, **26**, 239.
11. Ortega, A., Maldonado, E., Toscano, R. A., Díaz, E. and Reynolds, W. F., *Magnetic Resonance Chemistry*, (in press).
12. Rodríguez-Hahn, L., Esquivel, B. and Cárdenas, J., *Progress in Chemistry of Organic Natural Products*, 1994, **63**, 107.

13. Esquivel, B., Ochoa, J., Ćardenas, J., Ramamoorthy, T. P. and Rodríguez-Hahn, L., *Phytochemistry*, 1988, **27**, 483.
14. Sánchez, A. A., Esquivel, B., Ramamoorthy, T. P. and Rodríguez-Hahn, L., *Phytochemistry*, 1995, **38**, 171.
15. Cárdenas, J., Esquivel, B., Rodríguez-Hahn, L., Jankowski, K. and Van Calsteren, M. R., *Magnetic Resonance Chemistry*, 1994, **32**, 321.
16. Cárdenas, J., Esquivel, B., Toscano, R. A. and Rodríguez-Hahn, L., *Heterocycles*, 1988, **27**, 1809.