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ABIETANE DITERPENOIDS FROM *SALVIA PUBESCENS**

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Key Word Index—*Salvia pubescens*; Labiatae; diterpenoids; new abietane derivatives; 3β-hydroxy-demethyl cryptojaponol; 19(4 → 3)*abeo*-*O*-demethyl cryptojaponol.

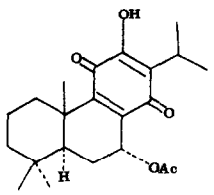
Abstract—From the aerial parts of *Salvia pubescens*, two new abietane diterpenoids were isolated and their structures established by spectroscopic means. The known 7α-acetoxy-royleanone, conacytone, nemorone and desacetylnemorone were also isolated.

INTRODUCTION

Salvia L. constitutes the largest genus of the Labiatae family with 900 species widespread throughout the world [1]. This genus has been divided [2] into four subgenera according to their morphological characteristics: Sclarea, Salvia, Leonia and Calosphace. Most of the American species belong to the Calosphace subgenus which is represented in México by *ca* 300 species [3].

Recently we have undertaken a systematic study of the diterpenoid compounds of Mexican *Salvia* spp, studying different species of the same Section. Almost 80% of spp. studied up to now, contain diterpenoid compounds with a *neo*-clerodane skeleton [**4**–**6** among others]. *Neo*-clerodane diterpenoids have been also found in brasilian *Salvia* spp. (subgenus Calosphace) [7, 8].

Salvia pubescens has been classified [2] in the Section *Erytrostachys* (Subgenus Calosphace); *S. sessei* and *S. regla* (México) and *S. libanensis* (Colombia) are also included in this Section. A phytochemical study of *S. sessei* [M. Jiménez, E. Portugal, A. Lira, private communication] and *S. regla* [9], revealed in these species the presence of abietane quinone diterpenoids. Herein we describe the diterpenoid constituents of the aerial parts of a population of *S. pubescens* collected in Huajuapan de León (Oaxaca, México). In addition to oleanolic acid, the known diterpenoid abietane quinones 7α-acetoxy royleanone (**1**) [10], nemorone (**2**) and desacetyl-nemorone (**3**) [11], and conacytone (**4**) [12], two new abietane diterpenoids were isolated, whose structures were established as 3β-hydroxy-demethyl cryptojaponol (**7**) and 19[4 → 3] *abeo*-*O*-demethyl cryptojaponol (**5**).



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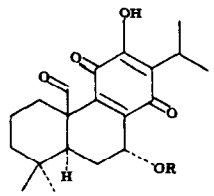

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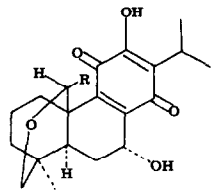
3 R=H



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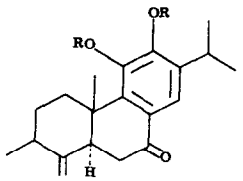

5 R=H
6 R=Ac

RESULTS AND DISCUSSION

The abietane diterpenoid **5** was isolated as an unstable oily product which showed in its IR spectrum phenolic (3600, 3540 cm^{-1}) and arylketone (3016, 1674, 1614, 1565 cm^{-1}) absorptions. Its ^1H NMR spectrum (Table 1) showed a deshielded aromatic proton at δ7.71 which was assigned to H-14, while two broad singlets at δ4.66 and 4.95 were attributed to the exocyclic methylene double bond protons. In the methyl region it showed the signals of an isopropyl group bound to an aromatic ring, of a methyl group attached to a fully substituted carbon atom and a doublet at δ1.15 (3H, J = 7 Hz) which was assigned to the secondary Me-19 bound to C-3 (Table 1).

Acetylation of **5** gave the crystalline diacetate **6**, $C_{24}H_{30}O_5$, which showed in its IR spectrum phenolic acetate (1775 cm^{-1}), aryl ketone (1687, 1606 cm^{-1}) and exocyclic double bond (902 cm^{-1}) absorptions. In its ^1H NMR spectrum (Table 1), the two phenolic acetate

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Table 1. ^{1}H NMR data of compounds **5**–**8** (80 MHz, $CDCl_3$, TMS as int. standard)

H	5	6	7	8
3			3.25 *m*	4.52 *m*
14	7.71 *s*	8.0 *s*	7.60 *s*	8.0 *s*
15	3.25 *spt*	2.90 *spt*	3.00 *spt*	2.91 *spt*
	(6)	(7)	(7)	(7)
Me-16	1.31 *d*	1.20 *d*	1.28 *d*	1.20 *d*
	(6)	(7)	(7)	(7)
Me-17	1.31 *d*	1.20 *d*	1.28 *d*	1.22 *d*
	(6)	(7)	(7)	(7)
Me-18 or	4.95 *br s*	4.88 *br s*	1.05 *s*	1.02 *s*
CH_2-18	4.66 *br s*	4.63 *br s*		
Me-19	1.15 *d*	1.05 *d*	0.94 *s*	0.95 *s*
	(7)	(7)		
Me-20	1.28 *s*	1.1 *s*	1.38 *s*	1.34 *s*
OH	6.45 *br*		5.73 2H	
OAc		2.28 *s* 6H		2.31 *s* 6H
				2.0 *s* 3H

Coupling constants in Hz are in parentheses.

Table 2. ^{13}C NMR data for compounds **6** and **8** (20 MHz, $CDCl_3$, TMS as int. standard)

C	6	8
1	36.4 (*t*)	35.1 (*t*)*
2	32.8 (*t*)	24.1 (*t*)
3	38.2 (*d*)	79.1 (*d*)
4	151.7 (*s*)	38.1 (*s*)
5	48.2 (*d*)	49.2 (*d*)
6	37.8 (*t*)	34.9 (*t*)*
7	196.5 (*s*)	196.8 (*s*)
8	130.8 (*s*)	130.5 (*s*)
9	142.7 (*s*)	143.5 (*s*)
10	41.2 (*s*)	40.0 (*s*)
11	140.4 (*s*)	140.8 (*s*)
12	145.5 (*s*)	145.6 (*s*)
13	140.6 (*s*)	140.3 (*s*)
14	124.2 (*d*)	124.4 (*d*)
15	27.8 (*d*)	27.7 (*d*)
16	22.8 (*q*)	22.6 (*q*)†
17	22.8 (*q*)	22.7 (*q*)†
18	105.9 (*t*)	16.2 (*q*)
19	20.8 (*q*)*	27.7 (*q*)
20	20.3 (*q*)*	21.2 (*q*)
OCOMe	167.9 (*s*)	167.4 (*s*)
	167.4 (*s*)	168.0 (*s*)
		170.5 (*s*)
OCOMe	18.1 (*q*)	20.9 (*q*)
	17.3 (*q*)	20.3 (*q*)
		20.3 (*q*)

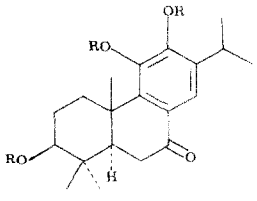
*,† Values on any vertical column may be interchaged.

methyl groups were responsible for a singlet (6H) observed at δ2.28. Catalytic hydrogenation of **6** gave the crystalline dihydroderivative **9**. The β-axial orientation assigned to the secondary methyl group at C-4 was based on steric considerations.

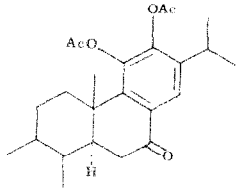
The ^{13}C NMR spectrum of **6** (Table 2) was in complete agreement with the structure and stereochemistry proposed for it. The assignments were made by comparison with those of related structures [13] and calculation of the theoretical chemical shifts expected for the substituted aryl-ketone moiety [14]. The β-equatorial orientation assigned to the secondary methyl group at C-3, was deduced from the spectral data and comparison with data of similar structures [15]. Thus, **5** can be considered as 19[4 → 3]*abeo*-demethyl-*O*-cryptojaponol.

The second new abietane diterpenoid **7**, was isolated as a crystalline product with a molecular formula $C_{20}H_{28}O_4$. Its IR spectrum showed free and hydrogen bonded hydroxyl groups (3544, 3191 *br* cm^{-1}) and aryl ketone (1655, 1589 cm^{-1}) absorptions. Its ^{1}H NMR spectrum (Table 1) showed signals for an isopropyl group attached to an aromatic ring and three methyl groups bound to fully substituted carbon atoms. The aromatic H-14 appeared as a singlet at δ7.60. A multiplet observed at δ3.25 was assigned to the proton geminal to the secondary hydroxyl group at C-3.

Acetylation of **7** gave a triacetate derivative, whose spectral data were in agreement with the structure (**8**) proposed for it. Thus the IR spectrum showed bands for phenolic acetate groups (1775 cm^{-1}) and acetate groups bound to an sp^3 carbon atom (1729 cm^{-1}). Its ^{1}H NMR spectrum (Table 1) showed the phenolic acetate methyl groups as a singlet (6H) at δ2.31, the secondary acetate methyl group was observed at δ2.0 (s, 3H). A multiplet at δ4.52 (1H) was assigned to the secondary acetate geminal proton, H-3. The width ($W_{1/2}$ = 20 Hz) of this signal is only possible if H-3 is axial, hence the acetate group at C-3 must be equatorially oriented. The ^{13}C NMR assignments for **8** (Table 2) were made by comparison with similar structures [13] and calculations of the ^{13}C chemical shifts of the aryl ketone moiety [14]. Therefore, compound **7** can be named as 3β-hydroxy-*O*-demethyl-cryptojaponol. *O*-Demethylcryptojaponol has been isolated [16] from an European *Salvia* spp.



7 R=H
8 R=Ac



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Compound **7** could be considered as a biogenetic precursor of the abietane diterpenoid **5**. This type of biogenetic relationship has been described for compounds isolated from *Coleus* species [15].

The diterpenoids isolated from *S. pubescens* are all abietane derivatives, as are the products previously isolated from *S. sessei* [M. Jiménez *et al.*, unpublished work] and *S. regla* [9]. Mexican *Salvia* species classified [2] in the same Section *Erythrostachys*. As stated earlier, the American species of *Salvia*, subgenus Calosphace, studied up to now [4–8] contain clerodane type diterpenoids. The only other Section of *Salvia*, Subgenus Calosphace, in which abietane and rearranged abietane diterpenoids have been found [12, 17], is *Salvia*, Sect. *Tomentellae*. The

finding of abietane type diterpenoids in *Salvia*, Section *Erytrostachys*, is of phytogeographic interest because European and Asiatic species [*Salvia*, subgenera *Salvia* and *Sclarea*] have yielded similar abietane type diterpenoids [10, 11, 16].

EXPERIMENTAL

Mps: uncorr; MS: direct inlet, 70 eV; ^{1}H NMR and ^{13}C NMR: 80 and 20 MHz, respectively, $CDCl_3$, TMS as internal standard unless otherwise stated. Assignments of ^{13}C NMR chemical shifts were made with the aid of off-resonance and noise decoupling ^{13}C NMR spectra. Plant material was collected at 4 km NE of Huajuapan de León, Oaxaca (México) and a voucher specimen (TPR 4740) is deposited at the Herbarium of the Instituto de Biología, UNAM.

Isolation of diterpenoids from S. pubescens. Dried aerial parts of *S. pubescens* (2910 g) were extracted with Me_2CO (18 l) at room temp. for 6 days. The solvent was removed under red. press. and the gummy residue obtained (166.8 g) was chromatographed over silica gel (1 kg deactivated with 10% H_2O) using, as eluents, petrol–EtOAc mixtures of increasing polarity. Fractions of 500 ml were collected. Elution with petrol–EtOAc (19:1, fractions 22–43) afforded 7α-acetoxyroyleanone **1** (1.51 g, 0.052% dry wt), identical by comparison with literature data [10]. Fractions 44–57, eluted with petrol–EtOAc (9:1), were combined (22.8 g) and rechromatographed over silica-gel (600 g) using petrol–EtOAc mixtures of increasing polarity as eluents. Elution with petrol–EtOAc (9:1) afforded 4.178 g (0.14%, dry wt) of a crystalline mixture, which on flash chromatography (C_6H_6–Me_2CO, 49:1) gave 2.8 g nemorone (**2**) (0.10% dry wt) and 1.1 g deacetylnemorone (**3**) (0.04% dry wt), both compounds were identified by comparison with literature data [11].

Fractions 60–73 from the original column (petrol–EtOAc 9:1) were combined (11.8 g). Repeated 'flash' chromatographies afforded 3.434 g (0.118% dry wt) **5**, as an unstable oily product, which was acetylated (C_5H_5N–Ac_2O) to yield **6**, as a crystalline product, mp 135–136°, from CH_2Cl_2–petrol; $[\alpha]_D^{20}$ 126 ($CHCl_3$, *c* 0.44); UV λ_{max}^{EtOH} nm (ε): 214 (33372), 256 (11542), 296 (1792.8); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775, 1687, 1606, 902; ^{1}H NMR (80 MHz): Table 1; ^{13}C NMR (20 MHz): Table 2; MS *m/z* (rel. int.): 398 $[M]^+$ (15), 356 (5), 316 (19), 315 (17), 314 (100), 299 (17), 43 (28). $C_{24}H_{30}O_5$ requires $[M]^+$ at *m/z* 398.

From the first 10 fractions eluted with petrol–EtOAc (4:1), 11.4 g oleanolic acid were isolated which was identified as its methyl ester derivative by comparison with an authentic sample. Further elution with petrol–EtOAc (4:1) of the original column gave, 757.3 mg (0.025%, dry wt) of a yellow crystalline product which was identified as conacytone (**4**) by comparison with an authentic sample [12].

Elution with petrol–EtOAc (1:4) afforded after extensive chromatographic purification, 500 mg **7**, as a crystalline product, mp 233–235°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3544, 3191, 1655, 1588; ^{1}H NMR (80 MHz): Table 1; MS *m/z* (rel. int.): 333 (31), 332 (100), 317 (32), 300 (18), 299 (62), 273 (18), 257 (18), 245 (15), 232 (22), 231 (67), 219 (15), 205 (12), 203 (11), 189 (12), 179 (20), 177 (14), 115 (15), 91 (15), 77 (13), 69 (10), 57 (13), 55 (13), 43 (28), 41 (17). $C_{20}H_{28}O_4$ requires $[M]^+$ at *m/z* 332.

Acetylation of **7**. A soln of **7** (126.1 mg) in C_5H_5N (1 ml) was treated with Ac_2O (1 ml) for 1 hr at room temp. Usual work-up of the reaction mixture afforded 96.7 mg **8**, Mp 230–232°, from petrol–Me_2CO; $[\alpha]_D^{20}$ 70 ($CHCl_3$, *c* 0.21); UV λ_{max}^{EtOH} nm (ε): 218 (20700), 262 (7068), 290 (9163); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775, 1729, 1686, 1605; ^{1}H NMR (80 MHz): Table 1; ^{13}C NMR (20 MHz): Table 2; MS *m/z* (rel. int.): 460 (1), 459 (5), 458 (18), 416 (25), 383 (19), 376 (17), 375 (88), 374 (100), 359 (10), 356 (7), 342 (7), 341 (30), 315 (11), 314 (37), 300 (7), 299 (33), 270 (10), 119 (6), 43 (35). $C_{26}H_{34}O_7$ requires $[M]^+$ at *m/z* 458.

Hydrogenaton of **6**. Compound **6** (26 mg) in EtOAc (5 ml) was hydrogenated using Pd/C (5%, 10 mg) as catalyst for 17 hr. After usual work-up 20 mg **9** were isolated. Mp: 179–180°; $[\alpha]_D^{20}$ 41 ($CHCl_3$, *c* 0.17); UV λ_{max}^{EtOH} nm (ε): 216 nm (21719), 258 (8565); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1772, 1686, 1602; ^{1}H NMR (80 MHz) δ: 7.95 (1H, *s*, H-14), 2.83 (1H, *sept*, H-15), 2.31 (6H, *s*, 2 OCOMe), 1.18–1.28 (15H, Me × 5); MS *m/z* (rel. int.): 400 (20), 398 (20), 383 (10), 356 (12), 341 (16), 317 (30), 316 (300), 315 (18), 314 (44), 301 (34), 300 (65), 232 (23), 231 (15), 43 (63). $C_{24}H_{32}O_5$ requires $[M]^+$ at *m/z* 400.

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