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FURTHER *ENT*-CLERODANE DITERPENOIDS FROM *SALVIA MELISSODORA*

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Key Word Index—*Salvia melissodora*; Labiatae; *ent*-clerodanes; diterpenoids.

Abstract—From the aerial parts of *Salvia melissodora*, ten *ent*-clerodane diterpenoids were isolated. One of the compounds had been isolated previously from another population of the same plant. However, seven of the compounds constitute new natural products. The structures of these compounds were established by spectroscopic and chemical means. Oleanolic acid and sitosterol were also found in this species.

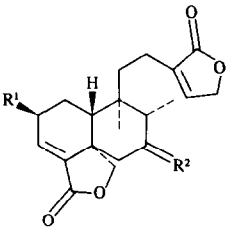
INTRODUCTION

Salvia melissodora Lag. (*Salvia*, Section *Scorodonia* of sub-genus Calosphace) is a perennial shrub, endemic to Mexico [1]. Previous studies on this plant [2, 3], indicate that it is a rich natural source of *ent*-clerodane diterpenoids. As a continuation of our systematic study of this species for the presence of new *ent*-clerodanes with potential antifeeding activity [3], we have studied a population of *S. melissodora* from the state of San Luis Potosí (México). In addition to sitosterol and oleanolic acid, ten *ent*-clerodane diterpenoids (**1**–**7** and **10**–**12**) were isolated. Compound **3** was previously isolated [3] from a population of the same species collected in the state of Hidalgo (México). The other nine compounds constitute new natural products, whose structures were established on the basis of spectroscopic data and chemical correlation.

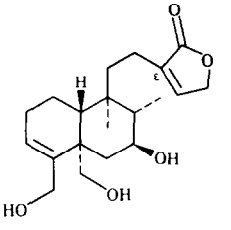
RESULTS AND DISCUSSION

Compound **1**, $C_{22}H_{28}O_7$ (MS and elemental analysis), was the major component isolated. Its IR spectrum exhibited characteristic absorptions for α,β-unsaturated-γ-lactone functions (1772 cm^{-1}), hydroxyl groups (3610, 3483 cm^{-1}), ester carbonyl (1741 cm^{-1}) and double bonds (1656, 1602 cm^{-1}). The UV spectrum [λ_{max}^{MeOH} nm (ε): 202 (22 143)] supports these assignments.

The ^1H NMR spectrum of **1** (Table 1) showed a doublet at δ6.7 ($J=6$ Hz) which was ascribed to the olefinic β-proton of an α,β-unsaturated γ-lactone group coupled to a methine moiety, it was assigned to H-3. An AB system at δ4.8 and 3.9 ($J=8$ Hz) was attributed to the C-19 methylene group. The *pro-S* diastereotopic proton of this group (δ3.9) is also ω-coupled ($^4J=2$ Hz) with the C-6β proton, indicating an axial orientation for C-19 and the absence of a C-6β substituent [4–7].



	R^1	R^2
1	OH	αOAc, βH
2	H	βOH, αH
3	H	βH, αOH
4	H	βH, αOAc
5	OH	βH, αOH
6	OAc	βH, αOH
7	H	O
8	OAc	H_2
9	OAc	O
10	OH	H_2
11	OH	O



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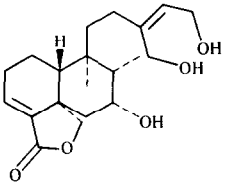

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The chemical shift of the *pro*-19*R* proton can be used as a diagnostic signal, since it is deeply influenced by the substitution pattern at C-7. For example, an average chemical shift of δ5.30 is observed for this proton when an α-axial hydroxyl group is present at C-7 [8–10]. On the other hand, an average of δ4.85 [11] is expected for an α-axial acetate group bound to C-7. A change in hybridization at C-7 also influences the chemical shift of the *pro*-19*R* proton, so an average of δ4.0 is observed [3, 8] in 7-oxo-derivatives. In the absence of the above mentioned factors, an average of δ4.35 is expected for the *pro*-19*R* proton.

The chemical shift observed for the *pro*-19*R* (δ4.8) proton in the ^1H NMR spectrum of **1**, indicated the

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Table 1. ^{1}H NMR (80 MHz) data for compounds

H	1	2	3	4	5
2	4.65 *dt* (6, 2)				4.60 *dt* (7, 2)
3	6.70 *d* (6)	6.80 *dd* (7, 3.5)	6.70 *dd* (7, 3)	6.70 *dd* (7, 2)	6.7 *d* (7)
7	5.35 *m* $W_{1/2}=8$	3.65 *td* (11, 5)	4.1 *m* $W_{1/2}=6$	5.30 *m* $W_{1/2}=8$	4.1 *m* $W_{1/2}=6$
14	7.2 *br s*	7.10 *br s*	7.1 *br s*	7.1 *br s*	7.15 *br s*
15 (2H)	4.8 *br d* (2)	4.8 *br d* (2)	4.75 *br d* (2)	4.75 *br d* (2)	4.8 *br d* (2)
19 *pro S*	3.90 *dd* (8, 2)	3.90 *dd* (8, 2.5)	3.85 *dd* (8, 2)	3.90 *dd* (8, 2)	3.85 *dd* (8, 2)
19 *pro R*	4.8 *d* (8)	4.25 *d* (8)	5.25 *d* (8)	4.80 *d* (8)	5.25 *d* (8)
17 (3H)	0.95 *d* (7)	1.1 *d* (8)	1.05 *d* (7)	0.95 *d* (7)	1.1 *d* (8)
20 (3H)	0.80 *s*	0.70 *s*	0.85 *s*	0.85 *s*	0.85 *s*
MeCOO	2.1 *s*		—	2.1 *s*	—
18 (2H)	—	—	—	—	—

[a]These signals could be interchanged.

Table 2. ^{13}C NMR data for compounds **1**, **2**, **5**, **7** and **12** (20 MHz)

C	1*	2†	5†	7*	12‡
1	27.4 (*t*)	18.9 (*t*)	27.0 (*t*)	20.3 (*t*)	17.6 (*t*)
2	63.8 (*d*)	27.0 (*t*)	62.5 (*d*)	27.4 (*t*)	26.2 (*t*)
3	132.4 (*d*)	135.8 (*d*)	132.3 (*d*)	137.3 (*d*)	128.4 (*d*)
4	141.8 (*s*)	137.7 (*s*)	141.1 (*s*)	136.2 (*s*)	144.9 (*s*)
5	44.8 (*s*)	45.1 (*s*)	44.60 (*s*)	43.8 (*s*)	43.9 (*s*)
6	37.5 (*t*)	42.9 (*t*)	39.8 (*t*)	50.7 (*t*)	40.3 (*t*)
7	73.7 (*d*)	68.0 (*d*)	70.8 (*d*)	209.2 (*s*)	68.5 (*d*)
8	39.5 (*d*)[a]	44.1 (*d*)	39.15 (*d*)[a]	51.5 (*d*)	44.0 (*d*)
9	37.7 (*s*)	38.7 (*s*)	37.16 (*s*)	48.1 (*s*)	39.8 (*s*)
10	39.2 (*d*)[a]	47.1 (*d*)	39.9 (*d*)[a]	47.9 (*d*)	46.0 (*d*)
11	36.6 (*t*)	35.4 (*t*)	35.96 (*t*)	35.8 (*t*)	36.4 (*t*)
12	18.5 (*t*)	18.4 (*t*)	18.02 (*t*)	19.5 (*t*)	19.3 (*t*)
13	134.0 (*s*)	132.1 (*s*)	132.7 (*s*)	133.4 (*s*)	134.4 (*s*)
14	145.4 (*d*)	146.8 (*d*)	146.1 (*d*)	144.8 (*d*)	144.2 (*d*)
15	70.6 (*t*)	70.4 (*t*)	70.3 (*t*)	70.3 (*t*)	70.3 (*t*)
16	175.1 (*s*)	174.0 (*s*)	174.0 (*s*)	174.0 (*s*)	174.3 (*s*)
17	11.3 (*q*)	10.8 (*q*)	12.06 (*q*)	7.7 (*q*)	10.9 (*q*)
18	169.1 (*s*)	168.5 (*s*)	169.3 (*s*)	167.8 (*s*)	63.9 (*t*)
19	71.3 (*t*)	71.8 (*t*)	71.2 (*t*)	71.2 (*t*)	65.6 (*t*)
20	18.9 (*q*)	18.4 (*q*)	19.0 (*q*)	18.9 (*q*)	19.9 (*q*)
21	169.9 (*s*)	—	—	—	—
22	21.2 (*q*)	—	—	—	—

*Run in $CDCl_3$.
†DMSO-d_6.
‡$CDCl_3$ + DMSO-d_6.
SFORD multiplicities are in parenthesis.
[a]Values in any vertical column could be exchangeable.

1–12 ($CDCl_3$, TMS as int. standard)

6	7	8	9	12
5.35 *m*		5.35 *dt*	5.4 *dt*	
		(7, 3)	(7, 3)	
6.7 *d*	6.85 *dd*	6.70 *d*	6.85 *d*	5.70 *br t*
(7)	(7, 3)	(7)	(7)	(4)
4.15 *m*	—		—	3.50 *m*
$W_{1/2}=8$				
7.05 *t*	7.15 *br s*	7.05 *t*	7.1 *t*	7.05 *br s*
(2)		(2)	(2)	
4.75 *br d*	4.80 *br d*	4.75 *br d*	4.75 *br d*	4.75 *br d*
(2)	(2)	(2)	(2)	(2)
3.85 *dd*	3.85 *dd*	3.90 *dd*	3.85 *dd*	3.95 *d*[a]
(8, 2)	(7, 2)	(8, 1)	(8, 2)	(6)
5.30 *d*	4.00 *d*	4.30 *d*	4.05 *d*	3.85 *d*[a]
(8)	(7)	(8)	(8)	(6)
1.05 *d*	1.05 *d*	0.90 *d*	1.05 *d*	1.00 *d*
(7)	(6)	(6)	(7)	(6)
0.9 *s*	0.65 *s*	0.65 *s*	0.65 *s*	0.80 *s*
2.10 *s*	—	2.10 *s*	2.1 *s*	—
—	—	—	—	3.9 AB
				(12)

presence of an α-axial acetate group at C-7, whose geminal proton was responsible for a multiplet at δ5.35 ($W_{1/2}=8$ Hz). A singlet at δ2.1 was attributed to the methyl group of this ester.

A three-proton singlet at δ0.8 was assigned to the C-20 methyl group and a three-proton doublet at δ0.95 ($J=7$ Hz) to the C-17 methyl group. These data indicated that **1** possesses a B ring identical to the B ring present in Kerlinolide, an *ent*-clerodane diterpenoid isolated from *Salvia keerlii* Benth [11].

The ^{1}H NMR spectrum of **1** also exhibited a double triplet ($J=6$, 2 Hz) at δ4.65, which was assigned to a geminal proton of an allylic hydroxyl group [5, 7]. The coupling constants of this signal and its chemical shift, led us to locate the hydroxyl group at C-2 with a β-axial orientation. The A ring of **1** is therefore identical to that present in articulin from *Baccharis articulata* [7] and semiatrin from *S. semiathrata* Zucc. [5]. The β-axial orientation for the hydroxyl group is supported by the chemical shift of C-10 in the ^{13}C NMR spectrum of **1** (Table 2).

Other relevant signals in the ^{1}H NMR spectrum of **1** are those due to an α-substituted butenolide group. A broad singlet at δ7.2 was assigned to H-14 and a two-proton broad doublet at δ4.8 ($J=2$ Hz) to the C-15 methylene group. The presence of an α-substituted butenolide is a common feature in all the diterpenoids isolated from this population of *S. melissodora* (Table 1).

The ^{13}C NMR spectrum (Table 2) of **1** supports the structure proposed for it. The chemical shift of the 17 and 20 methyl groups indicated an A/B *trans* ring fusion by comparison with related compounds [8, 11]. The A/B *trans* ring fusion is present in all the diterpenoids isolated until now from *S. melissodora* [2, 3].

In agreement with the previous discussion compound **1** must be named 7α-acetoxy-2β-hydroxy-*ent*-clerodan-3,13-dien-18,19:16,15-diolide.

Compound **2**, $C_{20}H_{26}O_5$ (MS), had a similar IR spectrum to **1**, except for the absence of ester carbonyl bands.

The ^{1}H NMR spectrum of **2** confirms the presence of an α-substituted butenolide group and an α,β-unsaturated γ-lactone group (Table 1). A double doublet at δ6.8 ($J=7$, 3.5 Hz) was attributed to H-3. The multiplicity of this signal indicated the absence of a substituent at C-2. A one-proton triple doublet at δ3.65 ($J=11$ and 5 Hz) was ascribed to the geminal proton of an hydroxyl group. The J values of this signal indicated an equatorial orientation for the hydroxyl group. The chemical shift observed for C-17 (δ10.8 *q*) in the ^{13}C NMR spectrum of **2** (Table 2) allowed us to place the hydroxy group at C-7. The equatorial orientation of this hydroxy group, explains the lack of deshielding effect on *pro*-19*R* proton, which is observed at δ4.25. In agreement with the previous discussion, compound **2** must be named 7β-hydroxy-*ent*-clerodan-3,13-dien-18,19:16,15-diolide.

Compound **3** was identified by spectroscopic data as the 7α-hydroxy-*ent*-clerodan-3,13-dien-18,19:16,15-diolide, which was previously isolated from another population of *S. melissodora* [3]. The identity of this compound was confirmed by comparison with an authentic sample.

The structure proposed for **4**, 7α-acetoxy-*ent*-clerodan-3,13-dien-18,19:16,15-diolide, was deduced from its spectral data. Its ^{1}H NMR spectrum (Table 1) is almost identical to that of **3**. The presence of a three proton singlet at δ2.1 and a multiplet with $W_{1/2}=8$ Hz at δ5.30, as well as the chemical shift observed for the *pro*-19*R* proton (δ4.8, $J=8$ Hz) indicated the presence of an acetate group α-axially oriented at C-7. Attempts to acetylate the 7α-hydroxy group in product **3** by standard procedures were unsuccessful due to the steric hindrance exerted by the α-axial C-20 methyl and the C-19 methylene groups.

The mass spectrum of **5** is consistent with the molecular formula, $C_{20}H_{26}O_6$. Its IR spectrum exhibited hydroxyl bands (3688 and 3500 cm^{-1}) and α,β-unsaturated γ-lactone absorption (1751 cm^{-1}). The ^{1}H NMR spectrum of **5** (Table 1) shows a doublet at δ6.7 ($J=7$ Hz) assigned

to H-3, therefore one of the hydroxy groups must be placed at C-2; its geminal proton is responsible for a double triplet ($J=7$ and 2 Hz) at δ4.6 as found in compound **1**. The second hydroxy group is a α-axially bound to C-7 exerting a deshielding effect the *pro*-19*R* proton which is observed as a doublet ($J=8$ Hz) at δ5.25. Compound **5** was thus identified as 2β,7α-dihydroxy-*ent*-clerodan-3,13-dien-18,19:16,15-diolide.

Compound **6** has the molecular formula, $C_{22}H_{28}O_7$ (MS and elemental analysis), and is an isomer of **1**. The IR spectrum of **6** indicated the presence of the same functional groups as in **1**. The ^{1}H NMR spectrum of **6** is very similar to that of **1** except for the chemical shift of H-2 (*m*, δ5.35), H-7 (*m* at δ4.15, $W_{1/2}=8$ Hz) and the *pro*-19*R* proton (*d*, δ5.3, $J=8$ Hz). These facts led to the location of an α-axially oriented hydroxy group at C-7 and a β-axial acetate group at C-2. In accordance with the previous discussion, **6** must be named 2β-acetoxy-7α-hydroxy-*ent*-clerodan-3,13-dien-18,19:16,15-diolide.

Treatment of **5** with acetic anhydride in pyridine in mild conditions, afforded **6**.

The IR spectrum of **7** showed the characteristic absorptions due to α,β-unsaturated-γ-lactone functions (1757 cm^{-1}), a cyclohexanone carbonyl group (1710 cm^{-1}), double bonds (1664 cm^{-1}) and the absence of hydroxy groups. The ^{13}C NMR spectrum (Table 2) was decisive in assigning the structure **7** to this product. A singlet at δ209.2 was attributed to C-7 by comparison with literature data [12] and biogenetic considerations. This assignment was supported by the chemical shifts observed for C-6 (δ50.7, *t*), C-8 (δ51.5, *d*) and C-9 (δ48.1, *s*), C-17 was found at δ7.7 (*q*). The chemical shifts of the rest of the signals in the ^{13}C NMR spectrum of **7**, are in agreement with the structure and relative stereochemistry proposed for this product. Oxidation of **2** and **3** with Jones reagent afforded a product identical to **7**, which must be therefore, 7-oxo-*ent*-clerodan-3,13-dien-18,19:16,15-diolide.

Some fractions of medium and high polarity (see Experimental) of the crude extract were acetylated in the usual manner in order to facilitate the separation of their components. In this way we were able to obtain products **8** and **9** as crystalline substances after extensive chromatographic purification. Since there was no evidence of the presence of acetate groups in the original mixture (IR and ^{1}H NMR spectra), we may assume that compounds **10** and **11** are present in the plant as natural products.

The acetate **8** possesses a molecular formula, $C_{22}H_{28}O_6$ (MS and elemental analysis). Its ^{1}H NMR spectrum (Table 1) indicated the presence of an α-substituted butenolide and an α,β-unsaturated γ-lactone bound to ring A. A double triplet at δ5.35 ($J=7$ and 3 Hz) was assigned to the geminal proton of an allylic acetate group β-axially oriented, i.e. H-2, by comparison with the signals of **6**. The chemical shift of the C-19 methylene protons (δ3.9, *dd*, $J=8$, 1 Hz and δ4.30, *d*, $J=8$ Hz) indicated the absence of a substituent at C-7. A three protons singlet at δ0.65 and a three protons doublet at δ0.9 ($J=7$ Hz) were asssigned to the C-20 and C-17 methyl groups. These data indicate an α-orientation for both methyl groups consistent with an A/B *trans* rings fusion in **8**, which must be therefore named 2β-acetoxy-*ent*-clerodane-3,13-dien-18,19:16,15-diolide. The related natural product **10** was identified as the 2β-hydroxy-derivative of **8**.

The structure proposed for compound **9** was easily deduced by comparison with the structures already discussed (*vide supra*). The mass spectrum of **9** is consistent with the molecular formula, $C_{22}H_{26}O_7$. Its IR spectrum indicated the presence of cyclohexanone, ester carbonyl and α,β-unsaturated γ-lactone groups (1713, 1757 and 1773 cm^{-1}). The ^{1}H NMR spectrum of **9** showed, in addition to the signals due to the α-substituted butenolide and the α,β-unsaturated 18:19 olide (Table 1), a singlet at δ2.1 ascribed to the methyl group of an acetate ester, whose geminal proton was responsible for a double triplet at δ5.4 ($J=7$ and 3 Hz). The chemical shift found for this proton indicated its allylic nature, therefore the acetate group must be attached to C-2 with a β-axial orientation (J values). The cyclohexanone carbonyl was ascribed to C-7 based on the chemical shift observed for the *pro*-19*R* proton (Table 1). Compound **9** possesses, therefore, identical A and B rings as those present in 2β-acetoxy bacchasmacranone, an *ent*-clerodane isolated from *Baccharis macraei* [12]. This comparison supports the structure proposed for **9**, which must be named 2β-acetoxy-7-oxo-*ent*-clerodane-3,13-dien-18,19:16,15-diolide. Its parent compound **11** is, therefore, the 2β-hydroxy derivative.

Further purification of the ethyl acetate fractions from the chromatography of the crude extract, yielded compound **12**, which analysed for a $C_{20}H_{30}O_5$. Its IR spectrum showed characteristic absorptions for hydroxy groups (3600 and 3300 cm^{-1}), an α,β-unsaturated γ-lactone group (1754 cm^{-1}) and double bonds (1653 cm^{-1}). The ^{1}H NMR spectrum of **12** (Table 1) indicated the presence of an α-substituted butenolide and the absence of the characteristic signals of an α,β-unsaturated γ-lactone bound to ring A, common to the clerodanes of *Salvia* species [3–5, 11]. Instead, the signals for two hydroxymethylene groups were observed at δ 3.85 which could be assigned to the C-18 and C-19 methylene protons. A broad triplet at δ5.7 ($J=4$ Hz) was attributed to H-3 and a one-proton multiplet at δ3.5 was ascribed to the geminal proton of an hydroxy group, which was located at C-7 based on the chemical shift observed for C-17 (δ10.9 *q*) in the ^{13}C NMR spectrum of **12** (Table 2). Oxidation of **12** with manganese dioxide in methylene chloride, afforded a product identical to **2**. Comparison of the ^{13}C NMR spectrum of **2** with those reported for related substances, support the A/B *trans* ring fusion proposed for it. Product **12** is therefore, 7β,18,19-trihydroxy-*ent*-clerodan-3,13-dien-16,15-olide and can be considered a biogenetic precursor of **2**.

It is interesting to note that the presence of an α-substituted butenolide group is a rare feature in the clerodanes isolated from *Salvia* species, the β-substituted butenolide group being frequently found. In Mexican *Salvias* the presence of *ent*-clerodanes with an α-substituted butenolide is at present restricted to *S. melissodora* [3] and *S. breviflora* [13], both species belong to *Salvia*, section *Scorodonia* of subgenus *Calosphace*. From a population of *S. melissodora* collected in the State of Mexico (Mexico) portulide C (**13**) was isolated [3]. It could be considered a common precursor of α and β-substituted butenolides.

The variety and abundance (see Experimental) of the *ent*-clerodane diterpenoids found in *S. melissodora*, is of interest since these type of compounds frequently exhibit antifeedant properties [14]. Bioassays to evaluate the antifeedant properties of compounds **1**–**10**, are now in progress.

EXPERIMENTAL

Mp uncorr. For general details on methods see ref [4]. Plant material was collected in August 1986 in the state of San Luis Potosí, México, and a voucher specimen (TPR 4859) is deposited at the Herbarium of the Instituto de Biología, UNAM.

Isolation of components: Dried aerial parts of *Salvia melissodora* Lag. (2.89 kg) were extracted twice (2 × 20 l) with Me_2CO at room temp. for one week. The solvent was removed under red. pres. to yield 218.3 g of a gummy residue, which was chromatographed over silica gel (1400 g, deactivated with 10% H_2O). Mixtures of petrol–EtOAc and EtOAc–MeOH of increasing polarity were used as eluents.

Elution with petrol–EtOAc (9:1) afforded sitosterol (490 mg), mp 135–137°, identified by comparison with an authentic sample. The fractions eluted with petrol–EtOAc (4:1) gave a triterpenoid acid (690 mg) identified as oleanolic acid by comparison of its methyl ester derivative (ethereal CH_2N_2) with an authentic sample (mp, mmp, IR and ^{1}H NMR spectra).

Fractions eluted with petrol–EtOAc (3:2) were divided into two groups (RLC). From the first one 9.6 g (0.331% dry wt) of compound **1** were obtained. The mother liquors of **1** (17.4 g), were chromatographed over 500 g of silica gel, to yield 128 mg (0.004% dry wt) of compound **2**, and 4.3 g (0.159% dry wt) of substance **5**. From the second group of fractions, after extensive chromatographic purifications, compounds **4** (294 mg, 0.0101% dry wt), **3** (320 mg, 0.011 dry wt), **6** (297 mg, 0.0103% dry wt) and **7** (48 mg, 0.0023% dry wt) were isolated as crystalline products. In this process, an oily mixture was also obtained (655 mg). The IR spectrum of this mixture indicated the presence of hydroxy groups (3493 and 3610 cm^{-1}) and the ^{1}H NMR spectrum showed the absence of acetate groups. On this basis, the mixture was acetylated (Ac_2O–C_5H_5N), affording compound **8** (346 mg) as a crystalline product.

Several fractions eluted with EtOAc, were combined (25 g) (TLC) and chromatographed over 400 g of silica gel, to yield 154 mg (0.0053% dry wt) of compound **12** and 5.5 g of an oily mixture from which, compound **10** (4.3 g) was isolated after acetylation.

7α-Acetoxy-2β-hydroxy-ent-*clerodan*-3,13-*dien*-18,19:16,15-*diolide* (**1**). Mp 236–239° (Me_2CO–hexane); $[\alpha]_D^{25} = -123.9$ ($CHCl_3$, *c* 0.184); UV λ^{MeOH} nm (ε): 202 (22 143); IR ν^{CHCl_3} cm^{-1}: 3610, 3483, 1772, 1741, 1656, 1602, 1246, 1036, 830; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; MS *m/z* (rel. int.): 406 (0.1), 405 (0.7), 404 $[M]^+$ (3), 386 (0.86), 344 (7), 199 (20), 193 (18), 165 (32), 111 (8), 105 (12), 97 (7), 55 (22), 43 (100), 41 (26). $C_{22}H_{28}O_7$ requires C, 65.01; H, 6.98. Found: C, 65.33; H, 6.97.

7β-Hydroxy-ent-*clerodan*-3,13-*dien*-18,19:16,15-*diolide* (**2**). Mp 196–197° (EtOAc–MeOH); $[\alpha]_D^{20} = -93.28$ (MeOH; *c* 0.149); UV λ^{MeOH} nm (ε): 204 (19 214); IR ν^{KBr} cm^{-1}: 3572, 3499–3429, 1746, 1653, 1206, 1029; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; CIMS *m/z* (rel. int.): 348 (7), 347 (21), 329 (25), 328 (3), 311 (100), 310 (8), 284 (16), 283 (65), 273 (4), 205 (9), 153 (13), 139 (9). $C_{20}H_{26}O_5$ requires $[M+1]^+$ at *m/z* 347.

7α-Hydroxy-ent-*clerodan*-3,13-*dien*-18,19:16,15-diolide (**3**). Mp 178–179° (Me_2CO–*iso*-propyl ether); $[\alpha]_D^{20} = -154.54$ ($CHCl_3$; *c* 0.165); UV λ^{MeOH} nm (ε): 205 (19 540); IR ν^{CHCl_3} cm^{-1}: 3626, 3493, 3043, 3005, 1757, 1660, 1190, 1077; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; MS *m/z* (rel. int.): 346 (1.1), 328 (3.7), 316 (20), 298 (19), 217 (11), 205 (100), 159 (35), 111 (8), 97 (6), 91 (50), 83 (10), 41 (26). $C_{20}H_{26}O_5$ requires C, 69.34; H, 7.70. Found: C, 68.86; H, 7.57.

7α-Acetoxy-ent-*clerodan*-3,13-*dien*-18,19:16,15-*diolide* (**4**). Mp 178–179° (CH_2Cl_2–*iso*-propyl ether); $[\alpha]_D^{20} = -110.65$ ($CHCl_3$; *c* 0.169); UV λ^{MeOH} nm (ε): 205 (21 719); IR ν^{CHCl_3} cm^{-1}: 1757, 1662, 1242, 1187; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; CIMS *m/z* (rel. int.): 390 (1), 389 (8), 330 (5), 329 (23), 328 (3), 312 (20), 311 (100), 298 (4), 293 (4), 284 (10), 153 (18). $C_{22}H_{28}O_6$ requires C, 68.02; H, 7.27. Found: C, 68.02; H, 7.55.

2β,7α-Dihydroxy-ent-*clerodan*-3,13-*dien*-18,19:16,15-*diolide* (**5**). MP 190–192° (Me_2CO–hexane); $[\alpha]_D^{20} = -177.56$ (MeOH; *c* 0.205); UV λ^{MeOH} nm (ε): 205 (20 634); IR ν^{nujol} cm^{-1}: 3488, 1753, 1724, 1663, 1092; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; MS *m/z* (rel. int.): 362 (2.8), 345 (6.6), 344 (19), 326 (5), 313 (39), 223 (28), 185 (42), 165 (34), 140 (60), 119 (45), 111 (32), 105 (59), 97 (32), 55 (71), 44 (94), 43 (100). $C_{20}H_{26}O_6$ requires $[M]^+$ at *m/z* 362.

2β-Acetoxy-7α-hydroxy-ent-*clerodan*-3,13-*dien*-18,19:16,15-*diolide* (**6**). Mp 197–199° (CH_2Cl_2-iso-propyl ether); $[\alpha]_D^{20} = -207$ ($CHCl_3$; *c* 0.167); UV λ^{MeOH} nm (ε): 204 (20 160); IR ν^{CHCl_3} cm^{-1}: 3626, 3497, 3043, 3005, 1756, 1657, 1238, 1047, 830; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; MS *m/z* (rel. int.): 405 (3), 404 (9), 387 (9), 362 (19), 344 (32), 313 (23), 199 (48), 157 (39), 111 (12), 97 (15), 93 (22), 91 (69), 83 (13), 43 (100), 41 (23). $C_{22}H_{28}O_7$ requires C, 65.33; H, 6.98. Found: C, 65.31; H, 7.07.

7-Keto-ent-*clerodan*-3,13-*dien*-18,19:16,15-*diolide* (**7**). Mp 89–93° (EtOAc); $[\alpha]_D^{20} = -168.42$ ($CHCl_3$; *c* 0.152); UV λ^{MeOH} nm (ε): 204 (18 479); IR ν^{CHCl_3} cm^{-1}: 3014, 1757, 1710, 1664, 1252, 1073, 830; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; MS *m/z* (rel. int.): 345 (1), 344 (4.2), 255 (12), 233 (80), 203 (51), 175 (58), 135 (25), 119 (23), 111 (9), 105 (33), 97 (8), 91 (100), 83 (10), 77 (51), 41 (61), 39 (32). $C_{20}H_{24}O_5$ requires $[M]^+$ at *m/z* 344.

2β-Acetoxy-ent-*clerodan*-3,13-*dien*-18,19:16,15-diolide (**8**). Mp 202–204° (CH_2Cl_2–MeOH); $[\alpha]_D^{20} = -209.49$; ($CHCl_3$, *c* 0.158); UV λ^{MeOH} nm (ε): 203 (19 929); IR ν^{CHCl_3} cm^{-1}: 1756, 1655, 1602, 1075, 1053, 830; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; MS *m/z* (rel. int.): 389 (1.5), 388 (5.5), 346 (12), 328 (18), 297 (18), 159 (69), 111 (8), 105 (30), 98 (18), 97 (8), 91 (56), 83 (11), 43 (100), 41 (33). $C_{22}H_{28}O_6$ requires C, 68.02; H, 7.27. Found: C, 68.09; H, 7.37.

7β-18,19-Trihydroxy-ent-*clerodan*-3,13-*dien*-16,15-*diolide* (**12**). Mp 149–150° (Me_2CO–*iso*-propyl ether); $[\alpha]_D^{20} = -53.75$ ($CHCl_3$; *c* 0.16); UV λ^{MeOH} nm (ε): 201.5 (13 000); IR ν^{CHCl_3} cm^{-1}: 3613, 3360, 3280, 3068, 3039, 1754, 1653, 1075, 1049, 830; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; MS *m/z* (rel. int.): 301 (1.4), 299 (0.4), 285 (1.2), 284 (5), 283 (8), 185 (13), 173 (100), 171 (19), 111 (15), 107 (18), 105 (35), 97 (8), 91 (32), 79 (20), 55 (20), 43 (18), 41 (32). $C_{20}H_{30}O_5$ requires C, 68.54; H, 8.63. Found: C, 68.51; H, 8.63.

2β-Acetoxy-7-keto-ent-*clerodan*-3,13-*dien*-18,19:16,15-*diolide* (**9**). Mp 238–240° (CH_2Cl_2–hexane); $[\alpha]_D^{20} = -239.76$ ($CHCl_3$; *c* 0.171); UV λ^{MeOH} nm (ε): 204 (17 815); IR ν^{CHCl_3} cm^{-1}: 3035, 1779, 1757, 1713, 1657, 1235, 830; ^{1}H NMR see Table 1; MS *m/z* (rel. int.): 402 (1.4), 384 (2), 360 (1.1), 342 (0.9), 253 (17), 145 (11), 105 (16), 97 (7), 91 (38), 93 (9), 43 (100), 41 (22). $C_{22}H_{26}O_7$ requires $[M]^+$ at *m/z* 402.

Acetylation of **5**. Compound **5** (150 mg) in C_5H_5N (1 ml) was treated with Ac_2O (1.5 ml) for 55 min at room temp. After the usual work-up, 156 mg of **6** were obtained.

Treatment of **3** *with Jones reagent*. Compound **3** (150 mg) in Me_2CO (5 ml) at 0° (ice bath), was treated with Jones reagent. After the usual work-up, compound **7** was obtained.

Oxidation of **2** *with Jones reagent*. Compound **2** (88 mg) in Me_2CO (6 ml) was treated with Jones reagent at 0°. After the usual work-up, a crystalline product was obtained which was identical to compound **7** and to the product obtained on treatment of **3** with Jones reagent (see above).

Oxidation of **12** *with* MnO_2. Compound **12** (50 mg) in CH_2Cl_2 (7 ml) was stirred for 54 hr at room temp. in the presence of 500 mg of active MnO_2. The mixture was filtered through celite and the crude product obtained (28 mg) was purified by flash

chromatography (Me_2CO–CH_2Cl_2, 1:4), to yield, in low yield, a product identical to compound **2** (mp, TLC, IR).

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REFERENCES

1. Epling, C. (1939) *Report. Spec. Nov. Regni. Veg.* **110**, 1.
2. Rodríguez-Hahn, L., Martínez, G. and Romo J. (1973) *Rev. Latinoam. Quím.* **4**, 93.
3. Esquivel, B., Vallejo, A., Gaviño, R., Cárdenas, J., Sánchez, A. A., Ramamoorthy, T. P. and Rodríguez-Hahn, L. (1988) *Phytochemistry* **27** (in press).
4. Esquivel, B., Cárdenas, J., Ramamoorthy, T. P. and Rodríguez-Hahn, L. (1986) *Phytochemistry* **25**, 2381.
5. Esquivel, B., Hernández, M., Ramamoorthy, T. P., Cárdenas, J. and Rodríguez-Hahn, L. (1986) *Phytochemistry* **25**, 1484.
6. Bruno, M., Savona, G., Pascual, C. and Rodríguez, B. (1981) *Phytochemistry* **20**, 2259.
7. Stapel, G., Mensser, H. G. and Snatzke, G. (1980) *Planta Med.* **39**, 366.
8. Herz, W., Pilotti, A. M., Soderholm, A. C., Shuhame, I. K. and Vichnewski, W. (1977) *J. Org. Chem.* **42**, 3913.
9. Ohsaki, A., Ohno, M., Shibata, K., Tokorogama, T. and Kubota, T. (1986) *Phytochemistry* **25**, 2414.
10. Tonn, C. E. and Giordano, O. S. (1980) *An. Asoc. Quím. Argentina* **67**, 1.
11. Esquivel, B., Méndez, A., Ortega, A., Soriano-García, M., Toscano, A. and Rodríguez-Hahn, L. (1985) *Phytochemistry* **24**, 1769.
12. Gambaro, V., Chamy, M. C., Garbarino, J. A., San-Martin, A. and Castillo, M. (1986) *Phytochemistry* **25**, 2175.
13. Cuevas, G., Collera, O., García, F., Cárdenas, J., Maldonado, E. and Ortega, A. (1987) *Phytochemistry* **26**, 2019.
14. Van Beck, T. A. and de Groot, Ac. (1986) *Reveil Trav. Chim. Pays-Bas.* **105**, 513.