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Clerodane diterpenes from *Baccharis sagittalis*: insect antifeedant activity

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Abstract

Two clerodane-type diterpene glycosides esters, which were studied as peracetyl derivatives, together with the known diterpene marrubiagenine, were isolated from the aerial part of *Baccharis sagittalis* (Less). Their structures were established by spectroscopic methods. Antifeedant activity toward *Tenebrio molitor* larvae of the isolated compounds along with six other diterpenes was evaluated and some structure–antifeedant bioactivity relationships are reported.
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Keywords: *Baccharis sagittalis*; Asteraceae; Clerodane-type glycosides; β-D-Galactopyranosides; Marrubiagenine; *Tenebrio molitor* L.; Insect antifeedant; Structure–activity relationships

1. Introduction

Baccharis constitutes the largest genus of the family Asteraceae with more than 400 species distributed in the American continent. Several diterpenes with the clerodane skeleton have been reported to occur in this genus (Merrit and Ley, 1992). In previous papers, the feeding-deterrent activities exhibited toward *Tenebrio molitor* (L.) (Coleoptera:Tenebrionidae) larvae by some *neo*-clerodane diterpenoids isolated from natural sources were reported (Sosa et al., 1994). Computer-assisted conformational and electronic studies (Enriz et al., 1994, 2000), as well as analysis of the role that hydrophobicity play in the bioactivity (Luco et al., 1994), have also been carried out.

In continuation of the investigation on the distribution of clerodane diterpenes within the genus *Baccharis* (Cifuente et al., 2001), the constituents of *B. sagittalis* (Less) DC. growing in the Mendoza province in the semi-arid western region of Argentina were studied. The isolation and structural determinations of two new clerodane-type diterpene galactosides (**1**–**2**) as peracetyl derivatives, and marrubiagenine (**3**) (Tschesche and Plenio, 1973) are reported in this study. Additionally, some new structure–antifeedant bioactivity relationships toward *T. molitor* larvae using diterpenes **4**–**9** are discussed.

2. Results and discussion

Air-dried aerial parts of *B. sagittalis* were extracted with Me_2CO at room temperature and the dark-green extract subjected to flash chromatography. Each fraction was purified by Si gel, Sephadex LH-20 and RP 18 column chromatography to afford a mixture of the non-acetyl derivatives of **1**–**2** and marrubiagenine (**3**). Several attempts to separate the glycosides were unsuccessful, hence the fractions were acetylated. The peracetyl derivatives were then isolated after Si gel column chromatography.

Preliminary examination of the ^1H and ^{13}C NMR spectra suggested that compounds **1** and **2** were diterpene glycosides of the clerodane-type with compound **1** being obtained as an amorphous powder. The NMR spectral data for the non-sugar substructure (Table 1) assigned by a combination of 1D and 2D NMR

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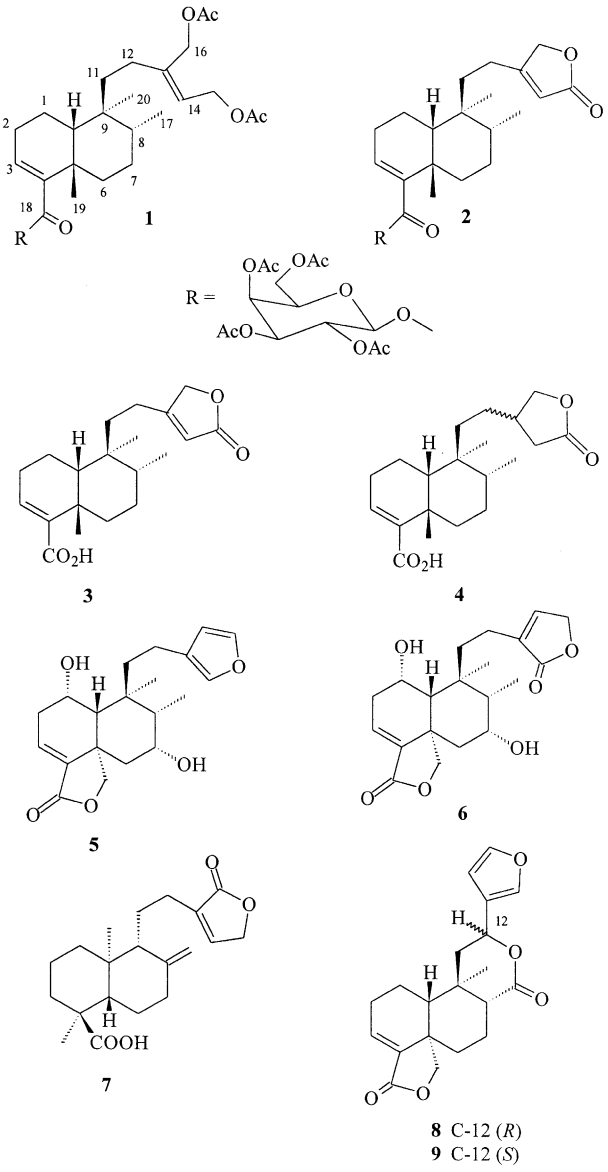
Table 1
^{1}H and ^{13}C NMR spectral data (δ in ppm, J in Hz) for compounds **1–4** in $CDCl_3$

	Compound **1**		Compound **2**		Compound **3**		Compound **4**	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1 (2H)	16.5 *t*	–	16.4 *t*	–	16.9 *t*	2.05–1.85 *m*[a]	16.8 *t*	–
2A	24.0 *t*	2.10– 2.20 *m*[a]	21.8 *t*	2.40–1.70 *m*[a]	22.2 *t*	2.35 *dd* (J = 12.0, 4.3)	24.3 *t*	2.30– 1.80 *m*[a]
2B	–	–	–	–	–	2.10 *m*[a]	–	–
3	142.8 *d*	6.80 *t* (J = 4.2)	142.4 *d*	6.81 *t* (J = 4.2)	142.1 *d*	6.83 *t* (J = 4.3)	142.2 *d*	6.82 *t* (J = 4.3)
4	136.3 *s*	–	136.0 *s*	–	137.4 *s*	–	137.5 *s*	–
5	40.1 *s*	–	39.8 *s*	–	40.3 *s*	–	40.1 *s*	–
6A	36.3 *t*[b]	–	36.1 *t*[b]	–	36.7 *t*[b]	2.60 *m*[a]	36.7 *t*[b]	–
6B	–	–	–	–	–	0.95 *m*[a]		–
7	28.2 *t*[b]	–	23.7 *t*[b]	2.0– 1.25 *m*[a]	24.3 *t*[b]	2.35 *m*[a]	26.5 *t*[b]	–
8	37.5 *d*	1.45 *m*[a]	37.3 *d*	1.42 *m*[a]	37.9 *d*	1.50 *m*[a]	37.7 *d*	1.25 *m*[a]
9	36.2 *s*	–	36.3 *s*	–	36.3 *s*	–	36.3 *s*	–
10	45.1 *d*	1.70 *m*[a]	44.9 *d*	1.40 *m*[a]	45.5 *d*	1.40 *m*[a]	45.3 *d*	1.30 *m*[a]
11	36.1 *t*[b]	–	34.7 *t*[b]	–	35.2 *t*[b]	–	34.7 *t*[b]	–
12	28.4 *t*[b]	–	28.0 *t*[b]	–	28.5 *t*[b]	2.40–2.20 *m*[a]	28.5 *t*[b]	–
13	140.2 *s*	–	173.6 *s*	–	173.1 *s*	–	36.3 *d*	2.4 *m*[a]
14	123.5 *d*	5.54 *t* (J = 6.8)	114.6 *d*	5.85 *br s*	115.2 *d*	5.82 *t* (J = 2.0)	35.7 *t*	2.20 *ddd* (J = 16.5,8.3,1) 2.70 *dd* (J = 16.6, 8.2)
15	60.2 *t*[b]	4.63 *d* (J = 6.8)	170.7 *s*	–	170.6 *s*	–	177.2 *s*	–
16	61.5 *t*[b]	4.60 *br s*	72.7 *t*	4.79 *t* (J = 1.5)	72.9 *t*	4.72 *t* (J = 2.0)	73.4 *t*	3.92 *t* (J = 12.5) 4.45 *dd* (J = 10.4, 8.3)
17	15.8 *q*	0.75 *d* (J = 6.2)	15.6 *q*	0.81 *d* (J = 6.0)	16.0 *q*	0.75 *d* (J = 6.2)	15.9 *q*	0.72 *d* (J = 6.2)
18	164.5 *s*	–	164.2 *s*	–	172.9 *s*	–	172.9 *s*	–
19	32.9 *q*	1.20 *s*	32.6 *s*	1.21 *s*	33.4 *q*	1.25 *s*	33.3 *q*	1.22 *s*
20	17.7 *q*	0.70 *s*	17.3 *q*	0.75 *s*	17.9 *q*	0.82 *s*	17.9 *q*	0.80 *s*
1′	91.2 *d*	5.80 *d* (J = 8.0)	91.0 *d*	5.82 *d* (J = 8.4)	–	–	–	–
2′	70.0 *d*[b]	5.30 *m*[b]	69.8*d*[b]	5.35 *m*[b]	–	–	–	–
3′	72.6 *d*[b]	5.20 *m*[b]	72.3 *d*[b]	5.20 *m*[b]	–	–	–	–
4′	67.8 *d*[b]	5.10 *m*[b]	67.6 *d*[b]	5.15 *m*[b]	–	–	–	–
5′	72.5 *d*[b]	3.88 *br d* (J = 16.0)	72.2 *d*[b]	3.90 *br d* (J = 16.8)	–	–	–	–
6′A	61.4 t	4.10 *dd* (J = 12.6, 2.0)	61.2 *t*	4.11 *dd* (J = 12.6, 2.0)	–	–	–	–
6′B	–	4.30 *dd* (J = 12.6, 4.5)	–	4.31 *dd* (J = 12.6, 4.5)	–	–	–	–
$\underline{CH_3}CO$	20.1, 20.3, 20.4, 20.5, 20.7, 20.8	1.98, 2.00, 2.04, 2.05, 2.09, 2.11; all (*s*)	20.1, 20.2, 20.3, 20.4	1.99, 2.05, 2.07, 2.10; all (*s*)	–	–	–	
$CH_3\underline{CO}$	168.8, 169.2, 169.9, 170.4, 170.5, 170.6	–	168.5, 169.0, 169.6, 170.1	–	–	–	–	–

[a] Overlapped signal, J unresolved.
[b] These signals might be interchangeable within the same column, J unresolved.



spectroscopic techniques (DEPT, HETCOR and COLOC) revealed the presence of two tertiary methyl groups singlets at δ_H 1.20 (δ_C 32.9) and δ_H 0.70 (δ_C 17.7) assigned to H-19 and H-20, respectively, the secondary methyl group (H-17) on the other hand, had a resonance at δ_H 0.75 (*d*, $J=6.2$) (δ_C 15.8). A signal at δ_H 6.80 (*t*, $J=4.2$) (δ_C 142.8) clearly coupled from the COSY spectrum with a methylene group as overlapped multiplet signals at δ_H 2.10–2.20, was assigned to H-3. Additionally, a signal at δ_H 4.63 (2H, *d*, $J=6.8$) (H-15), along with a two proton broad singlet at δ_H 4.60 (H-16) and one olefinic proton signal at δ_H 5.54 (*t*, $J=6.8$) (δ_C 123.5) (H-14) were in agreement with an allylic diol with an acetyl ester as part of the C-9 side chain. The chemical shifts and multiplicities for H-15/H-16 (Rojatkar and Nagasampagi, 1994) as well as biogenetic considerations (Akhila et al., 1991) indicated the *Z* configuration for the C-13–C-14 double bond.

The utility of the ^{13}C NMR chemical shift of the C-5 methyl group (C-19) to differentiate *cis* and *trans*-A/B ring fusion in clerodanes has been reported (de Rosa et al., 1976; Achari et al., 1990).The presence of a C-19 methyl carbon resonance in the region of 30 ppm in the ^{13}C NMR spectrum of clerodane diterpenes suggested a *cis*-geometry, while values in the 15–20 ppm range are typically observed in related *neo*-clerodanes and *ent-neo*-clerodanes which show a *trans*-ring fusion (Martin et al., 1996). In the ^{13}C NMR spectrum of compound **1** (Table 1), C-19 showed resonance at δ_C 32.9 (*q*) which was in agreement with an A/B *cis*-fusion in the decalin moiety.

On the other hand, four additional three-proton singlets in the δ_H range 2.11–1.98 suggested the presence of an acetylated hexose unit as a sugar substructure. An ABX pattern of signals at δ_H 4.10 (*dd*, $J=12.6$, 2.0) and δ_H 4.30 (*dd*, $J=12.6$, 4.5), both clearly coupled with a broad doublet centered at δ_H 3.88 ($W_{1/2}=16.0$ Hz) from the COSY spectrum, was assigned to a H-6′A, H-6′B, and H-5′proton system. A complex three-proton multiplet in the range of δ_H 5.10–5.30 was in agreement with H-2′, H-3′, and H-4′ resonances. In addition, a signal at δ_H 5.80 (*d*, $J=8.0$) (δ_C 91.2) for the anomeric carbon was in accordance with an esterified C-18 carboxyl group (Ybarra et al., 1997). This conclusion was supported by clear correlation of H-1′ with C-18 (^3J) in the 2D COLOC spectrum. Moreover, inspection of this spectrum furnished evidence of cross-peaks between H-19 with C-4, C-5, C-6, and C-10; H-20 with C-8, C-10 and C-11; H-3 with C-1; H-16 with C-13, and C-15, as key long range correlations.

After acid hydrolysis, the sugar residue of compound **1** (TMSi-ether derivative) was identified as D-galactose by comparison with an authentic sample. Finally, in the positive FABMS an intense fragment ion peak *m*/*z* 420 M-galactosyl(Ac)$_4^+$ was observed. Thus, the structure of **1** was elucidated as the peracetylated derivative of the *cis*-cleroda-15,16-dihydroxy-3,13(*Z*)-dien-18-*O*- β-D-galactopyranosil ester.

Compound **2** was obtained as an amorphous powder. The ^{13}C NMR spectral data of **2** (Table 1) showed signals for six carbonyl groups; seven sp^3 methines (one as a ketal carbon); eight methylenes, with two of these bearing an oxygen atom; seven methyl groups; and four quaternary carbons, two of these sp^2. The non-sugar substructure gave a signal at δ_H 6.81 (*t*, $J=4.2$) (δ_C 142.4) due to H-3. The tertiary methyl groups C-20 and C-19 showed resonances at δ_H 0.75 (*s*) (δ_C 17.3) and δ_H 1.21 (*s*) (δ_C 32.6), respectively. A signal at δ_H 0.81 (*d*, $J=6.0$) was assigned to H-17. On the other hand, a low-field broad singlet at δ_H 5.85 ($J=2.0$) (δ_C 114.6), together with signals at δ_C 173.6 (*s*), 114.6 (*d*), 170.7 (*s*), and 72.7 (*t*) were considered to be in accordance with a β-substituted butenolide function on the C-9 side chain (Zdero et al., 1991). From the C-19 resonance at δ_C 32.6

(*s*) the A/B ring fusion was identical to compound **1** (Martin et al., 1996).

An AB system at δ_H 4.11 (*dd*, $J = 12.6$, 2.0) and 4.31 (*dd*, $J = 12.6$, 4.5), together with one proton multiplets at δ_H 5.35, 5.20, and 5.15, suggested that the acetylated sugar part was a hexose similar to that described for compound **1**. A doublet at δ_H 5.82 ($J = 8.4$) (δ_C 91.0) was attributed to an anomeric carbon as part of an ester function. COLOC correlations supported the connectivities proposed for the glycoside under study. Long range correlations for H-3, H-16, H-19, and H-20 were identical to those discussed above for **1**. Both the H-1′ with C-18 (^3J) correlation and the coupling constant for the anomeric proton permitted determination the position and stereochemistry of the sugar linkage. Furthermore, acid hydrolysis of the peracetylated derivative yielded D-galactose as a sugar residue.

Finally, in the positive FABMS an intense fragment ion peak at *m*/*z* 331 M-galactosyl$(Ac)_4^+$ was observed. Accordingly, compound **2** was assigned the *cis*-cleroda-3,13(14)-dien-15,16-olide-18-*O*-[β-D-galactopyranosyl]-peracetylester structure. Thus compound (**2**) is a new derivative of marrubiagenine (**3**), for which only the C-18 β-D-glucopyranosyl derivative has been reported (Tschesche und Plenio, 1973).

The structure of marrubiagenine **3** (Tschesche und Plenio, 1973; Zdero et al., 1991) has been deduced previously by spectroscopic means (UV, IR, ^{1}H NMR, MS). However, no ^{13}C NMR spectral data for it were reported.

Thus, extensive NOESY experiments were carried out on compound **3** to investigate the biogenetical basis of the relative stereochemistries of the aglycone moiety in compounds **1** and **2**. It revealed that C-17 and C-20 were α-oriented showing NOE correlation. On the other hand, cross-peaks between H-3/H-20; H-2α/H-20; H-7α/H-20, and H-6/H-19, were in agreement with the proposed relative stereochemistry for each chiral centers (Tori et al., 1993).

Table 2
Antifeedant activities and active centers distance for compounds **3**–**9**

Compound	Non-choice test[a]	(SD)	Calculated distance[c] (Å)
3	30.4[b]	2.6	10.50
4	42.3	3.2	10.36 (C-13 *R* isomer) 10.07 (C-13 *S* isomer)
5	29.5[b]	2.5	10.01
6	41.6	3.8	9.52
7	43.4	4.1	10.67
8	25.5[b]	2.3	10.65
9	43.0	3.3	9.25

[a] Statistical significance determined by Anova.
[b] Compound deemed as active.
[c] Distance between the oxygen atom of the side chain heterocycle and C-18.

The insect antifeedant activity associated with diterpenes possessing a clerodane skeleton encouraged us to test a series of compounds against *T. molitor* larvae in order to search for possible new structure–activity relationships.

Since **2** and **3** have a C-9 side chain with a 15,16-butenolide moiety identical to that of the potent antifeedant ajugarin I and related compounds (Ley, 1990; Simmonds et al., 1996), a comparative study of the influences of this functionality was carried out. Compound **3** was subjected to catalytic hydrogenation (C/Pd) under controlled conditions to give derivative **4**. The ^{1}H and ^{13}C NMR spectra of compound **4** (Table 1) were very similar to that of **3**, except that the two proton signals at C-16 appeared at δ_H 4.45 (*dd*, $J = 10.4$, 8.3) and 3.92 (*t*, $J = 12.5$) and the olefinic proton signal at C-14 were absent. Two new signals at δ_C 35.7 and δ_C 36.3 attributable to C-14 and C-13 respectively, along with the expected upfield shifts of C-15 (δ_C 177.2), were observed. COSY, HETCOR, COLOC, and NOESY experiments established the same connectivities and stereochemistry previously discussed for the decalin moiety. Although, the stereochemistry at C-13 was not determined, one diastereomer was recovered as the principal derivative. This surprising result may be due to the chirality of the decaline framework, which could induce the observed high diastereoselectivity.

The β-substituted butenolide **3** showed (Table 2) antifeedant activity toward *T. molitor* (L) larvae similar to bacrispine (**5**) (Ceñal et al., 1997), which possesses a β-substituted furane ring at C-12. In the same test, compound **6** (Ceñal et al., 1997) with an α-substituted-15,16-butenolide function, was inactive. The same results were obtained using the labdane **7** (Carreras et al., 1998) with similar connectivity at C-9, as well as with the butanolide **4**. However, compound **8** (Gallardo et al., 1996), having a *R* configuration at the C-12 stereogenic center, was the most active diterpene of the series. A dramatic loss of antifeedant activity was observed for the C-12 *S* configuration of compound **9** (Simirgiotis et al., 2000).

The above results suggested that the presence of a β-substituted furan ring or a β-substituted butenolide function on the side chain could be necessary for antifeedant bioactivity. On this basis, the loss of activity shown by compounds **4** and **6** could be explained. Additionally, compound **9** did not have the necessary structural requirements for eliciting the measured bioactivity. This observation had been made previously by comparison of the structure-activity relationship of several *neo*-clerodanes and *ent-neo*-clerodanes possessing an A/B *trans*-junction (Sosa et al., 1994).

In order to elucidate some general trends in structure–activity relationship, theoretical calculations on the assayed compounds were carried out. The common feature of the calculated Molecular Electrostatic Potential surfaces (MEPs) for the active compounds was the

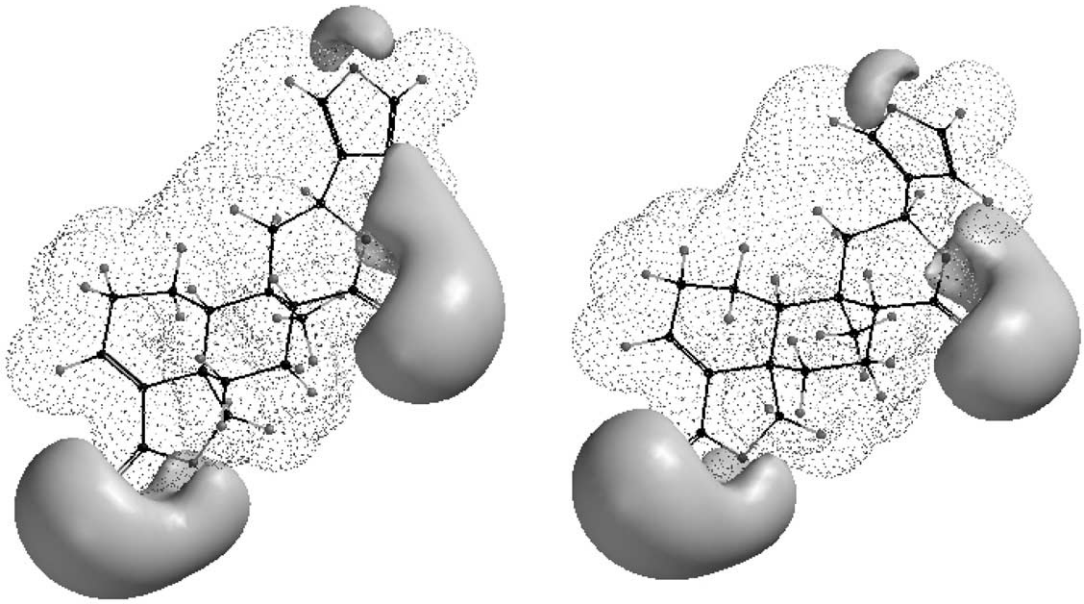


Fig. 1. MEPs for bacchotricuneatin A (**8**) and for 12-*epi*-bacchotricuneatin A (**9**).

existence of localised minima in the vicinity of the side chain heterocycle, along with the α,β-unsaturated carbonyl system on the decalin moiety. This could suggest a relationship between the bioactivity of the compounds and the distance between the two possible active centres. The distance between the heterocyclic side chain oxygen and C-18 for the compounds under study are shown in Table 2. These results suggest that the antifeedant effect on the *T. molitor* larvae model is related to the interatomic distances between C-18 and the side chain heterocycle oxygen in a range of 9.8–10.8 Å (Enriz et al., 1994, 2000).

Compounds **8** and **9** show a rigid structure as evidenced by conformational analysis. All the conformations obtained basically differ in the position of the sidechain furyl group. MEPs (Fig. 1) were in agreement with the epimeric relationships, suggesting that the negative charge density localised over the furyl group, in the compound-receptor interaction is of importance. This is because the rigidity of the structure would contribute to emphasize the effect of the chiral centre. Hence, both the molecular connectivity as well as stereoelectronic factors seem to play an essential role in antifeedant activity.

Finally, since clerodane-type diterpenoids [having an α-substituted-γ-butanolide on the sidechain, together with an α,β-unsaturated-γ-lactone in the decalin system (similar to compound **6**)] have been reported as being antifeedants towards other insect model such as *Spodoptera littoralis* (Boisd.) (Simmonds et al., 1996), the interspecies differences in the response of test insects must be taken into account.

3. Experimental

3.1. General

^{1}H NMR spectra were recorded in $CDCl_3$ at 200.13 MHz, whereas ^{13}C NMR spectra were obtained at 50.23 MHz on a Bruker AC-200. COSY, NOESY, HETCOR and COLOC experiments were obtained using standard software. EIMS were collected at 70 eV on a Finnigan-Mat GCQ-plus instrument. HREIMS were obtained with a VG-ZAB-BEQ9 spectrometer and positive FABMS on a ZAB-SEQ4F mass spectrometer at LANAIS-EMAR-CONICET, Universidad de Buenos Aires. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter, and IR spectra were recorded on a Bruker IFS-2S spectrometer. CC were performed on silica gel G 70–230 mesh and 60 H, Sephadex LH-20 and LiChroprep RP-18. TLC were carried out on silica gel 60 F_{254} (0.2 mm-thick plates) and DC-Alufolien RP-18. GC analyses of the sugars as TMSi derivatives were performed using SE-52 column with FID detector. $T_1 = 160$ °C, $T_2 = 200$ °C ($\Delta t = 8$ °C/min).

3.2. Plant material

Baccharis sagittalis (Less) DC., was collected during March 1999 in Potrerillos, Mendoza, Argentina. A voucher specimen is deposited at the Herbarium of the Universidad Nacional de San Luis (L.A. del Vitto No. 8841).

3.3. Extraction and isolation

Dried aerial parts (1600 g) of *B. sagittalis* were extracted three times with acetone (5,000 ml) at room temperature for 2 weeks. The organic extract (54 g) was subjected to flash chromatography on silica gel, eluting with *n*-hexane–EtOAc gradient to afford 13 fractions. The non-polar fractions were purified by several column chromatography on Si gel eluting with *n*-hexane, *n*-hexane–EtOAc with increasing polarity mixtures and EtOAc–MeOH (97:3) to give six fractions. Each fractions obtained was monitored by TLC (C_6H_6–dioxane–AcOH 30:5:1). Fractions 4 and 5 were separated and purified by CC to furnish compound **3** (90 mg). The more polar fractions were purified by Sephadex LH-20 and RP-18 CC, eluted with a MeOH–H_2O gradient to yield 10 fractions. Fractions 2 and 4 were acetylated dissolving in 1 ml Ac_2O-pyridine (1:1) and left overnight at room temp. After usual work-up the mixture was purified by silica gel CC using *n*-hexane–EtOAc gradient to yield the peracetyl derivatives **1** (40 mg) and **2** (60 mg).

3.4. cis-Cleroda-15,16-dihydroxy-3,13(Z)-dien-18-O-[β-D-galactopyranosyl]-peracetylester (1)

Amorphous solid; $[\alpha]_D^{25}$ −27.8° (*c* 0.28, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2966, 1760, 1642, 1450, 1371, 1225, 1037, 908, 600; for ^1H and ^{13}C NMR spectra, see Table 1. FABMS *m/z* 420 M-galactosyl$(Ac)_4^+$

3.5. cis-Cleroda-3,13(14)-dien-15,16-olide-18-O-[β-D-galactopyranosyl]-peracetylester (2)

Amorphous solid; $[\alpha]_D^{25}$ −31.6° ($CHCl_3$; *c* 0.88); IR ν_{max}^{KBr} cm^{-1}: 2964, 1755, 1639, 1542, 1379, 1223, 908, 899, 600; for ^1H and ^{13}C NMR spectra, see Table 1. Combustion analysis: Found C, 63.15; H, 7.20; $C_{34}H_{46}O_{12}$ requires: C, 63.15; H, 7.12. FABMS *m/z* 331 M-galactosyl$(Ac)_4^+$

3.6. Acid hydrolysis of 1 and 2

The corresponding glycoside (35 mg) was hydrolyzed with 2 N HCl in dilute MeOH–H_2O (9:1) (25 ml) at 100 °C for 2 h. The reaction mixture was diluted with H_2O and neutralized with Ag_2CO_3. Solids were removed by filtering through Celite and the filtrate was extracted with EtOAc. The aqueous layer was lyophylized to give the sugar residue. TMSi derivatives were prepared and subjected to GC analysis (flame detector). Standards of D-glucose (rt = 6.8 min) and D-galactose (rt = 6.0 min) were run in the same conditions. The retention time of the sugar analysis was in agreement with D-galactose derivative (rt = 6.04).

3.7. Marrubiagenine (cis-cleroda-3,13(14)-dien-15,16-olide-18-oic acid) (3)

Mp, IR, EIMS, HREIMS data (*m/z* 332.19892 M^+, calc. For $C_{20}H_{28}O_4$ *m/z* 332.1987), were identical to previously reported. $[\alpha]_D^{25}$ −58° ($CHCl_3$; *c* 2.3); for ^1H and ^{13}C NMR spectra, see Table 1.

3.8. 13,14-Dihydro-marrubiagenine (4)

Compound **4** was prepared by catalytic hydrogenation of **3** (30 mg) in EtOAc, shaking with hydrogen over palladized charcoal (5%, 30 mg) for 2 h at room temp. Removal of catalyst (Celite) and solvent left an oil (24 mg) which was purified by prep.TLC to give compound **4** (21 mg). Crystalline solid; mp 145–147; $[\alpha]_d^{25}$ −28.9° (Me_2CO; *c* 0.61); IR ν_{max}^{KBr} cm^{-1}: 2956, 2928, 1778, 1759, 1677, 1461, 1382, 1224, 1039, 603; for ^1H and ^{13}C NMR spectra, see Table 1. EIMS *m/z* (relative intensity): 334 M^+ (1), 203 (12), 175 (16), 149 (34), 121 (60), 107 (33), 95 (51), 81 (100), 69 (36).

3.9. Biological evaluation

The antifeedant activity of the clerodane diterpenes, **3–9** was assayed against *Tenebrio molitor* L. (Coleoptera:Tenebrionidae) larvae using a non-choice test at a concentration of 100 ppm in a mixture of H_2O:MeOH:Me_2CO (95:5:5) containing Triton CS-7 (0.1% by volume) (Sosa et al., 1994). Compounds **5** and **6** were previously isolated from *Baccharis crispa* Sprengel (Ceñal et al., 1997). Compound **7** was obtained from *Eupatorium buniifolium* H. et A. (Carreras et al., 1998) From *Baccharis spicata* (Lam.) Beill. (Gallardo et al., 1996), and *Laennecia sophiifolia* (Kunth) G.L. Nesom (Simirgiottis et al., 2000) compounds **8** and **9**, were isolated respectively. The activity was expressed as percentage of feeding inhibition, PFI = [(% Treated)/(% Treated + % Untreated)] × 100. A test result of 50 indicates equal consumption of treated and untreated carrot slices while a lower number indicate antifeedant activity. This experiment was repeated six times in duplicate for each of the compounds assayed. The test data were subjected to analysis of variance (block design-Anova) followed by mean comparisons, and the results are shown in Table 2.

3.10. Theoretical calculations

In vacuum conformational theoretical study of compounds **3–9** was carried out using the facilities of PC Spartan PRO® software. The conformational search was performed using a Monte Carlo method and MMFF94 mechanics model. For compounds **3–7** dihedral angles of the side chain C-12, C-13, and C-14 atoms were varied each 30° as well as the C-14 dihedral angle for compounds **8–9**.

For compounds **3**, **4**, and **7** dihedral angles of C-1, C-6, C-10, and C-18 were varied each 120°. For compounds **5**–**6** similar rotation was used for C-1, C-6, C-10, and C-19. Finally, in compounds **8**–**9** a variation of the same order was applied for C-1, C-6, C-7, C-11, C-17, and C-19. The lowest energy conformation was selected and minimized using the AM1 model. The obtained geometries were used for Molecular Electrostatic Potential (MEP) surfaces calculation.

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References

Achari, B., Chaudhuri, C., Saha, C.R., Dutta, P.K., Pakrashi, S.C., 1990. A clerodane diterpene and other constituents of *Clerodendron inerme*. Phytochemistry 29, 3671–3673.

Akhila, A., Rami, K., Thakur, R.S., 1991. Biosynthesis of the clerodane furano-diterpene lactone skeleton in *Tinospora cordifolia*. Phytochemistry 30, 2573–2576.

Carreras, C.R., Rossomando, P.C., Giordano, O.S., 1998. *Ent*-labdanes in *Eupatorium buniifolium*. Phytochemistry 48, 1031–1034.

Ceñal, J.P., Giordano, O.S., Rossomando, P.C., Tonn, C.E., 1997. Neoclerodanes diterpenes from *Baccharis crispa*. J. Nat. Prod. 60, 490–492.

Cifuente, D.A., Tonn, C.E., Giordano, O.S., 2001. Two new labdane diterpene glycosides from *Baccharis medullosa* DC. Nat. Prod. Lett. 15, 425–431.

De Rosa, S., Minale, L., Riccio, R., Sodano, G., 1976. The absolute configuration of avarol, a rearranged sesquiterpenoid hydroquinone from a marine sponge. J.C.S. Perkins I 1408–1414.

Enriz, R.D., Baldoni, H.A., Jáuregui, E.A., Sosa, M.E., Tonn, C.E., Giordano, O.S., 1994. Structure–activity relationships of clerodane diterpenoids acting as antifeedant agents. J. Agric. Food Chem. 11, 2958–2963.

Enriz, R.D., Baldoni, H.A., Zamora, M.A., Jáuregui, E.A., Sosa, M.E., Tonn, C.E., Luco, J.M., Goraliza, M., 2000. Structure–antifeedant activity relationships of clerodane diterpenoids. Comparative study with withanolides and azadirachtin. J. Agric. Food Chem. 48, 1384–1392.

Gallardo, V.O., Tonn, C.E., Nieto, M., Morales, G., Giordano, O.S., 1996. Bioactive *neo*-clerodane diterpenoids toward *Tenebrio molitor* larvae from *Teucrium nudicaule* H. and *Baccharis spicata* (Lam.). Nat. Prod. Lett. 8, 189–197.

Ley, S.V., 1990. Synthesis of antifeedants of insects: novel behaviour-modifying chemicals from plants. In: Bioactive Compounds from Plants. Ciba Foundation Symposium 154. John Wiley & Sons, Chichester, pp. 80–98.

Luco, J.M., Sosa, M.E., Cesco, J.C., Tonn, C.E., Giordano, O.S., 1994. Molecular connectivity and hydrophobicity in the study of antifeedant activity of clerodane diterpenoids. Pestic. Sci. 41, 1–6.

Martin, T.S., Ohtani, K., Kasai, R., Yamasaki, K., 1996. Furanoid diterpene glucosides from *Tinospora rumphii*. Phytochemistry 42, 153–158.

Merrit, A.T., Ley, S.V., 1992. Clerodane diterpenoids. Nat. Prod. Rep. 9, 243–287.

Rojatkar, S.R., Nagasampagi, B.A., 1994. Diterpenes form *Cipadessa fruticosa*. Phytochemistry 37, 505–507.

Simmonds, M.S.J., Blaney, W.M., Esquivel, B., Rodriguez-Hahn, L., 1996. Effect of clerodane type diterpenoids isolated from *Salvia* spp. on the feeding behaviour of *Spodoptera littoralis*. Pestic. Sci. 47, 17–23.

Simirgiotis, M.J., Favier, L.S., Rossomando, P.C., Giordano, O.S., Tonn, C.E., Padrón, J.I., Trujillo, V.J., 2000. New diterpenes from *Laennecia sophiifolia*. Phytochemistry 55, 721–726.

Sosa, M.E., Tonn, C.E., Giordano, O.S., 1994. Insect antifeedant activity of clerodane diterpenoids. J. Nat. Prod. 57, 1262–1265.

Tschesche, R., Plenio, H.U., 1973. Uber marrubiasid und marrubialacton, zwei diterpenderivate mit umgelagertem labdangerust aus *Leonurus marrubiastrum* L. Chem. Ber. 106, 2929–2942.

Tori, M., Masuya, T., Asakawa, Y., 1993. Three clerodane-diterpenoids from the liverwort *Schistochila nobilis*. Phytochemistry 32, 1229–1233.

Ybarra, M.I., Borkosky, S.A., Catalan, C.A., Cerda-García-Rojas, C.M., Joseph-Nathan, P., 1997. Diterpenes from *Hyalis argentea*. Phytochemistry 44, 479–483.

Zdero, C., Bohlmann, F., King, R.M., 1991. Diterpenes and norditerpenes from the *Aristeguetia* group. Phytochemistry 30, 2991–3000.