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Effects of ajugarins and related neoclerodane diterpenoids on feeding behaviour of *Leptinotarsa decemlineata* and *Spodoptera exigua* larvae

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Abstract

Three naturally occurring ajugarins and seven semisynthetic derivatives of them, possessing different functionalities in the decalin part, together with two natural furoneoclerodane diterpenes, have been assessed as feeding behavior modifying agents of larvae of the generalist *Spodoptera exigua* and a specialist like *Leptinotarsa decemlineata*. Ajugarin I and some of its derivatives exhibited a significant antifeedant activity against larvae of *S. exigua* in both choice and no-choice assays. Conversely, the furoneoclerodane diterpenes only presented antifeedant activity against larvae of *L. decemlineata*. These results indicate that the biological action of the tested substances is strongly modulated by minimal structural variations, which are also responsible for the specificity of action.

Keywords: Diterpenes; Ajugarins; Furoneoclerodanes; Antifeedant activity; *Leptinotarsa decemlineata*; *Spodoptera exigua*

1. Introduction

Clerodanes are a large group of naturally occurring diterpenoids isolated mainly from Compositae and Labiatae plants (Merritt and Ley, 1992; Rodríguez-Hahn et al., 1994). These compounds have attracted interest in the last years on account of their biological activities against some economically important lepidopteran (Simmonds and Blaney, 1992; Rodríguez et al., 1999), coleopteran (Ortego et al., 1995; López-Olguín et al., 1999) and orthopteran (Hanson et al., 1982) pests. Among these substances, ajugarins I (**1**) (Kubo et al., 1976; Savona et al., 1984; Beauchamp et al., 1996) and II (**2**) (Kubo et al., 1976; Beauchamp et al., 1996; Bruno et al., 2000) and deacetylajugarin II (**3**) (Savona et al., 1984; Fontana et al., 1998) are neoclerod-13-en-15,16-olide derivatives that have been shown to have significant insect antifeedant properties against larvae of the lepidopteran *Spodoptera littoralis* (Boisduval) (Kubo et al., 1976; Simmonds et al., 1989; Cole et al., 1990; Simmonds and Blaney, 1992; Bremner et al., 1998), *S. frugiperda* (J. E. Smith) (Simmonds and Blaney, 1992; Bruno et al., 2000), *S. exempta* (Walker) (Kubo et al., 1976) and *Helicoverpa* (*Heliothis*) *armigera* (Hübner) (Simmonds et al., 1989; Bruno et al., 2000).

The interesting insect antifeedant action of ajugarins, together with the uncertain knowledge available on the structure–activity relationships of these substances (Kubo et al., 1983; Simmonds and Blaney, 1992), prompted us to undertake some chemical transformations of **2** and **3** in order to establish how the functionalities of their decalin part modulate feeding behavior of larvae of the specialist *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) and the generalist *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). In addition to the neoclerod-13-en-15,16-olide derivatives **1**–**10**, we have also tested teumassilenins A and C (**11** and **12**, respectively), recently isolated (Fontana et al., 1998) from *Teucrium massiliense* L. (Labiatae), whose structures possess a β-

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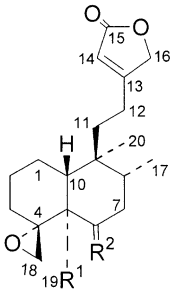
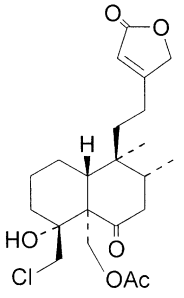


	R^1	R^2
1	CH_2OAc	α-OAc, β-H
2	CH_2OAc	α-OH, β-H
3	CH_2OH	α-OH, β-H
4	CH_2OH	α-OAc, β-H
5	CHO	α-OH, β-H
6	CH_2OAc	O
7	CHO	α-OAc, β-H



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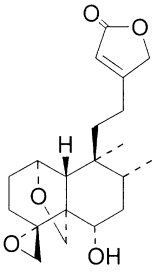

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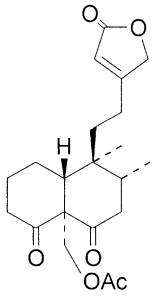

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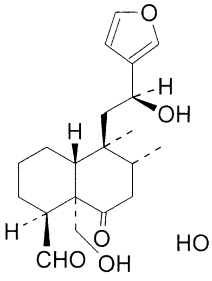

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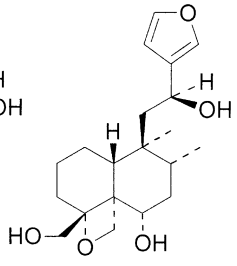

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substituted furan ring instead of the α,β-unsaturated γ-lactone of **1**–**10**.

2. Results and discussion

Deacetylajugarin II (**3**) (Savona et al., 1984; Fontana et al., 1998), one of the major constituents of the acetone extract of the aerial parts of *Teucrium massiliense* (0.73% on dry plant material), was used as the starting material for obtaining compounds **1**, **2** and **4**–**10**. Ajugarin I (**1**) was prepared by treatment of **3** with acetic anhydride-pyridine at room temperature for 24 h (Savona et al., 1984). Reaction of **3** with the same reagent for 1.5 h yielded a mixture of three compounds, which were easily separated by column chromatography giving unreacted **3** (41%), ajugarin II (**2**, 32%) (Kubo et al., 1976; Beauchamp et al., 1996; Bruno et al., 2000) and compound **4** (24.5%). Selective oxidation of **3** with the Dess–Martin periodinane (Dess and Martin, 1991) gave the aldehyde **5** in almost quantitative yield (96%), whereas oxidation of **2** with chromium trioxide-pyridine (Poos et al., 1953) yielded the expected 6-keto derivative **6** (79%). Treatment of **4** with the Dess–Martin periodinane gave the 19-aldehyde **7** in poor yield (30%), probably due to the steric hindrance caused by the acetoxyl group at the C-6α position. Best results (60% yield of **7**) were achieved by using 4-methylmorpholine-*N*-oxide (NMO) and catalytic amounts of tetrapropylammonium perruthenate (TPAP) as an oxidizing reagent (Ley et al., 1994). The chlorohydrin derivative **8** was obtained in poor yield (22%, see Experimental) by treating ajugarin II (**2**) with pyridinium chlorochromate (PCC), which produces the oxidation of the C-6α hydroxyl group and the oxirane-opening by attack of the nucleophile (Cl^-) on the primary center of the epoxide (C-18) (Rodríguez et al., 1994). Remote oxidation of **3** with iodobenzene diacetate (IBD)-iodine (Majetich and Wheless, 1995) gave the 1α,19-epoxy derivative **9** in 72% yield. Treatment of ajugarin II (**2**) with an excess of Jones' reagent (Bowers et al., 1953) gave the 18-nor-neoclerodane derivative **10** (56%), via oxidation of the C-6α hydroxyl group and opening of the 4α,18-oxirane, followed by oxidation of the resulting glycol to an α-hydroxycarboxylic acid, which undergoes decarboxylation to yield **10** (Chen et al., 1995).

The structures of all the above compounds are strongly supported by their ^1H and ^{13}C NMR, IR and mass spectra, and other analytical data (see Experimental Section). The derivatives **4**–**10** have not been described previously.

Among the compounds tested (**1**–**12**) we have found an outstanding differential antifeedant pattern depending on the insect species. The two naturally occurring furoneoclerodane diterpenes (**11** and **12**), possessing a β-substituted furan ring in which the C-13–C-16 carbon atoms are involved, showed antifeedant activity against last instar larvae of the specialist *L. decemlineata*, but no activity was detected on *S. exigua* (Table 1). On the contrary, compounds possessing a β-substituted α,β-unsaturated γ-lactone instead of the furan ring, like natural ajugarin I (**1**) and the semisynthetic derivatives **4**, **8** and **10**, only showed antifeedant effect on the fifth instar larvae of the generalist *S. exigua* (Table 1). These results indicated that the antifeedant action on *L. decemlineata* larvae is associated with the presence of a furan ring in the molecule of the active compounds (**11** and **12**), whereas the rest of the chemical structure does not play a significant role for such biological effect. The 12*S*-hydroxyl group of **11** and **12**, however, could also be an important structural feature for the mode of action of these compounds at a molecular level, because it modifies drastically the reactivity of the furan.

Ajugarin I (**1**) showed antifeedant activity against larvae of *S. exigua* in both choice and no-choice assays (Table 1). Deacetylation at C-19 (**4**) resulted in a decrease of the activity and no antifeedant effect was observed when there is an α-hydroxyl function (**2**) or a ketone (**6**) at the C-6 position, or when both C-6α and C-19 sub-

Table 1
Effect of compounds **1–12** on the feeding behaviour of *Leptinotarsa decemlineata* and *Spodoptera exigua* larvae

Compound[a]	*Leptinotarsa decemlineata*			*Spodoptera exigua*		
	No-choice bioassay AI[b]	Choice bioassay AI[c]	Choice/ no choice SI[d]	No-choice bioassay AI[b]	Choice bioassay AI[c]	Choice/ no choice SI[d]
1	5.30±6.27	26.78±8.64	−7.62±7.77	70.04±6.78[e]	67.90±17.18	15.51±8.88
2	−12.70±9.01	31.39±17.72	6.87±6.98	8.30±9.83	25.12±9.75	−8.79±19.81
3	11.25±8.43	13.70±11.84	−12.78±9.74	−22.39±7.45	9.67±6.63	−31.68±6.30
4	5.30±5.60	26.78±5.43	−7.62±5.04	30.97±9.15	57.12±9.11[e]	−11.34±9.12
5	9.03±17.64	46.14±7.98	−23.00±7.33	24.53±14.08	38.46±13.82	−10.82±15.28
6	−9.22±7.25	39.92±7.05	−18.06±5.90	34.29±22.98	25.80±12.21	23.04±19.27
7	16.11±5.52	8.62±11.62	7.95±8.42	14.90±16.82	39.46±8.78	37.33±9.91
8	29.36±11.74	33.06±16.26	25.76±18.04	29.95±16.60	91.36±6.20[e]	1.96±16.23
9	5.51±12.93	28.49±12.57	−16.04±26.00	−16.11±19.15	26.07±14.18	−22.14±12.23
10	−13.77±6.26	36.42±15.95	2.24±18.06	9.88±19.56	72.87±13.36[e]	−8.78±11.23
11	75.62±8.58[e]	88.19±6.35[e]	12.03±9.21	−26.25±9.56	10.45±10.45	−3.04±7.18
12	67.64±12.30[e]	73.71±19.74[e]	43.80±11.24	15.99±7.34	13.74±9.14	−23.89±10.35

[a] At 1000 ppm.
[b] Average antifeedant index $[(C-T)/C]\times 100\% \pm$ standard error ($n = 10$). C and T represent consumption of control and treatment disks, respectively.
[c] Average antifeedant index $[(C-T)/C+T)]\times 100\% \pm$ standard error ($n = 10$). C and T represent consumption of control and treatment disks, respectively, within each arena.
[d] Average suppression index $[(C-T)/C]\times 100\% \pm$ standard error ($n = 10$). T represents consumption of both treated and untreated disks in test arenas and C in control arenas that contain untreated leaf disks only.
[e] Antifeedant activity considered for indexes over 50%.

stituents are deacetylated (**3**) (Table 1). Moreover, transformation of the C-19 acetoxyl group into an aldehyde (**5** and **7**) or a 1α,19-ether function (**9**), together with deacetylation at the C-6α position in the case of **5** and **9**, also caused the loss of the activity (Table 1). These results suggest that both acetoxyl groups at the C-6α and C-19 positions play a major role in the antifeedant activity of ajugarin I (**1**) against this species. However, when the C-19 acetoxyl group was maintained, but the functionality at C-6 was a ketone and the 4α,18-epoxide was replaced by a chlorohydrin (**8**) or it was eliminated giving the 18-nor-4-oxo derivative **10**, a very high antifeedant activity was found in choice assays for these compounds (Table 1). This suggests that **8** and **10** act predominantly as feeding deterrents of *S. exigua* in a choice situation, but the larvae will feed on it when other resources are not available, as compared with ajugarin I (**1**). The effect of ajugarins **1**–**10** on the feeding behavior of *S. exigua* larvae (Table 1) establishes that the activity is strongly dependent on the functionality of the *trans*-decalin part of the molecule, as has been suggested previously (Kubo et al., 1983). In addition, the furoneoclerodanes **11** and **12** have no activity on *S. exigua* (Table 1), as reported for some related diterpenoids tested against other lepidopteran species (Simmonds and Blaney, 1992).

The data presented in this study on the antifeedant activity of compounds **1**–**12** against *L. decemlineata* and *S. exigua* larvae, reveal that minimal structural changes in the substance can account for large variations in the activity and cause remarkable differences of specificity between both insect species.

3. Experimental

3.1. General

Mps uncorr. ^{1}H NMR chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.25). ^{13}C NMR chemical shifts are reported with respect to solvent signals (δ_{CDCl_3} 77.00). ^{13}C NMR assignments were determined by gHSQC and gHMBC spectra. MS were recorded in the positive EI mode and no fragments below m/z 50 were registered. Merck Si gel no. 7734 (70-230 mesh) deactivated with 15% H_2O, w/v, was used for column chromatography.

Deacetylajugarin II (**3**) and teumassilenins A (**11**) and C (**12**) were available from our previous studies on *T. massiliense* (Savona et al., 1984; Fontana et al., 1998). Ajugarin I (**1**) was obtained from **3** as described previously (Savona et al., 1984).

3.2. Preparation of 19-acetoxy-4α,18-epoxy-6α-hydroxyneoclerod-13-en-15,16-olide [ajugarin II (**2**)] and 6α-acetoxy-4α,18-epoxy-19-hydroxyneoclerod-13-en-15,16-olide (**4**) from deacetylajugarin II (**3**)

Treatment of **3** (1 g, 2.85 mmol) with Ac_2O-pyridine (1:1, 20 ml) at room temperature for 1.5 h yielded, after workup in the usual manner, a mixture of **2**, **4**, and starting material (**3**). Column chromatography of this mixture [gradient of elution from EtOAc–petroleum ether (2:1) to 100% EtOAc] gave, in order of increasing chromatographic polarity, **4** (275 mg, 0.70 mmol, 24.5%), **2** (362 mg, 0.92 mmol, 32.3%), and **3** (411 mg, 1.17 mmol, 41.1%).

Table 2
^{1}H NMR spectral data of compounds **2** and **4–10**[a]

	2	**4**	**5**	**6**	**7**	**8**	**9**	**10**
H								
1α	~1.55[b]	~1.54[b]	1.76, *qd*	~1.62[b]	[c]	[c]	–	1.91, *dddd*
1β	~1.55[b]	~1.54[b]	1.48, *dddd*	~1.62[b]	[c]	[c]	4.33, *br d*	~1.71[b]
2α	~2.00[b]	2.00, *ddddd*	2.02, *ddddd*	~1.98[b]	[c]	[c]	~1.56[b]	~2.16, *m*
2β	1.40, *qdd*	1.39, *ddddd*	1.36, *qt*	~1.40[b]	[c]	[c]	~1.56[b]	~1.69[b]
3α	~2.10[b]	2.35, *dddd*	2.60, *tdd*	~2.26[b]	2.51, *tdd*	[c]	~2.41[b]	2.62, *ddd*
3β	1.10, *ddd*	1.07, *ddd*	1.09, *ddd*	~1.01[b]	~1.06[b]	[c]	~1.18[b]	2.38, *ddd*
6β	35.2, *ddd*	4.77, *ddd*	3.65, *dd*	–	4.88, *dd*	–	3.65, *ddd*[d]	–
7α	~1.56[b]	~1.57[b]	1.67, *ddd*	2.76, *t*	[c]	2.49, *dd*	~1.56[b]	2.46, *dd*
7β	~1.56[b]	~1.57[b]	1.79, *ddd*	~2.24[b]	[c]	2.30, *dd*	~1.56[b]	2.39, *dd*
8β	~1.64[b]	~1.55[b]	~1.61[b]	~1.92[b]	[c]	[c]	~1.60[b]	~2.04[b]
10β	1.32, *dd*	1.32, *dd*	1.32, *ddd*	~1.80[b]	[c]	[c]	1.57, *br s*	2.05, *dd*
11A	~1.55[b]	~1.55[b]	~1.63[b]	~1.66[b]	[c]	[c]	[c]	~1.69[b]
11B	~1.55[b]	~1.55[b]	~1.63[b]	~1.66[b]	[c]	[c]	2.04, *ddd*	~1.69[b]
12A	~2.10[b]	2.10, *dddd*	2.09, *dddd*	~2.09[b]	~2.10[b]	[c]	2.28, *m*	~2.10[b]
12B	2.26, *dddd*	2.24, *dddd*	2.28, *dddd*	~2.25[b]	~2.25[b]	[c]	2.28, *m*	2.28, *dddd*
14	5.82, *quint*	5.83, *quint*	5.82, *quint*	5.84, *quint*	5.86, *quint*	5.87, *quint*	5.84, *quint*	5.84, *quint*
16 (2H)	4.72, *d*	4.72, *d*	4.72, *d*	4.73, *d*	4.75, *d*	4.76, *d*	4.73, *d*	4.72, *d*
Me-17	0.84, *d*	0.82, *d*	0.88, *d*	0.90, *d*	0.87, *d*	0.92, *d*	0.88, *d*	0.90, *d*
18A	2.42, *d*	2.24, *d*	2.54, *d*	2.25, *d*	2.34, *d*	3.88, *d*	2.70, *d*	–
18B	3.21, *dd*	2.92, *dd*	3.26, *dd*	3.37, *dd*	3.01, *dd*	3.95, *br d*	3.31, *dd*	–
19A	4.52, *d*	4.03, *ddd*[e]	10.22, *d*	4.79, *s*	10.20, *s*	4.56, *d*	4.07, *d*	4.60, *d*
19B	4.56, *br d*	4.30, *dd*[f]	–	4.79, *s*	–	4.83, *d*	4.16, *d*	4.76, *d*
Me-20	0.74, *s*	0.70, *s*	0.71, *s*	1.01, *s*	0.77, *s*	1.04, *s*	0.96, *s*	0.99, *s*
OAc	2.08, *s*	2.03, *s*	–	2.07, *s*	2.04, *s*	2.02, *s*	–	2.01, *s*
6α (OH)[g]	3.40, *s*	–	3.62, *s*	–	–	–	2.82, *d*	–
19 (OH)[g]	–	2.37, *dd*	–	–	–	–	–	–
$J_{H,H}$ (*Hz*)								
1α,1β	[b]	[b]	13.3	[b]	[b]	[b]	–	13.7
1α,2α	4.1	4.2	4.1	[b]	[b]	[b]	–	3.4
1α,2β	13.3	13.2	13.3	[b]	[b]	[b]	–	13.4
1α,10β	12.5	11.5	13.3	[b]	[b]	[b]	–	12.0
1β,2α	2.8	2.9	2.9	[b]	[b]	[b]	4.6	[b]
1β,2β	4.3	5.2	4.1	[b]	[b]	[b]	<0.3	[b]
1β,10β	3.4	4.4	4.2	[b]	[b]	[b]	<0.3	3.2
2α,2β	13.3	13.1	13.3	[b]	[b]	[b]	[b]	13.0
2α,3α	4.2	4.4	4.1	[b]	4.2	[b]	[b]	6.7
2α,3β	3.1	2.7	2.9	[b]	[b]	[b]	[b]	2.9
2β,3α	13.3	13.4	13.3	[b]	13.0	[b]	[b]	12.7
2β,3β	3.5	4.2	4.1	[b]	[b]	[b]	[b]	4.7
3α,3β	13.1	13.6	13.3	[b]	13.0	[b]	[b]	13.2
6β,7α	10.6	8.9	11.0	–	8.7	–	11.8	–
6β,7β	4.5	5.7	4.6	–	7.7	–	5.6	–
7α,7β	[b]	[b]	13.5	13.5	[b]	15.5	[b]	15.9
7α,8β	[b]	[b]	12.1	13.5	[b]	13.8	[b]	11.5
7β,8β	[b]	[b]	3.2	[b]	[b]	4.0	[b]	5.2
8β,17	6.2	6.3	6.7	6.7	6.0	6.7	6.8	6.8
11A,11B	[b]	[b]	[b]	[b]	[b]	[b]	12.6	[b]
11A,12A	[b]	9.8	12.8	[b]	[b]	[b]	[b]	[b]
11A,12B	4.5	4.5	5.0	[b]	[b]	[b]	[b]	4.6
11B,12A	[b]	4.5	3.2	[b]	[b]	[b]	4.8	[b]
11B,12B	9.8	9.8	9.5	[b]	[b]	[b]	7.1	8.1
12A,12B	13.6	13.5	13.7	[b]	[b]	[b]	[b]	14.0
12A(B),14	1.6	1.4	1.4	1.4	1.4	1.4	1.6	1.7
14,16A(B)	1.7	1.7	1.8	1.6	1.7	1.7	1.7	1.9
16A,16B	0	0	0	0	0	0	0	0
18A,18B	3.5	3.7	3.4	5.5	4.0	11.8	3.7	–
18B,3α	2.4	2.2	2.3	2.3	2.6	<0.6	2.1	–
19A,19B	12.0	12.0	–	0	–	11.7	9.4	12.0
19A,6β	0	1.5	0	–	0	0	0	–

(*continued on next page*)

Table 2 (*continued*)

	2	4	5	6	7	8	9	10
19B,6β	0.4	0	–	–	–	0	0	–
6β,6α (OH)[g]	0	–	0	–	–	–	1.6	–
19A,19 (OH)[g]	–	11.4	–	–	–	–	–	–
19B,19 (OH)[g]	–	1.4	–	–	–	–	–	–
19,10β	0	0	1.1	0	0	0	0	0

[a] At 500 MHz (**2**, **4, 5** and **10)** or 300 MHz **(6–9),** in $CDCl_3$ solution; chemical shifts are reported in δ values. All these assignments were in agreement with HSQC and HMBC spectra.
[b] Overlapped signal; approximate δ value was measured from the HSQC spectrum.
[c] Not measured due to overlapping.
[d] Collapsed into a *dd* ($J=$ 11.8, 5.6 Hz) after addition of D_2O.
[e] Collapsed into a *dd* ($J=$ 12.0, 1.5 Hz) after addition of D_2O.
[f] Collapsed into a *d* ($J=$ 12.0 Hz) after addition of D_2O.
[g] Interchangeable with D_2O.

Ajugarin II (**2**): colorless needles (EtOAc–petroleum ether), mp 179–181 °C; $[\alpha]_D^{23}$ +17.7° ($CHCl_3$,*c* 0.261); ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1785, 1755, 1640 (α,β-unsaturated γ-lactone), 1730, 1235 (OAc), 2960, 1450, 1390, 1375, 1325, 1165, 1040, 1020, 885, 850, 840; ^1H NMR: Table 2; ^{13}C NMR: Table 3; EIMS *m*/*z* (rel. int.): 392 $[M]^+$ (2), 362 (2), 361 (2), 332 (3), 320 (22), 319 (100), 301 (13), 189 (9), 167 (12), 165 (11), 147 (10), 123 (19), 121 (12), 119 (10), 107 (11), 105 (12), 98 (11), 95 (13), 93 (15), 91 (15), 81 (11), 79 (15), 69 (10), 63 (13), 55 (17). (Found: C, 67.51; H, 8.31. Calc. for $C_{22}H_{32}O_6$: C, 67.32; H, 8.22%.)

These data for **2** were in agreement with those reported for natural and semisynthetic ajugarin II [mp 188–189 °C, $[\alpha]_D^{24}$ +14.6° ($CHCl_3$,*c* 0.025)] (Kubo et al., 1976; Beauchamp et al., 1996; Bruno et al., 2000), and the present unequivocal and complete assignment of its ^1H and ^{13}C NMR spectra (Tables 2 and 3, respectively), based on two-dimensional NMR experiments, completes and corrects previous data reported by other authors (e.g. the chemical shifts of the C-7, C-8, C-13 and C-15 carbons) (Bruno et al., 2000).

Compound **4**: colorless needles (EtOAc–petroleum ether), mp 249–251 °C; $[\alpha]_D^{23}$ +5.5° ($CHCl_3$,*c* 0.272); ν_{max}^{KBr} cm^{-1}: 3620, 3590 (OH), 3130, 1795, 1760, 1630 (α,β-unsaturated γ-lactone), 3070 (oxirane), 1740, 1245 (OAc), 2960, 1455, 1370, 1170, 1125, 1080, 1040, 1010,

Table 3
^{13}C NMR spectral data of compunds **2** and **4–10**[a]

C	2	4	5	6	7	8	9	10
1	20.8, *t*	21.0, *t*	21.0, *t*	21.6, *t*	21.2, *t*	21.1, *t*	77.7, *d*	20.4, *t*
2	25.0, *t*	25.0, *t*	24.8, *t*	24.8, *t*	24.9, *t*	21.6, *t*	38.2, *t*	25.9, *t*
3	31.9, *t*	32.3, *t*	31.7, *t*	33.1, *t*	32.4, *t*	29.5, *t*	28.0, *t*	38.6, *t*
4	66.8, *s*	65.5, *s*	66.3, *s*	61.5, *s*	63.9, *s*	76.9, *s*	65.6, *s*	205.3, *s*
5	45.2, *s*	46.2, *s*	54.5, *s*	54.3, *s*	54.9, *s*	57.5, *s*	51.9, *s*	64.2, *s*
6	73.4, *d*	74.3, *d*	73.2, *d*	206.6, *s*	72.2, *d*	212.6, *s*	68.1, *d*	203.8, *s*
7	34.7, *t*	32.6, *t*	34.8, *t*	44.4, *t*	33.8, *t*	45.8, *t*	32.4, *t*	43.6, *t*
8	33.9, *d*	34.5, *d*	34.3, *d*	38.0, *d*	34.4, *d*	36.1, *d*	35.8, *d*	35.9, *d*
9	38.6, *s*	38.2, *s*	39.1, *s*	38.9, *s*	38.7, *s*	38.4, *s*	36.8, *s*	38.8, *s*
10	47.3, *d*	47.8, *d*	49.1, *d*	51.0, *d*	49.7, *d*	45.9, *d*	54.9, *d*	51.8, *d*
11	34.8, *t*	34.6, *t*	34.5, *t*	35.4, *t*	34.2, *t*	35.0, *t*	32.7, *t*	35.5, *t*
12	22.1, *t*	22.0, *t*	22.1, *t*	22.0, *t*	21.9, *t*	21.6, *t*	23.1, *t*	21.8, *t*
13	169.9, *s*	169.7, *s*	169.6 s	169.2, *s*	169.3, *s*	168.9, *s*	169.8, *s*	169.0, *s*
14	115.4, *d*	115.4, *d*	115.5, *d*	115.6, *d*	115.4, *d*	115.7, *d*	115.5, *d*	115.6, *d*
15	173.5, *s*	173.6, *s*	173.5, *s*	173.4, *s*	173.6, *s*	173.4, *s*	173.6, *s*	173.4, *s*
16	72.9, *t*	72.9, *t*	72.8, *t*	72.9, *t*	72.9, *t*	72.9, *t*	72.9, *t*	72.9, *t*
17	15.4, *q*	15.3, *q*	15.4, *q*	15.8, *q*	15.3, *q*	15.5, *q*	15.3, *q*	15.9, *q*
18	48.7, *t*	47.5, *t*	50.4, *t*	48.9, *t*	49.8, *t*	48.3, *t*	53.2, *t*	–
19	62.0, *t*	61.6, *t*	205.1, *d*	62.7, *t*	203.9, *d*	61.8, *t*	71.4, *t*	62.7, *t*
20	17.6, *q*	17.3, *q*	18.8, *q*	17.9, *q*	18.6, *q*	16.8, *q*	16.8, *q*	17.8, *q*
$CH_3\underline{C}OO$	171.0, *s*	169.5, *s*	–	171.0, *s*	170.1, *s*	170.0, *s*	–	170.6, *s*
$\underline{C}H_3COO$	21.1, *q*	21.3, *q*	–	20.8, *q*	21.1, *q*	21.0, *q*	–	20.7, *q*

[a] At 125.7 MHz, in $CDCl_3$ solution; chemical shifts are reported in, δ values. All these assignments were in agreement with HSQC and HMBC spectra. Multiplicities were determined from HSQC data.

960, 890, 855, 845; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3; EIMS *m/z* (rel. int.): 392 $[M]^+$ (1), 361 (12), 332 (3), 331 (5), 320 (21), 319 (100), 301 (17), 289 (23), 191 (11), 189 (13), 147 (8), 136 (8), 123 (12), 121 (11), 107 (10), 105 (10), 95 (10), 93 (12), 91 (14), 79 (14), 67 (11), 55 (15). (Found: C, 67.21; H, 8.13. $C_{22}H_{32}O_6$ requires: C, 67.32; H, 8.22%.)

3.3. Preparation of 4α,18-epoxy-6α-hydroxy-19-oxoneoclerod-13-en-15,16-olide (5) from deacetylajugarin II (3)

To a stirred solution of **3** (120 mg, 0.342 mmol) in dry CH_2Cl_2 (5 ml) an excess of the Dess–Martin periodinane (157 mg, 0.37 mmol) (Dess and Martin, 1991) was added at room temperature under Ar. After 20 h, the reaction mixture was filtered through a short column of Si gel and the solvent was evaporated under vacuum. The residue was then subjected to column chromatography (EtOAc as eluent) yielding pure **5** (115 mg, 0.330 mmol, 96.4%) as colorless needles (EtOAc–petroleum ether): mp 163–165 °C; $[\alpha]_D^{23}$ +25.1° ($CHCl_3$,*c* 0.422); ν_{max}^{KBr} cm^{-1}: 3460 (OH), 3110, 1775, 1750, 1645 (α,β-unsaturated γ-lactone), 3070 (oxirane), 2770, 1715 (aldehyde), 2970, 2880, 1460, 1395, 1330, 1260, 1135, 1060, 1020, 915, 880; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3; EIMS *m/z* (rel. int.): 349 $[MH]^+$ (1), 302 (2), 287 (4), 275(2), 231 (3), 211 (10), 191 (17), 189 (21), 173 (14), 171 (11), 163 (12), 161 (11), 159 (10), 149 (14), 147 (17), 145 (18), 138 (34), 136 (20), 135 (18), 123 (24), 122 (33), 121 (43), 98 (44), 95 (47), 93 (70), 91 (84), 81 (57), 79 (100), 77 (62), 67 (64), 55 (87). (Found: C, 69.09; H, 8.17. $C_{20}H_{28}O_5$ requires: C, 68.94; H, 8.10%.)

3.4. Preparation of 19-acetoxy-4α,18-epoxy-6-oxoneoclerod-13-en-15,16-olide (6) from ajugarin II (2)

To a solution of **2** (80 mg, 0.20 mmol) in dry pyridine (8 ml) a mixture of CrO_3 (200 mg) and pyridine (2 ml) was added, and the reaction mixture was stirred at room temperature for 20 h under Ar (Sarett oxidation procedure, Poos et al., 1953). Then, the reaction mixture was poured into H_2O (50 ml) and extracted with EtOAc (4×25 ml). The extracts were dried ($MgSO_4$) and evaporated to dryness. The residue was chromatographed (Si gel column, petroleum ether–Me_2CO from 20 to 40% in Me_2CO as eluent), giving unreacted **2** (12 mg, 0.03 mmol, 15%, less polar constituent) and **6** (63 mg, 0.16 mmol, 79.1%) as colorless needles (EtOAc–*n*-hexane): mp 147–148 °C; $[\alpha]_D^{22}$ +11.9° ($CHCl_3$, *c* 0.184); ν_{max}^{KBr} cm^{-1}: 3100, 1780, 1750 br, 1645 (α,β-unsaturated γ-lactone), 1740, 1250 (OAc), 1725 (ketone), 2960, 2950, 1450, 1370, 1215, 1140, 1035, 905, 885, 865; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3; EIMS *m/z* (rel. int.): $[M]^+$ absent, 330 $[M–AcOH]^+$ (1), 317 $[M–CH_2OAc]^+$ (100), 299 (13), 289 (20), 189 (17), 137 (13), 121 (11), 105 (10), 93 (10), 91 (15), 81 (11), 79 (14), 69 (13), 67 (11), 55 (15). (Found: C, 67.76; H, 7.61. $C_{22}H_{30}O_6$ requires: C, 67.67; H, 7.74%.)

3.5. Preparation of 6α-acetoxy-4α,18-epoxy-19-oxoneoclerod-13-en-15,16-olide (7) from compound 4

To a stirred solution of **4** (60 mg, 0.153 mmol) in dry CH_2Cl_2 (10 ml) NMO (20 mg, 0.170 mmol), TPAP (3 mg, 0.0084 mmol), and powdered molecular sieve (4 Å, 77 mg) were added (Ley et al., 1994). The reaction mixture was stirred at room temperature under Ar for 2 h, and then filtered through a pad of Celite. Removal of the solvent under vacuum, followed by column chromatography of the residue [EtOAc–petroleum ether (4:1) as eluent] yielded **7** (36 mg, 0.09 mmol, 60.3%) and unreacted **4** (9 mg, 0.02 mmol, 15%).

Oxidation of **4** with the Dess–Martin periodinane, as described above for **3**, gave **7** in 30% yield after 24 h of reaction.

Compound **7** was crystallized from EtOAc–*n*-hexane: colorless needles, mp 233–235 °C; $[\alpha]_D^{23}$ +38.1° ($CHCl_3$, *c* 0.063); ν_{max}^{KBr} cm^{-1}: 3130, 1790, 1750, 1635 (α,β-unsaturated γ-lactone), 2780, 1725 (aldehyde), 1740, 1240 (OAc), 2990, 2950, 2880, 1445, 1375, 1175, 1120, 1030, 1015, 1000, 890, 860; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3; EIMS *m/z* (rel. int.): $[M]^+$ absent, 330 $[M\text{-}AcOH]^+$ (2), 319 (64), 302 (32), 301 (30), 290 (55), 289 (100), 287 (25), 205 (23), 193 (29), 191 (48), 189 (46), 187 (30), 173 (47), 171 (32), 163 (39), 159 (49), 145 (44), 136 (59), 121 (62), 105 (55), 93 (51), 91 (72), 79 (68), 67 (47), 55 (62). (Found: C, 67.54; H, 7.82. $C_{22}H_{30}O_6$ requires: C, 67.67; H, 7.74%.)

3.6. Preparation of 19-acetoxy-18-chloro-4α-hydroxy-6-oxoneoclerod-13-en-15,16-olide (8) from ajugarin II (2)

To a stirred solution of **2** (76 mg, 0.19 mmol) in dry CH_2Cl_2 (5 ml) an excess of PCC (85 mg, 0.39 mmol) and powdered molecular sieve (4 Å, 30 mg) were added, and the reaction mixture was stirred under Ar at room temperature. After 5 days, the mixture was filtered through a short column of Si gel and the filtrate was evaporated to dryness. Then, the residue was subjected to column chromatography [EtOAc–petroleum ether (1:1) as eluent] yielding starting material (**2**, 36 mg, 0.09 mmol, 47.3%, most polar constituent) and compound **8** (18 mg, 0.04 mmol, 21.7%): colorless needles (EtOAc–*n*-hexane), mp 154–156 °C; $[\alpha]_D^{22}$ −41.7° ($CHCl_3$, *c* 0.285); ν_{max}^{KBr} cm^{-1}: 3490, 3460 (OH), 1790, 1755, 1635 (α,β-unsaturated γ-lactone), 1745, 1245 (OAc), 1700, 1690 (chelated ketone), 2960, 2930, 1450, 1385, 1335, 1180, 1130, 1035, 1010, 890, 790, 730, 630; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3; EIMS *m/z* (rel. int.): $[M]^+$ absent, 391 $[M\text{-}Cl]^+$ (12), 377 (12), 317 (100), 305 (18), 301 (35), 289 (15), 271 (11), 269 (10), 205 (14), 201 (11), 189 (23), 187 (13), 165 (21), 149 (31), 137 (24), 121 (21), 95 (23), 93 (20), 91 (27), 81 (30), 79 (29), 69 (32), 67 (26), 57 (25),

55 (41). (Found: C, 61.68; H, 7.41; Cl, 8.21. $C_{22}H_{31}ClO_6$ requires: C, 61.89; H, 7.32; Cl, 8.30%.)

3.7. Preparation of 1α,19;4α,18-diepoxy-6α-hydroxyneoclerod-13-en-15,16-olide (9) from deacetylajugarin II (3)

Compound **3** (100 mg, 0.286 mmol) was dissolved in dry benzene (40 ml) and treated with IBD (94 mg, 1 eq.) and I_2 (73 mg, 1 eq.) (Majetich and Wheless, 1995). The reaction mixture was heated at 50 °C under Ar while it was irradiated with a 200 W tungsten lamp. After 35 min, the reaction mixture was poured into an aqueous saturated solution of $Na_2S_2O_3$ to eliminate the excess of I_2, and extracted with EtOAc (4×30 ml). The organic extract was dried (Na_2SO_4) and the solvents removed under vacuum. The residue was then chromatographed (Si gel column, gradient of elution petroleum ether–EtOAc from 20 to 70% in EtOAc) to give **9** (72 mg, 0.207 mmol, 72.4%): colorless fine needles (EtOAc– *n*-hexane), mp 148–151 °C; $[\alpha]_D^{22}$ −15.9° ($CHCl_3$, *c* 0.169); $\nu_{max}^{KBr}cm^{-1}$: 3510, 3490 (OH), 3100, 1780, 1750, 1640 (α,β-unsaturated γ-lactone), 2940, 2880, 1455, 1445, 1365, 1320, 1160, 1130, 1050, 1015, 900, 880, 865, 640; ^1H NMR: Table 2; ^{13}C NMR: Table 3; EIMS *m*/*z* (rel. int.): $[M]^+$ absent, 316 $[M\text{-}CH_3OH]^+$ (1), 287 (3), 219 (5), 205 (6), 191 (8), 177 (9), 175 (10), 173 (16), 149 (15), 135 (17), 133 (18), 121 (28), 119 (25), 111 (43), 105 (40), 98 (38), 95 (39), 93 (39), 91 (68), 85 (36), 81 (54), 79 (65), 77 (50), 69 (59), 67 (69), 57 (34), 55 (100). (Found: C, 68.79; H 8.16. $C_{20}H_{28}O_5$ requires: C, 68.94; H, 8.10%.)

3.8. Preparation of 19-acetoxy-4,6-dioxo-18-nor-neoclerod-13-en-15,16-olide (10) from ajugarin II (2)

To a solution of **2** (60 mg, 0.153 mmol) in Me_2CO (10 ml) an excess (0.5 ml) of Jones' reagent (Bowers et al., 1953) was added at 0 °C with stirring. The reaction mixture was then stirred at room temperature for 2.5 h and the excess of Jones' reagent was destroyed by addition of EtOH (5 ml). Then, the reaction was diluted with H_2O (50 ml). Extraction with EtOAc (4×20 ml), and workup as usual gave a residue which was subjected to column chromatography [petroleum ether–EtOAc (7:3) as eluent] yielding **10** (32 mg, 0.085 mmol, 55.6%): colorless prisms (EtOAc– *n*-hexane), mp 176–177 °C; $[\alpha]_D^{22}$ +8.2° ($CHCl_3$, *c* 0.294); ν_{max}^{KBr} cm^{-1}: 3140, 1805, 1775, 1655 (α,β-unsaturated γ-lactone), 1750,1245 (OAc), 1740, 1715 (ketones), 2995, 1460, 1395, 1210, 1160, 1045, 1000, 910, 895; ^1H NMR: Table 2; ^{13}C NMR: Table 3; EIMS *m*/*z* (rel. int.): 376 $[M]^+$ (9), 346 (16), 334 (80), 329 (15), 316 (15), 305 (25), 303 (30), 288 (60), 275 (19), 274 (19), 260 (18), 224 (63), 206 (29), 205 (37), 177 (28), 169 (30), 165 (54), 137 (100), 123 (36), 121 (31), 119 (29), 109 (63), 105 (32), 98 (39), 95 (32), 69 (66), 67 (43), 55 (86). (Found: C, 66.87; H, 7.46. $C_{21}H_{28}O_6$ requires: C, 67.00; H,7.50%.)

3.9. Insects for the bioassays

Newly ecdysed fourth instar larvae of *L. decemlineata* were obtained from a colony reared on potato, *Solanum tuberosum*, cv. Kennebec, at 25±2 °C, 90±10% rh and 16:8 h (L:D) photoperiod in a environmental chamber. This colony is renewed annually with wild adults collected from potato fields. *S. exigua* eggs were obtained from the University of Leiden (The Netherlands). The larvae were reared on a semi-artificial diet (Poitout and Bues, 1970) under environmental conditions identical to those described for *L. decemlineata*.

3.10. Feeding experiments

Assays were performed in plastic Petri dishes (15×90 mm), coated on their bottom half with about 20 ml of a 2.5% agar solution (Escoubas et al., 1993). Potato and sugar beet leaf disks (1.77 cm^2) for *L. decemlineata* and *S. exigua*, respectively, were cut with a cork borer No. 15 and fit into holes punched in the agar layer.

The disks were treated on the upper surface with 20 μl of an acetone solution containing 1000 ppm of the test compound or the solvent carrier alone. After complete evaporation of the solvent, newly emerged fourth instar *L. decemlineata* larvae and fifth instar *S. exigua* were starved for 6 h, and placed in each dish in a growth chamber at 26±0.5 °C and 85±10% rh, where they were allowed to feed. In the choice assay, three treated and three control disks were alternately arranged in each arena. For the no-choice assays, six treated or control disks were used in each arena. Feeding was terminated after the consumption of 50% of the disks (control plus treated) in the choice assay and when larvae had ingested about 75% of the control disks in the no-choice assay. Ten replications per treatment were used in all assays.

Feeding indexes were calculated on a dry weight basis. For each assay, initial dry weight of the leaf disks was estimated with 100 leaf disks to calculate the ratio of fresh to dry weight. At the end of the experiment, the uneaten leaf disks were oven dried at 60 °C for 2 days and weighed. The antifeedant index (AI) was calculated in the no-choice assay by the equation $[(C-T)/C]\times100\%$ (Bentley et al., 1984), and in the choice assay by the equation $[(C-T)/(C+T)]\times100\%$ (Simmonds et al., 1989), where *C* and *T* represent the consumption in control and treated disks, respectively. The suppression index (SI) was determined by the equation $[(C-T)/C]\times100\%$, where *T* represents consumption of both treated and untreated leaf disks in choice arenas, and C represents the consumption in control arenas that contain untreated leaf disks only (Raffa and Frazier, 1988). No antifeedant activity was considered for indexes below 50%.

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