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Bioactive triterpene derivatives from latex of two *Euphorbia* species

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Abstract

We have investigated the antifeedant and toxic effects of 23 semisynthetic terpenoid derivatives obtained through chemical modifications of the major components of *Euphorbia resinifera* (α-euphol and α-euphorbol) and *E. officinarum* (obtusifoliol and 31-norlanostenol) latex on several insect species (*Spodoptera littoralis, Myzus persicae* and *Rhopalosiphum padi*), their selective cytotoxicity on insect Sf9 and mammalian CHO cells and their phytotoxic effects on *Lactuca sativa*. The conversions focused mainly on positions 3, 7, 11, and 24 with several oxidizing agents. A total of 18 compounds affected *S. littoralis* growth (IGR). Our results support the importance of the C-3 substituent, suggest the involvement of the C-7 substituent and indicate that the C-3 hydroxyl is not essential for the IGR effect. Overall, Sf9 cells were more sensitive to the active compounds than CHO cells. All of these compounds had non selective moderate phytotoxic effects on radicle elongation of *L. sativa*.
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Keywords: *Euphorbia*; Triterpene; Insect growth regulation; *Spodoptera littoralis*

1. Introduction

The coastal and sub coastal area of south-western Morocco (Anti-Atlas), is a unique and endangered botanical area dominated by the Argan (*Argania spinosa*), the gum tree (*Acacia gummifera*) and various *Euphorbia* shrubs (*E. obtusifolia* subsp. *regis-jubae*, *E. balsamifera*) or cactiform (*Euphorbia officinarum* subsp. *echinus*, *E. beaumierana*, or *E. resinifera*) (Médail and Quézel, 1999). The latex of *E. resinifera* (Euphorbium) and *E. officinarum* are used in traditional medicine (Appendino and Szallasi, 1997; Bellakhdar, 1997). The main active principle in Euphorbium is resiniferatoxin (Hergenhahn et al., 1975). These latexes are also rich in euphol, euphorbol, obtusifoliol and 31-norlanostenol triterpenes (Benharref and Lavergne, 1985; Daoubi et al., 2004). These compounds have been reported as anti-inflammatory and antiviral and also affected *Xenopus* cell division (Akihisa et al., 2002; Wang et al., 2003; Yasukawa et al., 2000).

As part of our ongoing valorisation of *Euphorbia* species native to the Anti-Atlas Mountains of Morocco, we have investigated the antifeedant and toxic effects of 23 semisynthetic terpenoid derivatives from the major components of *E. resinifera* (α-euphol and α-euphorbol) and *E. officinarum* (obtusifoliol and 31-norlanostenol) latex (Benharref and Lavergne, 1985; Daoubi et al., 2004, 2007; Mazoir et al., 2005) (Schemes 1 and 2) on several insect species with varying feeding adaptations (*Spodoptera littoralis, Myzus persicae* and *Rhopalosiphum padi*). We have also tested the selective cytotoxicity of these compounds on insect Sf9 and mammalian CHO cells. Additionally, we have tested their phytotoxic effects on *Lactuca sativa*.

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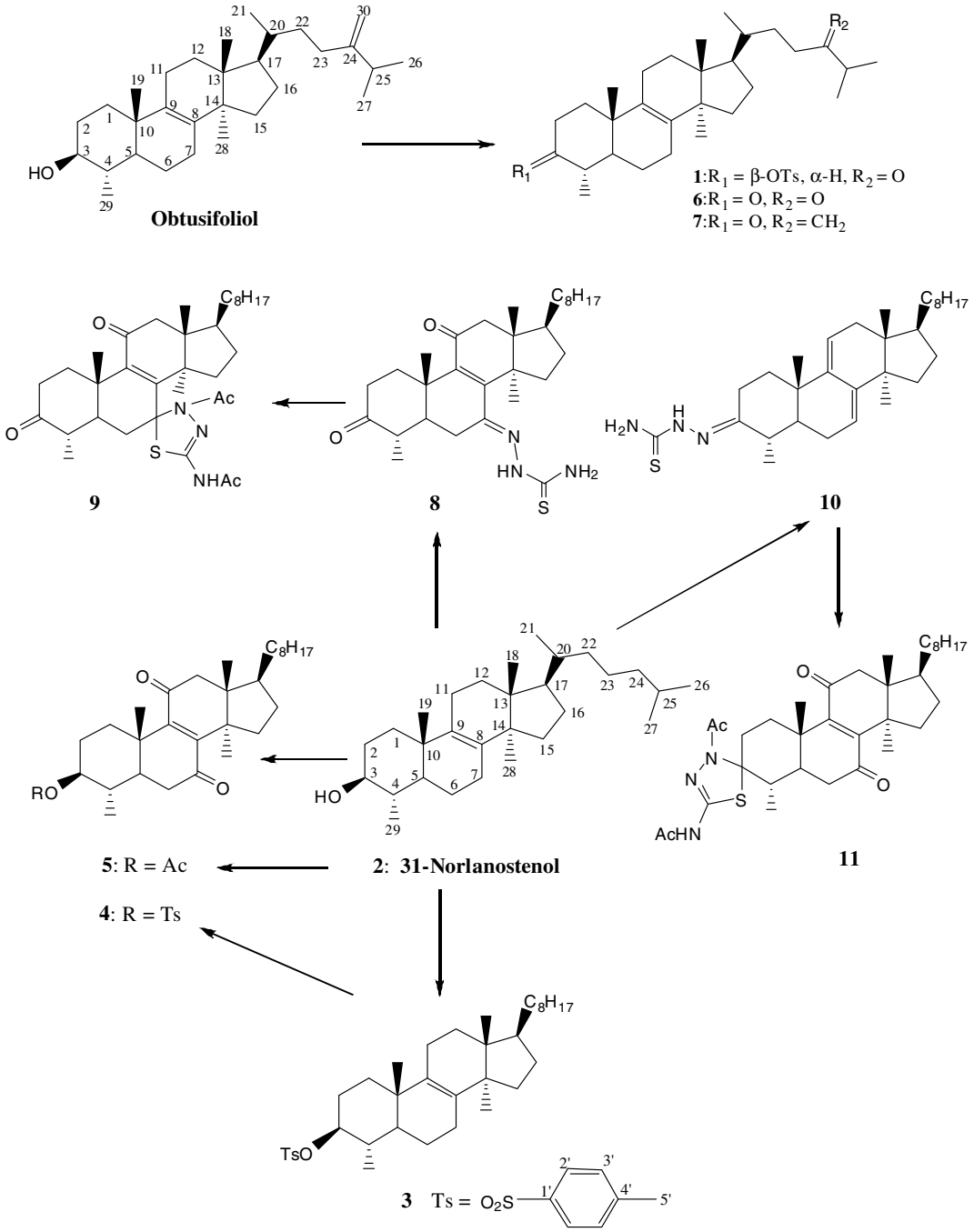
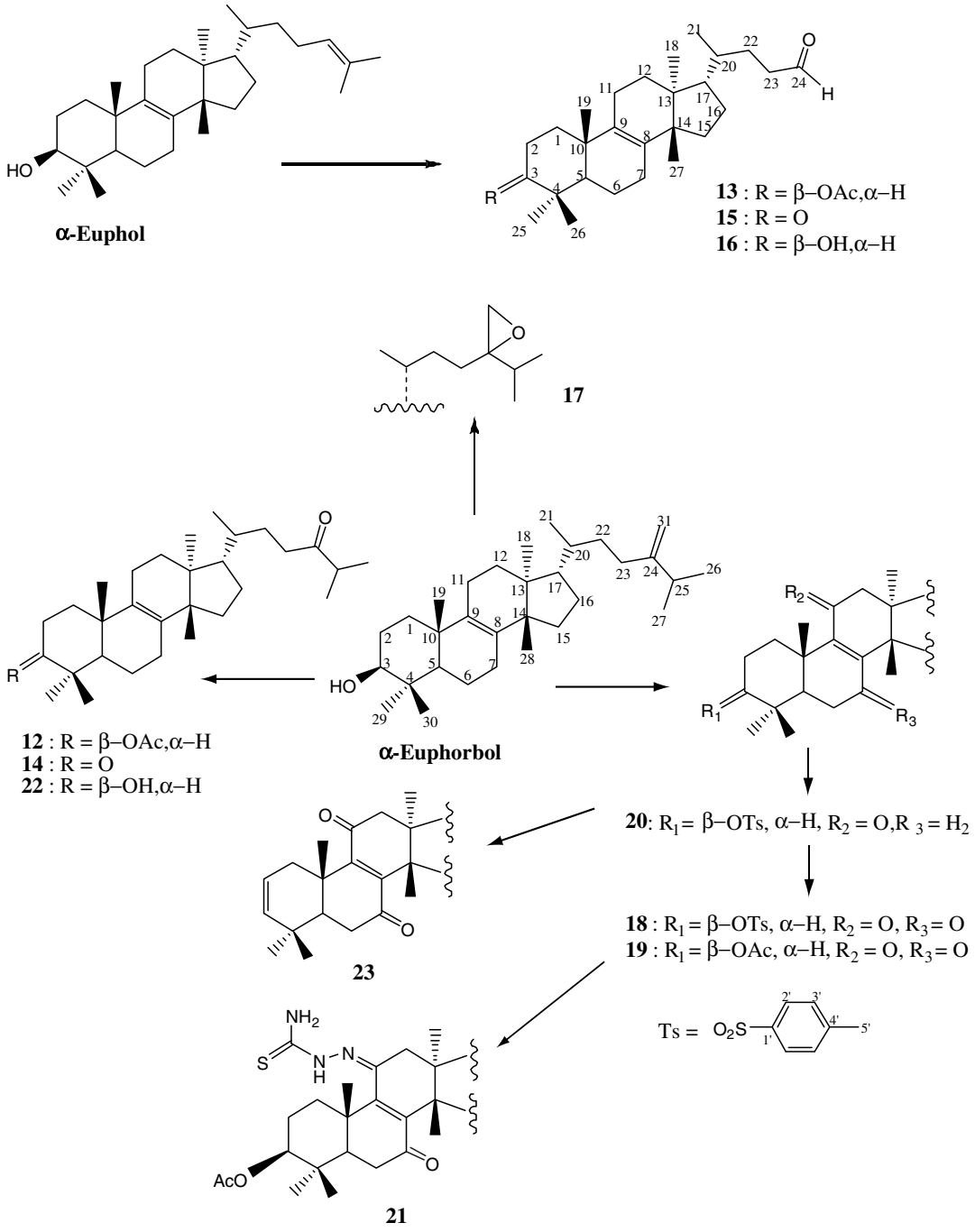
2. Results and discussion

The derivatives studied here were obtained through chemical modifications of natural triterpenes (Schemes 1 and 2). The conversions focused mainly on positions 3, 7, 11, and 24 with several oxidizing agents such as chromic anhydride (CrO_3) (Dakir et al., 2004; Mazoir et al., 2007), sodium periodate ($NaIO_4$) with ruthenium trichloride ($RuCl_3 \cdot 3H_2O$) (Mazoir et al., 2005) and *meta*-chloroperbenzoic acid (*m*-CPBA) (El Jamili et al., 2002) to synthesize new functionalized triterpene derivatives with a good yield and high chimioselectivity.

Our results showed that several of the test compounds were moderate antifeedants in choice tests when compared with the positive controls when tested at an initial dose of 50 μg/cm^2 (%SI = 98 for polygodial on *M. persicae* and *R. padi* and %FI = 100 for ryanodine on *S. littoralis* at the same dose). Specifically, 31-norlanostenol **2**, **4**, **6**, **8**, **12**, **13**, **14**, and **18**, were active to *M. persicae* (%SI between 73 ± 10 and 67 ± 9, $p < 0.05$ Wilcoxon Paired Test); com-



Scheme 1. Derivatives from *Euphorbia officinarum*.



Scheme 2. Derivatives from *Euphorbia resinifera*.

pounds **2**, **5**, **7**, **13**, **15**, and **16** were active to *R. padi* (% SI between 70 ± 12 and 65 ± 10; $p < 0.05$ Wilcoxon Paired Test) and **9** and **15** showed moderate-low antifeedant effects to *S. littoralis* (%FI of 55 ± 10 and 52 ± 9, respectively, $p < 0.05$ Wilcoxon Paired Test). Therefore, these effects did not merit further dose-response experiments (%FI/SI < 75).

The fact that these insect species showed some feeding behaviour response to the test compounds is not surprising. Several insect species including *S. littoralis* have deterrent taste receptors that can detect 20-hydroxyecdysone (Marion-Poll and Descoins, 2002) resulting in antifeedant effects. Antifeedant effects of ecdysteroids to aphids including *M. persicae* have been also reported (Liktor-Busa et al., 2007; Malausa et al., 2006).

A large number of the test substances (80%) had postingestive effects on *S. littoralis* (Table 1). Among these substances, **3**, **4**, **5**, **8**, **10**, **11**, **14**, and **16** resulted in negative values for biomass gain (ΔB) while significantly reducing ingestion (ΔI). Triterpenes **1**, **2**, and **13** reduced ΔB, but not ΔI. Treatment effects on ΔB did not disappear with covariance adjustment (ANCOVA2, Table 1)

Table 1
Postingestive effects of the test compounds on biomass gain (ΔB) and consumption (ΔI) of orally injected *S. littoralis* L6 larvae (40 μg/larvae)

Compound	ΔB[a]	ΔI[b]	pANCOVA2[c]	Sf9[d]	CHO[d]
1	57**	82	0.0002	80.87 (73.19, 89.37)	>100
2	76*	113	0.0001	94.69 (72.14, 124.3)	>100
3	−12**	22**	0.010	>100	>100
4	−7**	32**	0.033	≈100	>100
5	−17**	22**	0.093	$100 > LD_{50} > 50$	>100
6	103	108	–	15.67 (11.49, 21.37)	22.35 (21.15, 23.63)
7	52*	72*	0.018	14.48 (10.96, 21.86)	82.39 (80.45, 84.38)
8	−8**	31**	0.0007	14.05 (9.92, 19.93)	75.56 (71.18, 80.22)
9	37**	32**	0.054	25.99 (21.98, 30.73)	18.27 (15.23, 21.91)
10	−20**	24**	0.034	67.25 (57.76, 78.29)	>100
11	−5**	54**	0.00001	>100	>100
12	32**	53**	0.016	>100	≈100
13	55*	81	0.0001	17.69 (9.48, 33.0)	23.40 (19.08, 28.72)
14	−3**	28**	0.00001	14.41 (7.52, 27.63)	20.59 (13.12, 32.32)
15	53*	51*	0.102	≈50	28.30 (23.17, 34.57)
16	−4**	32**	0.00001	$10 > LD_{50} > 1$	35.86 (29.22, 44.01)
17	86	79*	–	$50 > LD_{50} > 25$	28.24 (23.58, 28.24)
18	92	85	–	26.92 (20.23, 35.82)	>100
19	99	100	–	2.79 (1.75, 4.46)	32.91 (29.36, 36.9)
20	98	94	–	>100	>100
21	1**	42**	0.002	9.04 (6.73, 12.15)	5.25 (3.98, 6.92)
22	39*	64*	0.00001	10.44 (6.62, 16.47)	$50 > LD_{50} > 25$
23	92	94	–	0.6 (0.32, 1.13)	13.92 (10.69, 18.14)
Rotenone[e]	56*	62*	<0.05	0.23 (0.15, 0.30)	38.14 (14.36, 101.16)

Cytotoxicity on insect and mammalian cells (Sf9 and CHO, respectively).
[a] ΔB: change in insect body weight (mg dry weight).
[b] ΔI: mg food consumed (mg dry weight).
[c] Treatment *p*-level, ANCOVA2 (ΔI as covariate).
[d] Concentration (μg/ml) needed to give 50% cell viability and 95% confidence limits (lower, upper).
[e] From González-Coloma et al. (2002).
* <0.05.
** <0.0005, ANCOVA1 (initial larval weight as covariate).

except for **5**, **9**, and **15**. Therefore, these compounds acted as strong postingestive toxicants, with **5**, **9**, and **15** being less potent. Derivative **17** had only a moderate effect on ingestion.

For *E. officinarum* derivatives (Scheme 1), we found that a C-3, C-11-oxo with a C-7 thiosemicarbazone (**8**) or thiadiazoline (**9**), C-3 tosylation (**3** vs. **2**) and C-7, C-11-oxo (**5**, **4**, and **11**) or a different pattern of B and C ring instauration (**10**) resulted in strong larval postingestive toxicity. A ketone substitution of the C-3 hydroxyl (**7**) or the modification of the lateral chain at C-24 (**1**) resulted in a moderate increase of activity. For *E. resinifera* derivatives (Scheme 2) hydroxyl/oxo group at C-3 plus ketone at C-24 (**12**, **14**, **22**, **13**, **15**, and **16**) are structural requirements for varying potencies of larval postingestive toxicity. Acetylation/tosylation at C-3 lowered the activity (**18**, **19**, and **20**) while the C-11 thiosemicarbazone (**21**) increased this effect. 2,3 Instauration of the A-ring resulted in inactivation (**23**). Compounds **1**, **22**, and **6**, **14** with antipodal configurations at chiral centres C-13, C-14 and C-17 of their D-rings showed different postingestive effects with a moderate increase between **1** and **22** and a drastic change between **6** (inactive) and **14** (strong effect).

Plant sterols such as peniocerol and macdougallin with structural similarities to 31-norlanostenol (**2**) acted as efficient insect growth regulators (IGR) on *S. frugiperda* and *Tenebrio molitor*. Conversion of the C-3, C-6 hydroxyl groups to the tosyl derivative resulted in a significant loss of activity (Céspedes et al., 2005). Our results support the importance of the C-3 substituent, suggest the involvement of the C-7 groups and indicate that a C-3 hydroxyl is not essential for the IGR effect of this class of compounds as suggested (Céspedes et al., 2005).

Overall, Sf9 cells were more sensitive to the active compounds than CHO (Table 1) maybe due to differences in membrane composition and/or receptor affinity between insect and mammalian cells (Hu et al., 2004; Marheineke et al., 1998). Rotenone (González-Coloma et al., 2002) had the strongest cytotoxic effect to Sf9, followed by **23 > 16**, **19 > 21**, **22 > 6**, **7**, **8**, **13**, **14 > 9**, **18** with **7**, **8**, and **18** being selective to this cell line (Table 1). These Sf9 cytotoxic compounds also had larval postingestive effects except for **23**, **19**, and **6**, suggesting metabolic detoxification of these terpenes as described for ecdysteroids in this insect (Webb et al., 1995). Compounds **21**, **23**, **6**, **13** and **14** had the strongest cytotoxicity to mammalian CHO cells (Table 1).

Insect cell lines have been shown to respond to ecdysone by clumping, generating filamentous extensions and increased mortality (Hu et al., 2004) and lanostane and euphane triterpenes have been reported as cytotoxic inhibiting DNA synthesis in cancer cells or arresting *Xenopus* cell division (Li et al., 2005; Ukiya et al., 2002; Wang et al., 2003).

Compounds **4**, **5**, **18**, **19**, **23**, **16** and **22** inhibited *L. sativa* germination moderately during the first 24 h of the experiment (Fig. 1). All the compounds tested inhibited *L. sativa* radicle elongation (average inhibition of 50%).

Sterols are ubiquitous components of the plant plasma membrane and play an important role in membrane fluidity and permeability (Hartmann, 1998; Schaller, 2003) and as precursors of brassinosteroids, a group of plant growth regulators (Clouse and Sasse, 1998; Clouse, 2002). Studies performed with obtusifoliol 14α-demethylase inhibitors, a key step in sterol biosynthesis (O'Brien et al., 2005), including 7-oxo-obtusifoliol derivatives, showed a good correlation between their inhibition constants and their herbicidal activities (Rahier and Taton, 1997). Furthermore, exogenous treatments with plant sterols including obtusifoliol, modulated the expression of genes involved in cell expansion or division acting as signaling molecules (He et al., 2003; O'Brien et al., 2005). However our test compounds had non-selective phytotoxic effects on *L. sativa* root elongation suggesting their interference with the plant membrane rather than acting as chemical signals and/or by inhibition of obtusifoliol 14DM.

In summary, a series of semisynthetic triterpene derivatives of α-euphol, α-euphorbol, obtusifoliol and 31-norlanostenol have been prepared with several oxidizing agents focusing on positions **3**, **7**, **11**, and **24**. We have found that only a few of the test compounds (18–20%) randomly distributed among the different structures acted as antifeedants on *M. persicae* and *R. padi*. A larger number of the test substances (80%) had postingestive effects on *S. littorallis,* affecting insect growth (IGR). Our results support the importance of the C-3 substituent, suggest the involvement of the C-7 substituent and indicate that a C-3 hydroxyl is not essential for the IGR effect as previously suggested. Overall, Sf9 insect cells were more sensitive to the active compounds than mammalian CHO. Among the larval toxicants, **3–5**, **7**, **8**, **10**, **11**, **12** and **22** were not cytotoxic to CHO cells. All of these compounds had phytotoxic effects on radicle elongation of *L. sativa*. Therefore, through simple oxidations of *E. resinifera* and *E. officinarum* major latex triterpenes we have obtained insect toxicants (IGRs) with moderate-low effect on mammalian cells and plants. Further research is needed in to asses the environmental risk of this class of compounds.

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on AMX2 Brüker 500 MHz using TMS as an internal standard and $CDCl_3$ as the solvent. Melting points were measured in a Buchi 510 apparatus. Chemical shifts are given in ppm. Mass spectra were obtained with an Autospect Instrument 70 ev spectrometer. Column chromatography was carried

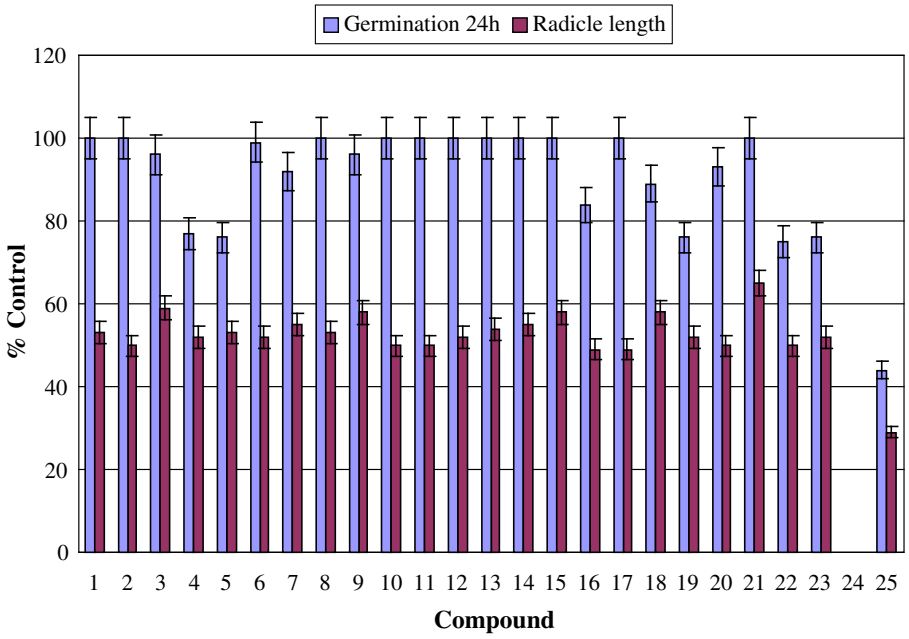


Fig. 1. Phytotoxic activity of triterpene derivatives (50 μg/cm^2) against *Lactuca sativa* germination and radicle length (expressed as % control). Kuskall Wallis test *H* and *p*-values were 68.42, 0.00003 for 24h; 33.95, 0.202 for 48 h and 124.90, <0.0001 for radicle length. Compound **25** is the positive control juglone.

out on silica gel (Merk Art. 15111, 7741), eluent: hexane–EtOAc. *p*-Toluenesulfonyl chloride (TsCl), chromic anhydride (CrO_3), sodium periodate ($NaIO_4$), ruthenium trichloride ($RuCl_3 \cdot 3H_2O$) and *m*-CPBA were from Aldrich.

3.2. Plant material

Latex from *E. resinifera* and *E. officinarum* were collected in the area of Demnat and Agadir (Morocco), respectively, and identified by Dr. A. Ouhammou (Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad, Marrakech, Morocco). Latex was obtained as described (Benharref and Lavergne, 1985; Daoubi et al., 2004, 2007; Mazoir et al., 2005). A voucher specimen has been deposited at the Cadi Ayyad University Herbarium (Voucher Number 5511 and 5512 for *E. resinifera* and *E. officinarum,* respectively).

3.3. Extraction and isolation

The major triterpenes of *E. resinifera* and *E. officinarum* latex were extracted and isolated as described (Benharref and Lavergne, 1985). *E. resinifera* afforded α-euphol (40.1% yield of latex) and α-euphorbol (17.9% yield of latex) (Benharref and Lavergne, 1985). *E. officinarum* afforded obtusifoliol (17.9% yield of latex) and 31-norlanostenol (19.7% yield of latex) (Benharref and Lavergne, 1985). These compounds were oxidized following the sequences described in Schemes 1 and 2.

3.4. Synthesis

3.4.1. 3β-Tosyloxy-4α,14α-dimethyl-5α-cholest-8-ene ($C_{36}H_{56}O_3S$) (**3**)

A mixture of 100 mg (0.24 mmol) of 31-norlanostenol (**2**) dissolved in 5 ml of pyridine and 184.2 mg (0.96 mmol) of *p*-toluenesulfonyl chloride (TsCl) was stirred at room temperature during 12 h and then 100 ml of cold water was added. The tosylated product obtained was filtered under reduced pressure and recrystallized from MeOH to give compound **3** (134.45 mg, 98% yield); m.p. 142–143 °C (MeOH); EIMS *m/z* $[M]^+$ 568 (80); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.08 (1H, *ddd*, *J* = 11.0, 11.0, 3.0 Hz, H-3), 0.68 (3H, *s*, H-18), 0.95 (3H, *s*, H-19), 0.84 (3H, *d*, *J* = 2.2 Hz, H-26), 0.85 (3H, *d*, *J* = 2.2 Hz, H-27), 0.87 (3H, *d*, *J* = 6.4 Hz, H-21), 0.86 (3H, *s*, H-28), 0.79 (3H, *d*, *J* = 6.4 Hz, H-29), 7.78 (2H, *d*, *J* = 8.8 Hz, H-2′), 7.31 (2H, *d*, *J* = 8.8 Hz, H-3′), 2.43 (3H, *s*, H-5′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.8 (*t*, C-1), 28.3 (*t*, C-2), 86.5 (*d*, C-3), 34.5 (*d*, C-4), 47.2 (*d*, C-5), 21.7 (*t*, C-6), 28.0 (*t*, C-7), 135.9 (*s*, C-8), 134.7 (*s*, C-9), 36.1 (*s*, C-10), 20.7 (*t*, C-11), 25.5 (*t*, C-12), 44.4 (*s*, C-13), 49.7 (*s*, C-14), 30.9 (*t*, C-15), 30.6 (*t*, C-16), 50.4 (*d*, C-17), 15.7 (*q*, C-18), 18.0 (*q*, C-19), 36.3 (*d*, C-20), 18.7 (*q*, C-21), 36.0 (*t*, C-22), 23.9 (*t*, C-23), 39.4 (*t*, C-24), 27.8 (*d*, C-25), 22.7 (*q*, C-26), 22.5 (*q*, C-27), 24.4 (*q*, C-28), 15.9 (*q*, C-29), 144.6 (*s*, C-1′), 134.3 (*d*, C-2′), 129.8 (*d*, C-3′), 127.5 (*s*, C-4′), 21.4 (*q*, C-5′).

3.4.2. 3β-Acetoxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione ($C_{31}H_{48}O_4$) (**5**), 4α,14α-dimethyl-5α-ergosta-8,24-dien-3-one ($C_{30}H_{48}O$) (**7**), 3β-acetoxy-24-methylen-elemo-lanosta-8,24-diene-7,11-dione ($C_{33}H_{50}O_4$) (**19**), 3β-tosyloxy-24-methylen-elemo-lanosta-8,24-dien-11-one ($C_{38}H_{56}O_4S$) (**20**), 3β-tosyloxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione ($C_{36}H_{52}O_5S$) (**4**), 3β-tosyloxy-24-methylen-elemo-lanosta-8,24-diene-7,11-dione ($C_{38}H_{54}O_5S$) (**18**)

The oxidation procedure with CrO_3 has been described in Mazoir et al. (2007). 31-Norlanostenol (**2**) gave derivative **5** (Mazoir et al., 2007) (0.72 g, 64% yield). Obtusifoliol gave **7** (0.84 g, 85% yield) [10] (Mazoir et al., 2005). α-Euphorbol gave derivatives **19** (Mazoir et al., 2004a) and **20** (0.78 and 0.47 g, 66 and 33% yield, respectively). Derivatives **3** and **20** gave compounds **4** (Mazoir et al., 2007) and **18** (Mazoir et al., 2004b) (0.90 and 1.08 g, 65 and 75% yield, respectively).

3.4.3. 3β-Acetoxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione ($C_{31}H_{48}O_4$) (**5**)

M.p. 117–119 °C (hexane); EIMS *m/z* $[M]^+$ 484 (65). For NMR data see Mazoir et al. (2007).

3.4.4. 4α,14α-Dimethyl-5α-ergosta-8,24-dien-3-one ($C_{30}H_{48}O$) (**7**)

M.p. 96–97 °C (hexane); EIMS *m/z* $[M]^+$ 424 (100). For NMR data see Mazoir et al. (2005).

3.4.5. 3β-Acetoxy-24-methylen-elemo-lanosta-8,24-diene-7,11-dione ($C_{33}H_{50}O_4$) (**19**)

M.p. 138–139 °C (hexane); EIMS *m/z* $[M]^+$ 510 (70). For NMR data see Mazoir et al. (2004a).

3.4.6. 3β-Tosyloxy-24-methylen-elemo-lanosta-8,24-dien-11-one ($C_{38}H_{56}O_4S$) (**20**)

M.p. 122–123 °C (hexane); EIMS *m/z* $[M]^+$ 608 (100); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.25 (1H, *dd*, *J* = 12.0, 4.0 Hz, H-3), 7.80 (2H, *d*, *J* = 8.1 Hz, H-2′), 7.25 (2H, *d*, *J* = 8.1 Hz, H-3′), 2.45 (3H, *s*, H-5′), 0.93 (3H, *s*, H-18), 1.60 (3H, *s*, H-19), 0.90 (3H, *d*, *J* = 6.1 Hz, H-21), 0.91 (3H, *d*, *J* = 2.0 Hz, H-26), 0.92 (3H, *d*, *J* = 2.0 Hz, H-27), 0.89 (3H, *s*, H-28), 1.30 (3H, *s*, H-29), 1.25 (3H, *s*, H-30), 4.63 (1H, *s*, H-31a), 4.68 (1H, *s*, H-31b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.5 (*t*, C-1), 37.4 (*t*, C-2), 83.5 (*d*, C-3), 44.5 (*s*, C-4), 49.3 (*d*, C-5), 21.8 (*t*, C-6), 24.5 (*t*, C-7), 162.4 (*s*, C-8), 139.3 (*s*, C-9), 35.8 (*s*, C-10), 201.5 (*s*, C-11), 25.5 (*t*, C-12), 44.5 (*s*, C-13), 49.6 (*s*, C-14), 30.8 (*t*, C-15), 30.5 (*t*, C-16), 50.3 (*d*, C-17), 15.8 (*q*, C-18), 17.4 (*q*, C-19), 36.3 (*d*, C-20), 18.6 (*q*, C-21), 36.1 (*t*, C-22), 31.1 (*t*, C-23), 155.5 (*s*, C-24), 33.6 (*d*, C-25), 21.7 (*q*, C-26), 21.9 (*q*, C-27), 23.4 (*q*, C-28), 16.5 (*q*, C-29), 21.2 (*q*, C-30), 106.5 (*t*, C-31), 144.9 (*s*, C-1′), 134.7 (*d*, C-2′), 130.1 (*d*, C-3′), 128.2 (*s*, C-4′), 22.5 (*q*, C-5′).

*3.4.7. 3β-Tosyloxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione ($C_{36}H_{52}O_5S$) (**4**)*

M.p. 124–125 °C (hexane); EIMS *m/z* $[M]^+$ 596 (85). For NMR data see Mazoir et al. (2007).

*3.4.8. 3β-Tosyloxy-24-methylen-elemo-lanosta-8,24-diene-7,11-dione ($C_{38}H_{54}O_5S$) (**18**)*

M.p. 140–141 °C (hexane); EIMS *m/z* $[M]^+$ 622 (62). For NMR data see Mazoir et al. (2004b).

*3.4.9. 3β-Tosyloxy-4α,14α-dimethyl-5α-ergost-8-en-24-one ($C_{36}H_{54}O_4S$) (**1**), 4α,14α-dimethyl-5α-ergost-8-en-3,24-dione ($C_{30}H_{48}O_2$) (**6**), 3β-acetoxy-25,26,27-trisnor(5α,13α,14β,17α)lanost-8-en-24-al ($C_{29}H_{46}O_3$) (**13**), 3-oxo-25,26,27-trisnor(5α,13α,14β,17α)lanost-8-en-24-al ($C_{27}H_{42}O_2$) (**15**), 3β-hydroxy-25,26,27-trisnor(5α,13α,14β,17α)lanost-8-en-24-al ($C_{27}H_{44}O_2$) (**16**), 3β-acetoxy-elemo-lanosta-8-en-24-one ($C_{31}H_{50}O_3$) (**12**), elemo-lanosta-8-ene-3,24-dione ($C_{31}H_{48}O_2$) (**14**), 3β-hydroxy-elemo-lanost-8-en-24-one ($C_{31}H_{50}O_2$) (**22**)*

The oxidation procedure with $NaIO_4$–($RuCl_3$, $3H_2O$) has been described in Mazoir et al. (2007). Obtusifoliol gave derivatives **1** (Mazoir et al., 2007) and **6** (0.79 and 0.90 g, 62 and 66% yield, respectively). α-Euphol gave derivatives **13** (Auduin and Levisalles, 1983), **15** and **16** (0.91, 0.77 and 0.79 g, 66, 62 and 64% yield, respectively). α-Euphorbol gave **12** (Auduin and Levisalles, 1983; Mazoir et al., 2006), **14** and **22** (1.08, 0.56 and 0.49 g, 74, 40 and 35% yield, respectively).

*3.4.10. 3β-Tosyloxy-4α,14α-dimethyl-5α-ergost-8-en-24-one ($C_{36}H_{54}O_4S$) (**1**)*

M.p. 145–146 °C (hexane); EIMS *m/z* $[M]^+$ 582 (40). For NMR data see Mazoir et al. (2007).

*3.4.11. 4α,14α-Dimethyl-5α-ergost-8-en-3,24-dione ($C_{30}H_{48}O_2$) (**6**)*

M.p. 190–191 °C (hexane); EIMS *m/z* $[M]^+$ 438 (95); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.76 (3H, *s*, H-18), 0.95 (3H, *s*, H-19), 1.00 (3H, *d*, $J = 2.0$ Hz, H-26), 1.01 (3H, *d*, $J = 2.0$ Hz, H-27), 0.94 (3H, *d*, $J = 6.2$ Hz, H-21), 1.02 (3H, *d*, $J = 6.4$ Hz, H-29); ^{13}C NMR (125 MHz, $CDCl_3$) δ 33.5 (*t*, C-1), 45.2 (*t*, C-2), 213.5 (*s*, C-3), 44.5 (*d*, C-4), 49.2 (*d*, C-5), 22.7 (*t*, C-6), 22.0 (*t*, C-7), 132.5 (*s*, C-8), 135.6 (*s*, C-9), 38.2 (*s*, C-10), 20.7 (*t*, C-11), 51.5 (*t*, C-12), 47.7 (*s*, C-13), 47.5 (*s*, C-14), 32.5 (*t*, C-15), 27.5 (*t*, C-16), 49.5 (*d*, C-17), 16.5 (*q*, C-18), 16.4 (*q*, C-19), 36.4 (*d*, C-20), 18.5 (*q*, C-21), 34.8 (*t*, C-22), 28.4 (*t*, C-23), 215.5 (*s*, C-24), 31.8 (*d*, C-25), 22.5 (*q*, C-26) 23.5 (*q*, C-27), 15.4 (*q*, C-28), 18.5 (*q*, C-29).

*3.4.12. 3β-Acetoxy-25,26,27-trisnor(5α,13α,14β,17α)lanost-8-en-24-al ($C_{29}H_{46}O_3$) (**13**)*

M.p. 136–137 °C (hexane); EIMS *m/z* $[M]^+$ 442 (65). For NMR data see Auduin and Levisalles (1983).

*3.4.13. 3-Oxo-25,26,27-trisnor(5α,13α,14β,17α)lanost-8-en-24-al ($C_{27}H_{42}O_2$) (**15**)*

M.p. 112–113 °C (hexane); EIMS *m/z* $[M]^+$ 398 (66); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.45 (2H, m, H-2), 0.80 (3H, *s*, H-18), 1.10 (3H, *s*, H-19), 2.50 (2H, m, H-23), 9.80 (1H, *s*, H-24), 1.01 (3H, *s*, H-25), 1.02 (3H, *s*, H-26), 0.90 (3H, *s*, H-27); ^{13}C NMR (125 MHz, $CDCl_3$) δ 34.5 (*t*, C-1), 35.6 (*t*, C-2), 219.0 (*s*, C-3), 51.6 (*s*, C-4), 51.4 (*d*, C-5), 26.5 (*t*, C-6), 26.8 (*t*, C-7), 132.8 (*s*, C-8), 134.7 (*s*, C-9), 36.4 (*s*, C-10), 25.0 (*t*, C-11), 24.9 (*t*, C-12), 32.5 (*s*, C-13), 32.8 (*s*, C-14), 22.5 (*t*, C-15), 22.7 (*t*, C-16), 44.3 (*d*, C-17), 15.0 (*q*, C-18), 21.2 (*q*, C-19), 35.1 (*d*, C-20), 20.2 (*q*, C-21), 22.4 (*t*, C-22), 34.4 (*t*, C-23), 203.2 (*d*, C-24), 21.2 (*q*, C-25), 22.0 (*q*, C-26), 21.0 (*q*, C-27).

*3.4.14. 3β-Hydroxy-25,26,27-trisnor(5α,13α,14β,17α)lanost-8-en-24-al ($C_{27}H_{44}O_2$) (**16**)*

M.p. 145–146 °C (hexane); EIMS *m/z* $[M]^+$ 400 (82); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.21 (1H, *dd*, $J = 11.4$, 4.5 Hz, H-3), 0.75 (3H, *s*, H-18), 1.12 (3H, *s*, H-19), 2.51 (2H, *m*, H-23), 9.77 (1H, *s*, H-24), 1.02 (3H, *s*, H-25), 1.03 (3H, *s*, H-26), 0.92 (3H, *s*, H-27); ^{13}C NMR (125 MHz, $CDCl_3$) δ 34.6 (*t*, C-1), 35.7 (*t*, C-2), 79.2 (*d*, C-3), 51.8 (*s*, C-4), 51.3 (*d*, C-5), 26.6 (*t*, C-6), 26.9 (*t*, C-7), 133.7 (*s*, C-8), 134.9 (*s*, C-9), 36.5 (*s*, C-10), 25.1 (*t*, C-11), 24.8 (*t*, C-12), 32.6 (*s*, C-13), 32.9 (*s*, C-14), 22.6 (*t*, C-15), 22.8 (*t*, C-16), 44.4 (*d*, C-17), 15.1 (*q*, C-18), 21.3 (*q*, C-19), 35.2 (*d*, C-20), 20.3 (*q*, C-21), 22.5 (*t*, C-22), 34.5 (*t*, C-23), 203.4 (*d*, C-24), 21.1 (*q*, C-25), 22.2 (*q*, C-26), 21.3 (*q*, C-27).

*3.4.15. 3β-Acetoxy-elemo-lanosta-8-en-24-one ($C_{31}H_{50}O_3$) (**12**)*

M.p. 110–111 °C (hexane); EIMS *m/z* $[M]^+$ 470 (74). NMR data in Auduin and Levisalles (1983) and Mazoir et al. (2006).

*3.4.16. Elemo-lanosta-8-ene-3,24-dione ($C_{31}H_{48}O_2$) (**14**)*

M.p. 134–135 °C (hexane); EIMS *m/z* $[M]^+$ 452 (35); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.42 (2H, *m*, H-2), 0.76 (3H, *s*, H-18), 0.86 (3H, *s*, H-19), 0.93 (3H, *d*, $J = 6.2$ Hz, H-21), 2.50 (2H, *m*, H-23), 1.25 (3H, *d*, $J = 2.0$ Hz, H-26), 1.26 (3H, *d*, $J = 2.0$ Hz, H-27), 0.80 (3H, *s*, H-28), 0.95 (3H, *s*, H-29), 1.05 (3H, *s*, H-30); ^{13}C NMR (125 MHz, $CDCl_3$) δ 35.8 (*t*, C-1), 41.2 (*t*, C-2), 215.4 (*s*, C-3), 50.5 (*s*, C-4), 50.4 (*d*, C-5), 34.9 (*t*, C-6), 31.0 (*t*, C-7), 133.0 (*s*, C-8), 135.0 (*s*, C-9), 37.8 (*s*, C-10), 30.4 (*t*, C-11), 30.1 (*t*, C-12), 37.4 (*s*, C-13), 36.5 (*s*, C-14), 27.0 (*t*, C-15), 27.8 (*t*, C-16), 47.6 (*d*, C-17), 18.8 (*q*, C-18), 20.6 (*q*, C-19), 34.5 (*d*, C-20), 20.1 (*q*, C-21), 28.3 (*t*, C-22), 44.5 (*t*, C-23), 218.2 (*s*, C-24), 21.3 (*d*, C-25), 21.5 (*q*, C-26), 21.2 (*q*, C-27), 16.4 (*q*, C-28), 17.4 (*q*, C-29), 22.4 (*q*, C-30).

*3.4.17. 3β-Hydroxy-elemo-lanost-8-en-24-one ($C_{31}H_{50}O_2$) (**22**)*

M.p. 75–76 °C (hexane); EIMS *m/z* $[M]^+$ 454 (45); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.21 (1H, *dd*, *J* = 12.0, 4.0 Hz, H-3), 0.76 (3H, *s*, H-18), 0.85 (3H, *s*, H-19), 0.93 (3H, *d*, *J* = 6.0 Hz, H-21), 2.41 (2H, *m*, H-23), 1.45 (3H, *d*, *J* = 2.0 Hz, H-26), 1.46 (3H, *d*, *J* = 2.0 Hz, H-27), 0.80 (3H, *s*, H-28), 0.95 (3H, *s*, H-29), 1.06 (3H, *s*, H-30); ^{13}C NMR (125 MHz, $CDCl_3$) δ 35.7 (*t*, C-1), 41.0 (*t*, C-2), 79.3 (*s*, C-3), 50.4 (*s*, C-4), 49.5 (*d*, C-5), 34.8 (*t*, C-6), 31.2 (*t*, C-7), 133.8 (*s*, C-8), 134.4 (*s*, C-9), 37.7 (*s*, C-10), 30.3 (*t*, C-11), 30.2 (*t*, C-12), 37.3 (*s*, C-13), 36.4 (*s*, C-14), 27.1 (*t*, C-15), 27.6 (*t*, C-16), 47.5 (*d*, C-17), 18.6 (*q*, C-18), 20.5 (*q*, C-19), 34.4 (*d*, C-20), 20.0 (*q*, C-21), 28.2 (*t*, C-22), 44.3 (*t*, C-23), 215.4 (*s*, C-24), 21.2 (*d*, C-25), 21.4 (*q*, C-26), 21.1 (*q*, C-27), 16.3 (*q*, C-28), 17.2 (*q*, C-29), 22.3 (*q*, C-30).

*3.4.18. 24-Methylen-elemo-lanost-8-en-24-epoxy-3β-ol ($C_{31}H_{52}O_2$) (**17**)*

An equimolecular mixture of *m*-CPBA (El Jamili et al., 2002) (0.5 g, 2.27 mmol) and α-euphorbol (1 g, 2.27 mmol) in CH_2Cl_2 (50 ml) was stirred at room temperature for 2 h and then washed successively with 10% saturated sodium bicarbonate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue obtained was chromatographed over a silica gel column eluted with hexane/EtOAc (95/5) to give compound **17** (0.78 g, 76% yield).

*3.4.19. 24-Methylen-elemo-lanost-8-en-24-epoxy-3β-ol ($C_{31}H_{52}O_2$) (**17**)*

M.p. 156–147 °C (hexane); EIMS *m/z* $[M]^+$ 456 (100); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.20 (1H, *dd*, *J* = 12.0, 4.0 Hz, H-3), 2.55 (2H, *m*, H-31), 0.75 (3H, *s*, H-18), 0.87 (3H, *s*, H-19), 0.93 (3H, *d*, *J* = 6.0 Hz, H-21), 1.00 (3H, *d*, *J* = 2.0 Hz, H-26), 1.02 (3H, *d*, *J* = 2.0 Hz, H-27), 0.80 (3H, *s*, H-28), 0.95 (3H, *s*, H-29), 1.05 (3H, *s*, H-30); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.3 (*t*, C-1), 37.2 (*t*, C-2), 78.4 (*d*, C-3), 44.3 (*s*, C-4), 49.1 (*d*, C-5), 21.6 (*t*, C-6), 24.3 (*t*, C-7), 134.1 (*s*, C-8), 133.4 (*s*, C-9), 35.6 (*s*, C-10), 30.1 (*t*, C-11), 25.3 (*t*, C-12), 44.2 (*s*, C-13), 49.4 (*s*, C-14), 30.6 (*t*, C-15), 30.3 (*t*, C-16), 50.1 (*d*, C-17), 15.6 (*q*, C-18), 17.2 (*q*, C-19), 36.1 (*d*, C-20), 18.4 (*q*, C-21), 36.0 (*t*, C-22), 31.1 (*t*, C-23), 50.9 (*s*, C-24), 33.4 (*d*, C-25), 21.9 (*q*, C-26), 21.7 (*q*, C-27), 23.2 (*q*, C-28), 16.3 (*q*, C-29), 21.0 (*q*, C-30), 62.7 (*t*, C-31).

*3.4.20. 24-Methylen-elemo-lanosta-2,8,24-trien-7,11-dione ($C_{31}H_{48}O_2$) (**23**)*

One gram (1.6 mmol) of compound **18**, dissolved in 30 ml of pyridine was heated at reflux during 12 h, concentrated under reduced pressure and extracted with dichloromethane (3 × 40 ml). The organic layer was dried over Na_2SO_4. The residue was purified on a silica gel column eluted with hexane/EtOAc (97/3) to give compound **23** (0.51 g, 74% yield).

*3.4.21. 24-Methylen-elemo-lanosta-2,8,24-trien-7,11-dione ($C_{31}H_{48}O_2$) (**23**)*

M.p. 97–98 °C (methanol); EIMS *m/z* $[M]^+$ 422(85); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.40 (1H, *d*, *J* = 4.0 Hz, H-3), 5.45 (1H, *m*, H-2), 0.96 (3H, *s*, H-18), 1.12 (3H, *s*, H-19), 0.91 (3H, *d*, *J* = 6.0 Hz, H-21), 0.88 (3H, *d*, *J* = 2.0 Hz, H-26), 0.89 (3H, *d*, *J* = 2.0 Hz, H-27), 1.35 (3H, *s*, H-28), 0.94 (3H, *s*, H-29), 4.63 (1H, *s*, H-31a), 4.68 (1H, *s*, H-31b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.5 (*t*, C-1), 121.7 (*d*, C-2), 137.9 (*d*, C-3), 44.7 (*s*, C-4), 49.5 (*d*, C-5), 21.9 (*t*, C-6), 200.5 (*s*, C-7), 150.5 (*s*, C-8), 151.4 (*s*, C-9), 35.9 (*s*, C-10), 202.1 (*s*, C-11), 25.6 (*t*, C-12), 44.6 (*s*, C-13), 49.7 (*s*, C-14), 30.7 (*t*, C-15), 30.6 (*t*, C-16), 50.2 (*d*, C-17), 15.9 (*q*, C-18), 17.5 (*q*, C-19), 36.4 (*d*, C-20), 18.7 (*q*, C-21), 36.2 (*t*, C-22), 31.2 (*t*, C-23), 155.5 (*t*, C-24), 33.7 (*d*, C-25), 21.8 (*q*, C-26), 21.6 (*q*, C-27), 23.7 (*q*, C-28), 16.5 (*q*, C-29), 21.5 (*q*, C-30), 106.5 (*t*, C-31).

*3.4.22. 4α,14α-Dimethyl-5α-cholest-8-ene-3,11-dione-7-thiosemicarbazone ($C_{30}H_{45}O_2N_3S$) (**8**), 4α,14α-dimethyl-5α-cholesta-7,9-diene-3-thiosemicarbazone ($C_{30}H_{49}N_3S$) (**10**), 3β-acetoxy-24-methylen-elemo-lanosta-8,24-diene-7-one-11-thiosemicarbazone ($C_{34}H_{53}O_3N_3S$) (**21**)*

Equimolecular quantities of thiosemicarbazide (TSC) (Brousse et al., 2002; Ourhriss et al., 2005) and substrate (2.27 mmol) were dissolved in ethanol with several drops of concentrated H_2SO_4. The mixture was heated at reflux during 5 h, and evaporated under reduced pressure. The residue was purified on a silica gel column with hexane/EtOAc. 31-Norlanostenol (**2**) gave compounds **8** and **10** (0.93 and 0.81 g, 80 and 74% yield, respectively). Derivative **19** gave **21** (1.1 g, 78% yield).

*3.4.23. 4α,14α-Dimethyl-5α-cholest-8-ene-3,11-dione-7-thiosemicarbazone ($C_{30}H_{45}O_2N_3S$) (**8**)*

M.p. 206–207 °C (hexane); EIMS *m/z* $[M]^+$ 511(76); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.55 (1H, *s*, NH), 7.08 (2H, *s*, NH_2), 8.87 (1H, *s*, SH), 0.83 (3H, *s*, H-18), 1.19 (3H, *s*, H-19), 0.89 (3H, *d*, *J* = 6.5 Hz, H-21), 0.86 (3H, *d*, *J* = 2.7 Hz , H-26), 0.87 (3H, *d*, *J* = 2.7 Hz, H-27), 1.48 (3H, *s*, H-28), 1.06 (3H, *d*, *J* = 6.6 Hz, H-29); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.1 (*t*, C-1), 36.5 (*t*, C-2), 211.1 (*s*, C-3), 51.7 (*d*, C-4), 49.3 (*d*, C-5, 39.7 (*t*, C-6), 153.1 (*s*, C-7), 148.0 (*s*, C-8), 146.0 (*s*, C-9), 36.7 (*s*, C-10), 200.4 (*s*, C-11), 37.6 (*t*, C-12), 44.6 (*s*, C-13), 36.5 (*s*, C-14), 28.4 (*t*, C-15), 29.8 (*t*, C-16), 48.7 (*d*, C-17), 17.8 (*q*, C-18), 18.9 (*q*, C-19), 37.8 (*d*, C-20), 17.5 (*q*, C-21), 27.7 (*t*, C-22), 26.3 (*t*, C-23), 27.5 (*t*, C-24), 29.1 (*d*, C-25), 16.6 (*q*, C-26), 17.3 (*q*, C-27), 24.2 (*q*, C-28), 15.5 (*q*, C-29), 180.1 (*s*, C=S).

*3.4.24. 4α,14α-Dimethyl-5α-cholesta-7,9-diene-3-thiosemicarbazone ($C_{30}H_{49}N_3S$) (**10**)*

M.p. 205–206 °C (hexane); EIMS *m/z* $[M]^+$ 483(62); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.20 (1H, *s*, NH), 7.17 (2H, *s*, NH_2), 8.81 (1H, *s*, SH), 5.42 (1H, *J* = 6.6 Hz, H-7),

5.38 (1H, $J = 6.1$ Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.2 (*t*, C-1), 36.7 (*t*, C-2), 159.1 (*t*, C-3), 44.5 (*d*, C-4), 49.7 (*d*, C-5), 23.1 (*t*, C-6), 118.5 (*d*, C-7), 142.5 (*s*, C-8), 143.6 (*s*, C-9), 36.4 (*s*, C-10), 119.1 (*d*, C-11), 25.4 (*t*, C-12), 39.6 (*s*, C-13), 30.9 (*s*, C-14), 15.6 (*t*, C-15), 29.8 (*t*, C-16), 49.9 (*d*, C-17), 15.7 (*q*, C-18), 18.2 (*q*, C-19), 36.5 (*d*, C-20), 18.5 (*q*, C-21), 36.6 (*t*, C-22), 24.3 (*t*, C-23), 38.8 (*t*, C-24), 28.1 (*d*, C-25), 22.8 (*q*, C-26), 22.5 (*q*, C-27), 24.5 (*q*, C-28), 12.5 (*q*, C-29), 179.7 (*s*, C=S).

3.4.25. 3β-Acetoxy-24-methylen-elemo-lanosta-8,24-diene-7-one-11-thiosemicarbazone ($C_{34}H_{53}O_3N_3S$) (**21**)

M.p. 208–209 °C (hexane); EIMS *m/z* $[M]^+$ 610 (40); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.45 (1H, *dd*, $J = 12.0$, 4.0 Hz, H-3), 6.81 (1H, *s*, NH), 7.08 (2H, *s*, NH_2), 8.89 (1H, *s*, SH), 2.05 (3H, *s*, $COOCH_3$), 0.94 (3H, *s*, H-18), 1.15 (3H, *s*, H-19), 0.80 (3H, *d*, $J = 6.2$ Hz, H-21), 0.78 (3H, *d*, $J = 2.0$ Hz, H-26), 0.79 (3H, *d*, $J = 2.0$ Hz, H-27), 1.00 (3H, *s*, H-28), 4.64 (1H, *s*, H-31a), 4.71 (1H, *s*, H-31b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 33.6 (*t*, C-1), 34.5 (*t*, C-2), 79.6 (*d*, C-3), 51.2 (*s*, C-4), 49.3 (*d*, C-5), 36.1 (*t*, C-6), 200.0 (*s*, C-7), 147.4 (*s*, C-8), 149.6 (*s*, C-9); 37.9 (*s*, C-10), 153.4 (*s*, C-11), 34.5 (*t*, C-12), 37.8 (*s*, C-13), 37.1 (*s*, C-14), 31.4 (*s*, C-15), 31.1 (*s*, C-16), 47.5 (*d*, C-17), 21.5 (*q*, C-18), 22.4 (*q*, C-19), 45.8 (*d*, C-20), 20.4 (*q*, C-21), 27.8 (*t*, C-22), 24.7 (*t*, C-23), 150.3 (*s*, C-24), 39.0 (*d*, C-25), 18.4 (*q*, C-26), 18.4 (*q*, C-27), 20.5 (*q*, C-28), 20.4 (*q*, C-29), 21.7 (*q*, C-30), 106.1 (*t*, C-31), 179.4 (*s*, C=S), 170.9 (*s*, $COOCH_3$), 22.5 (*q*, $COOCH_3$).

3.4.26. 4α,14α-Dimethyl-5α-cholest-8-ene-3,11-dione-7-thiadiazoline ($C_{34}H_{51}O_4N_3S$) (**9**), 4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione-3-thiadiazoline ($C_{34}H_{51}O_4N_3S$) (**11**)

Synthesis of 1,3,4-thiadiazolines: Thiosemicarbazones **8** and **10** (0.25 mmol) were dissolved in 0.5 ml of pyridine and 0.5 ml of acetic anhydride and the mixture was heated at 110 °C, stirred during 1.5 h and evaporated under reduced pressure. The residue obtained was purified on a silica gel column with hexane/EtOAc to give derivatives **9** and **11** (98.50 and 104.47 mg, 66 and 70% yield, respectively).

3.4.27. 4α,14α-Dimethyl-5α-cholest-8-ene-3,11-dione-7-thiadiazoline ($C_{34}H_{51}O_4N_3S$) (**9**)

M.p. 236–237 °C (hexane); EIMS *m/z* $[M]^+$ 597(35); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.39 (1H, *s*, NH), 2.11 and 2.12 (3H each, *s*, $COCH_3$), 0.86 (3H, *s*, H-18), 1.15 (3H, *s*, H-19), 0.87 (3H, *d*, $J = 6.0$ Hz, H-21), 0.84 (3H, *d*, $J = 2.0$ Hz, H-26), 0.85 (3H, *d*, $J = 2.0$ Hz, H-27), 0.98 (3H, *s*, H-28), 1.25 (3H, *d*, $J = 6.0$ Hz, H-29); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.6 (*t*, C-1), 37.2 (*t*, C-2), 212.4 (*s*, C-3), 52.9 (*d*, C-4), 52.3 (*d*, C-5), 39.0 (*t*, C-6), 84.0 (*s*, C-7), 157.5 (*s*, C-8), 142.4 (*s*, C-9), 38.2 (*s*, C-10), 200.4 (*s*, C-11), 37.9 (*t*, C-12), 43.7 (*s*, C-13), 48.2 (*s*, C-14), 32.3 (*t*, C-15), 30.6 (*t*, C-16), 50.1 (*d*, C-17), 15.9 (*q*, C-18), 18.3 (*q*, C-19), 36.5 (*d*, C-20), 18.7 (*q*, C-21), 29.9 (*t*, C-22), 29.5 (*t*, C-23), 29.2 (*t*, C-24), 36.2 (*d*, C-25), 21.7 (*q*, C-26), 21.8 (*q*, C-27), 27.7 (*q*, C-28), 16.2 (*q*, C-29), 143.2 (*s*, C=N), 168.4 and 169.8 (*s*, $COCH_3$), 23.1 and 23.3 (*q*, $COCH_3$).

3.4.28. 4α,14α-Dimethyl-5α-cholest-8-ene-7,11-dione-3-thiadiazoline ($C_{34}H_{51}O_4N_3S$) (**11**)

M.p. 232–233 °C (hexane); EIMS *m/z* $[M]^+$ 597(46); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.95 (1H, *s*, NH), 2.17 and 2.18 (3H each, *s*, $COCH_3$), 0.80 (3H, *s*, H-18), 1.35 (3H, *s*, H-19), 0.86 (3H, *d*, $J = 6.0$ Hz, H-21), 0.83 (3H, *d*, $J = 2.0$ Hz, H-26), 0.84 (3H, *d*, $J = 2.0$ Hz, H-27), 0.95 (3H, *s*, H-28), 1.25 (3H, *d*, $J = 6.0$ Hz, H-29); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.4 (*t*, C-1), 36.5 (*t*, C-2), 90.5 (*s*, C-3), 51.8 (*d*, C-4), 50.5 (*d*, C-5), 39.7 (*t*, C-6), 200.9 (*s*, C-7), 151.0 (*s*, C-8), 151.7 (*s*, C-9), 38.7 (*s*, C-10), 202.9 (*s*, C-11), 39.0 (*t*, C-12), 47.3 (*s*, C-13), 47.8 (*s*, C-14), 32.4 (*t*, C-15), 30.6 (*t*, C-16), 50.0 (*d*, C-17), 15.9 (*q*, C-18), 18.3 (*q*, C-19), 36.2 (*d*, C-20), 18.7 (*q*, C-21), 29.9 (*t*, C-22), 28.2 (*t*, C-23), 27.6 (*t*, C-24), 34.7 (*d*, C-25), 21.8 (*q*, C-26), 21.8 (*q*, C-27), 27.6 (*q*, C-28), 18.3 (*q*, C-29) 142.7 (*s*, C=N), 168.5 and 169.8 (*s*, $COCH_3$), 22.8 and 23.0 (*q*, $COCH_3$).

3.5. Insect bioassays

S. littoralis, M. persicae and *R. padi* colonies were reared on artificial diet, bell pepper (*Capsicum annuum*) and barley (*Hordeum vulgare*) plants, respectively, and maintained at 22 ± 1 °C, >70% relative humidity with a photoperiod of 16:8 h (L:D) in a growth chamber.

3.5.1. Choice feeding assays

These experiments were conducted with *M. persicae* and *R. padi* (apterous) adults and sixth-instar *S. littoralis* larvae. *Capsicum anuum* or *Hordeum vulgare* leaf disks/fragments (1.0 cm^2) were treated on the upper surface with 10 µl of the test substance. Two treated and two control disks were arranged alternatively on five agar-coated Petri dishes (9.0 cm diameter) with three insects (*S. littoralis*) and allowed to feed in a growth chamber (environmental conditions as described above). Each experiment was repeated three times. Feeding was terminated after the consumption of between 50 and 75% of the control disks. Feeding or settling inhibition (%FI or %SI) was calculated as $\%FI = [1-(T/C) \times 100]$, where T and C are the consumption of treated and control leaf disks, respectively, or $\%SI = [1-(\%T\ 7\ \%C)]$, where $\%C$ and $\%T$ are percent aphids settled on control and treated leaf disks, respectively (Gutierrez et al., 1997; Reina et al., 2001). Ryanodine and polygodial were included as positive controls for *S. littoralis* and the aphids, respectively (González-Coloma et al., 1999; Moreno-Osorio et al., 2008).

3.5.2. Oral cannulation

This experiment was performed with pre-weighed newly moulted *S. littoralis* L6-larvae. Each experiment consisted

of 20 larvae orally injected with 40 μg of the test compound in 4 μl of DMSO (treatment) or solvent alone (control) as described in Reina et al. (2001). At the end of the experiments (72 h), larval consumption and growth were calculated on a dry weight basis. A covariance analysis (ANCOVA1) of food consumption (ΔI) and biomass gains (ΔB) with initial larval weight (BI) as covariate (covariate $p > 0.05$) was performed to test for significant effects of the test compounds on these variables. An additional ANOVA analysis and covariate adjustment on ΔB with ΔI as covariate (ANCOVA2) was performed on those compounds significantly reducing ΔB to understand their postingestive mode of action (antifeedant and/or toxic) (Reina et al., 2001). Rotenone was included as a positive control (González-Coloma et al., 2002).

3.6. Phytotoxicity

These experiments were conducted with *Lactuca sativa* var. Carrascoy seeds as described (Moiteiro et al., 2006). The germination was monitored daily for 6 days and the radicle length measured at the end of the experiment (20 digitalized radicals randomly selected for each experiment) with the application mage J Version 1.37r, 2006 (http://rsb.info.nih.gov./ij/). A non parametric analysis of variance (ANOVA) was performed on germination and radicle length data. Juglone was included as a positive control (Burgueño-Tapia et al., 2007).

3.7. Cytotoxicity

Sf9 cells derived from *S. frugiperda* pupal ovarian tissue (European Collection of Cell Cultures, ECCC) and Mammalian Chinese hamster ovary cells (CHO, a gift from Dr. Pajares, I.C. Biomédicas, CSIC) were grown as previously described (González-Coloma et al., 2002). Cell viability was analyzed by an adaptation of the MTT colorimetric assay method (Mossman, 1983). The active compounds were tested in a dose-response experiment to calculate their relative potency (ED50 values, the effective dose to give 50% cell viability) which was determined from linear regression analysis (% cell viability on log dose).

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