Phytochem Rev (2011) 10:245–260
DOI 10.1007/s11101-010-9187-8

Triterpene-based plant defenses

Azucena González-Coloma · Carmen López-Balboa · Omar Santana · Matías Reina · Braulio M. Fraga

Received: 7 April 2010 / Accepted: 27 May 2010 / Published online: 11 June 2010

Abstract Pentacyclic triterpenes are abundant in the plant kingdom and have a wide array of pharmacological activities. They also have insect antifeedant effects and therefore apparently play a role in plant defense. In this paper, we describe the insecticidal activity of pentacyclic triterpenes of plant origin from different chemical classes on several insect pests (*Spodoptera littoralis*, *Leptinotarsa decemlineata* and *Myzus persicae*), their phytotoxic properties and their selective cytotoxic effects on insect-derived Sf9 and mammalian CHO cells. We also discuss the role they play in plant defense based on these activities.

Keywords Triterpenes · Bioactivity · Antifeedant · Phytotoxic · Cytotoxic

Abbreviations

FI	Feeding inhibition
CHO	Chinese hamster ovary cells
CPB	Colorado potato beetle
IGR	Insect growth regulation
Sf9	*Spodoptera frugiperda* pupal ovarian cell
ΔB	Biomass gains
ΔI	Food consumption
ANCOVA	Analysis of covariance
DMSO	Dimethylsulphoxide
GABA	γ-Aminobutiric acid
CNS	Central nervous system
Tig	Tigloyl group

A. González-Coloma · C. López-Balboa · O. Santana
Instituto de Ciencias Agrarias-CCMA, CSIC, 28016 Madrid, Spain
e-mail: azu@ccma.csic.es

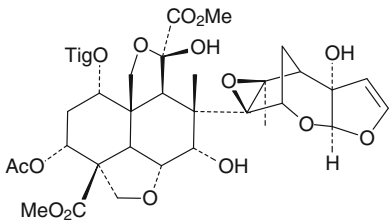
M. Reina · B. M. Fraga (✉)
Instituto de Productos Naturales y Agrobiología, CSIC, 38206 La Laguna, Tenerife, Canary Islands, Spain
e-mail: bmfraga@ipna.csic.es

Introduction

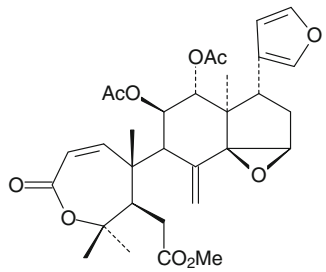
Triterpenes are part of the terpenoid family, an extensive group of natural products, which are abundant in the plant kingdom. These compounds possess pharmacological properties exhibiting anti-inflammatory (Fu et al. 2005), antibacterial (Katerere et al. 2003), antifungal (Yuan et al. 2008), antiviral (Zhu et al. 2001; Kuo et al. 2009), antitumor (Saxena et al. 2006; Kuo et al. 2009), antidiabetic (Wen et al. 2005), antiulcerogenic (De Andrade et al. 2008), anticariogenic (Segal et al. 2006), hepatoprotective (Liu 1995), neuroprotective (Lee and Kim 2001), antiparasitic (Danelli et al. 2009), analgesic (Tapondjou et al. 2003), antioxidant (D'Abrosca et al. 2005) and other effects. The pharmacological activities of natural triterpenoids and their therapeutic implications have been reviewed (Dzubak et al. 2006).

Some tetracyclic triterpenes possess anti-insect properties. It is well know that azadirachtin, a *seco*-ring

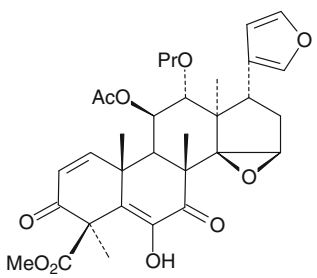
C limonoid isolated from the Indian neem tree *Azadirachta indica* (Butterworth and Morgan 1968), is a potent insect antifeedant with growth regulatory activity and reproductive effects. This compound, with an extremely complex structure, has recently been synthesized (Ley et al. 2008). The antifeedant properties of limonoids from *Melia azederach* (chinaberry), and related plants, have been reviewed (Nakatani 1999). Other tetranortriterpenes such as nymania-3 from *Dysoxylum malabaricum* (Govindachari et al. 1999), hirtin derivatives from *Trichilia palida* (Simmonds et al. 2001), munroniamide from *Munronya henryi* (Qi et al. 2003) and xyloccensins A-K and xylogranatins A-R from species of the *Xylocarpus* genus (Wu et al. 2008; Shen et al. 2009) act as feeding deterrents. Triterpenes of this type from the Meliaceae and Rutaceae groups have been tested showing that the orientation of the furan group and the hydroxylation of specific carbons affect their antifeedant properties against the fall armyworm (*Spodoptera litura*; Suresh et al. 2002).



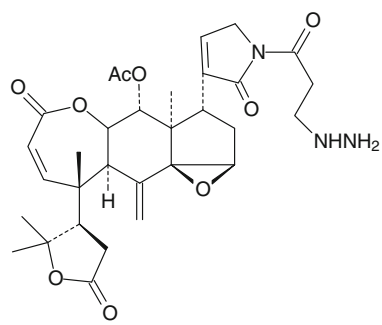
Azadirachtin



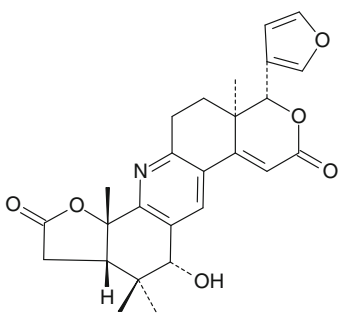
Nymania-3



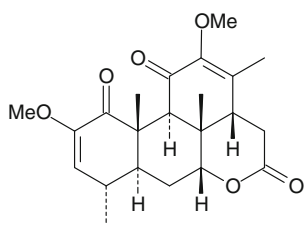
Hirtin



Munroniamide



Xylogranatin F

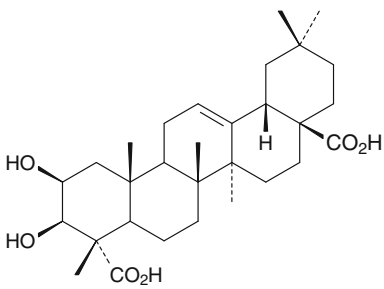


Quassin

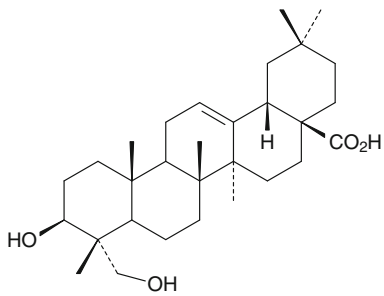
The quassinoids are another well-known nortriterpenes with insecticidal properties, which have been found in the Simarubaceae family (Polonsky 1985; Almeida et al. 2007). Components of Cucurbitaceae such as momordicine II obtained from *Momordica charantia*, showed also properties of this type (Chandravadana 1987; Yasui 2002). The food consumption, utilization,

and detoxification enzyme activity of the rice leaffolder larvae after treatment with cycloartane triterpenes have been studied (Nathan et al. 2007). Recently, the insect antifeedant and toxic effects of several tetracyclic triterpenes isolated from the latex of *Euphorbia* species have been described (Mazoir et al. 2008).

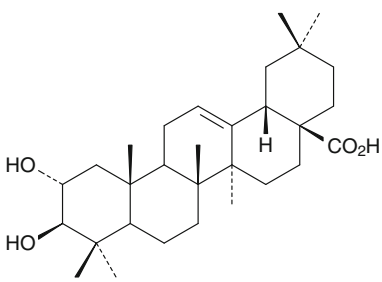
Pentacyclic triterpenes have also been shown to possess anti-insect properties. Thus, β-amyrin palmitate, isolated from *Santalum album*, acts as an insect growth inhibitor and exhibits chemosterilant properties (Shankaranarayana et al. 1980). Betulin derivatives are effective agents against bollworm larvae (*Heliothis zea*; Lugemwa et al. 1990) and Colorado potato beetle (*Leptinotarsa decemlineata*; Huang et al. 1995), while betulinic acid derivatives are active against *S. litura* (Jagadeesh et al. 1998). Oleanolic acid derivatives act as a feeding deterrent against *L. decemlineata* (Kubo et al. 1990; Hua et al. 1991), while ursolic and oleanolic acids and their fatty acid ester analogues, have been screened for their activity against *S. litura* (Mallavadhani et al. 2003). A triterpene glucoside of 2α,3β,6β,23α-tetrahydroxy-ursolic acid, with growth inhibitory activity against larvae of *Spilarctia obliqua*, was found in an extract of *Centella asiatica* (Shukla et al. 2000). The insecticidal and phytotoxic potential of friedelane triterpenes, derived from byproducts of the cork processing industry, has been reported (Moiteiro et al. 2006). Bioassay-guided fractionation of an extract from field peas (*Pisum sativum*) showed that dehydrosoyasaponin I was an active component against rice weevil (*Sitophilus oryzae*; Taylor et al. 2004). This insect was also used in a study of the antifeedant activity of maslinic acid, and other pentacyclic triterpenes, which had been isolated from *Junelia aspera* (Verbenaceae; Pungitore et al. 2005). The insecticidal activity of nortriterpene quinone methides obtained from *Maytenus* species against the codling moth (*Cydia pomonella*) has been reported (Avilla et al. 2000). Saponins containing hederagenin as aglycone also exhibited antifeedant activity. These compounds had been obtained from *Pometia eximia* (Sapindaceae), (Jayasinghe and Fujimoto 1999). Medicagenic acid, its C-3 glucoside and hederagenin monoglucoside obtained from alfalfa (*Medicago sativa*), were the most active triterpenes acting as plant growth inhibitors (Oleszek 1993). The deterrence and toxicity of the glycosyl derivatives of this acid against the pea aphid, *Acyrthosiphon pisum*, have also been determined (Golawska 2007). A review of the insecticidal and allelopathic activities of triterpene saponins from *Medicago* species has likewise been published (Tava and Avato 2006). Other pentacyclic triterpenes isolated from *Melilotus messanensis* (Macías et al. 1994, 1996, 1997) and *Sebastiana adenophora* (Macías-Rubalcava et al. 2007) have exhibited allelopathic properties.



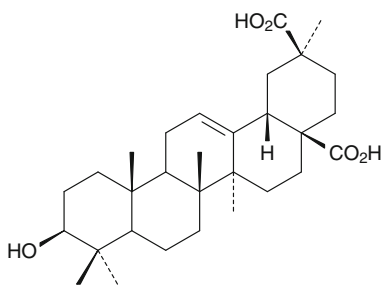
Medicagenic acid



Hederagenin



Maslinic acid



β-Glycyrrhetinic acid

In this work, we present published and unpublished data on the insect antifeedant and phytotoxic effects of several pentacyclic triterpenes of plant origin on *Spodoptera littoralis*, *Leptinotarsa decemlineata, Myzus persicae* and *Lactuca sativa*, and their selective cytotoxic effects on insect-derived Sf9 and mammalian CHO cells, which is followed by a discussion of their plant defensive role based on the above-mentioned results.

Results and discussion

The bioassayed triterpenes featured six different carbon skeleton: (a) *Lupane*: lupeol (**1**), lupeol acetate (**2**), betulin (**3**), betulin diacetate (**4**), betulinic acid (**5**) and betulinic acid acetate (**6**). (b) *Oleanane*: β-amyrin (**7**), β-amyrin acetate (**8**), oleanolic acid (**9**), oleanolic acid acetate (**10**), oleanolic acid methyl ester (**11**), 3-oxo-olean-12-en-28-oic acid (**12**), germanicol (**13**), and anagadiol (**14**). (c) *D:B*-friedo-*Oleanane*: glutinol (**15**). (d) *Friedelane* (*D:A*-friedo-*Oleanane*): epifriedelinol (**16**), epifriedelinol acetate (**17**), friedelinol (**18**), friedelinol acetate (**19**), and friedelin (**20**). (e) *Ursane*: uvaol (**21**) and uvaol diacetate (**22**). (f) *D:B*-friedo-*Ursane*: rhoiptelenol (**23**), rhoiptelenol acetate (**24**) and rhoiptelenone (**25**),

We compared their biological activities in order to study the structure–activity relationships, which showed that the activity of these compounds is related to the presence of different substituents at C-3 and C-28.

Antifeedant effects

The insect antifeedant effects of test compounds **1**–**25** are shown in Table 1. *L. decemlineata* (oliphagous) was the most sensitive insect to the triterpenes tested, followed by *S. littoralis* and *M. persicae* (polyphagous), according to their feeding ecologies. Rhoiptelenol (**23**) acted as an antifeedant in both choice and no-choice tests against *L. decemlineata*. Uvaol (**21**), glutinol (**15**), β-amyrin (**7**), rhoiptelenonone (**25**), lupeol acetate (**2**), epifriedelinol (**16**) and friedelin (**20**) exhibited antifeedant effects in choice tests. Uvaol (**21**), glutinol (**15**), rhoiptelenol (**23**) and β-amyrin (**7**) acted as strong antifeedants with effective doses within the silphinene range, a strong CPB sesquiterpene antifeedant (González-Coloma et al. 1997; Mullin et al. 1997). Oleanane (**10** and **11**) and ursane-type triterpenes (**21** and **22**) were antifeedants to *S. littoralis,* **21** being the most active. *M. persicae* feeding behaviour was only moderately affected by compounds **5**, **16**, **18** and **20**.

Amyrins and their derivatives have been described as defensive substances against phytophagous insects (Eigenbrode et al. 1991). Furthermore, several species of Doryphorina leaf beetles from Central and South America, a chrysomelid subtribe that includes *L. decemlineata*, produce oleanane triterpene glycosides in their defensive glands, using triterpenes of plant origin such as β-amyrin (**7**) as starting material (Laurent et al. 2003). Amyrins also act as feeding stimulants to the chrysomelid *Opharella communa* (Tamura et al. 2004). Other triterpenoids act as feeding stimulants on cucurbitaceous leaf beetles (Matsuda 1998) and as antifeedants (Chandravadana 1987; Abe and Matsuda 2000). Therefore, triterpenoids seem to be key structures in chrysomelid-plant interactions but the mechanisms that determine their antifeedant activity are not clear. It is possible that this biological effect is the result of their interaction with specific receptors (Huang et al. 1995).

In chrysomelid insects, a GABA mediated taste regulation has been proposed (Mullin et al. 1994). This study was supported by the molecular similarities between the silphinene antifeedant and the GABA antagonist picrotoxinin, and by silphinene activity on mammalian and insect CNS preparations (Bloomquist 2001, 2003; Bloomquist et al. 2008; González-Coloma et al. 2002a; Mullin et al. 1997). Furthermore, betulinic acid (**5**) binds angiotensine I and muscarinic receptors (Zhu et al. 1996), while triterpene acids such as oleanolic acid (**9**) and β-glycyrrhetinic acid are angiotensin blockers (Caballero-George et al. 2004). Betulin (**3**) binds GABA-A receptor sites in mice brain in vitro and has CNS activity in vivo (Muceniece et al. 2008). Therefore, the insect antifeedant mode of action of the pentacyclic triterpenes shown here could be neuroreceptor mediated involving GABA and other receptors.

Postingestive and cytotoxic effects

None of the test compounds abdominally injected into *L. decemlineata* were toxic (Table 2). No significant effects were observed when *S. littoralis*

Table 1 Antifeedant effects of different triterpenoids on *L. decemlineata* adults, *S. littoralis* larvae and *M. persicae* apterous adults (%C ± SE, %T ± SE in choice tests, 50 μg/cm^2)

Triterpene type	EC_{50} (μg/cm^2) 95% confidence limits (lower, upper)				
	L. decemlineata		*S. littoralis*	*M. persicae*	
	Choice	No-choice	Choice	%Ca	%T[b]
Lupane					
1	>50	>50	>50	53 ± 2,84	47 ± 2.84
2	3.38 (1.90–6.02)	>50	>50		
3	>50	>50	>50	49 ± 3.75	51 ± 3.75
4	>50	>50	>50		
5	>50	>50	>50	59 ± 3.82	41 ± 3.82*
6	35.48 (21.38–58.80)	>50	>50	–	–
Oleanane					
7	0.76 (0.20–2.83)	>50	>50	49 ± 3.69	51 ± 3.69
8	>50	>50	>50		
9	>50	–	>50	48 ± 3.26	52 ± 3.26
10	–	–	35.5 (21.4–58.8)	–	–
11	–	–	18.0 (15.3–32.8)	–	–
12	–	–	>50	–	–
13	–	–	–		
14	>50	>50	>50	–	–
D:B-*friedo*-oleanane					
15	0.69 (0.34–1.41)	$10 > EC_{50} > 0.2$	>50	56 ± 5.35	44 ± 5.35
Friedelane					
16	8.65 (2.38–31.43)	–	>50	65 ± 5.08	35 ± 5.08*
17	>50	–	>50	–	–
18	–		>50	66 ± 4.28	34 ± 4.28*
19	–	–	>50	–	–
20	14.41 (5.00–41.61)	–	>50	58 ± 3.07	42 ± 3.07*
Ursane		–			
21	0.20 (0.02–1.71)	>50	3.3 (1.6–6.8)	47 ± 3.48	53 ± 3.48
22	>50	>50	8.7 (4.9–15.5)	–	–
D:B-*friedo*-ursane					
23	2.04 (0.47–8.93)	13.74 (8.84–21.32)	>50	–	–
24	>50	>50	>50	–	–
25	3.18 (0.86–11.67)	>50	>50	–	–

* $P < 0.05$, Wilkoxon signed-rank test

[a] % aphids on control disk

[b] % aphids on treated disk

were orally injected with the test compounds at a dose of 20 μg/larvae. At a dosage of 40 μg/larvae, the friedelane group was the most toxic (**16**–**20**). Oleanolic acid methyl ester (**11**) and uvaol diacetate (**22**) had a significant effect on ΔI without affecting ΔB, suggesting moderate postingestive antifeedant effects. Betulin (**3**), betulinic acid (**5**), its acetate **6**, the friedelane derivatives **16**-**20** and uvaol (**21**)

significantly reduced both ΔI and ΔB without any further toxic effects (pANCOVA2 ≥ 0.05), suggesting strong postingestive antifeedant and/or insect growth regulator (IGR) effects for these substances.

Betulinic acid (**5**) and epifriedelinol (**17**) were selective toxicants to insect Sf9 cells (Table 3). Betulin (**3**) had the strongest cytotoxic effect on Sf9, followed by uvaol (**21**), betulinic acid (**5**), friedelin (**20**), epifriedelinol (**16**) and betulinic acid acetate (**6**). Lupeol (**1**) showed selective cytotoxic effects against mammalian CHO cells and betulin (**3**) showed the strongest cytotoxic effect to CHO, followed by uvaol diacetate (**22**) and uvaol (**21**; Table 3). Overall, Sf9 cells were more sensitive to the active compounds than CHO. This may be due to differences in membrane composition and/or receptor affinity between insect and mammalian cells (Hu et al. 2004; Marheineke et al. 1998).

Asiatic acid (**26**) reduced the consumption and growth of the rice grasshopper *Oxya fuscovita* and inhibited the digestive enzymes amylase and invertase (Sanjan and Partho 1993). Angiotensin blockers,

Table 2 Toxic effects of compounds **1**-**19** on *L. decemlineata* (hemolymph injection, 10 μg/insect) and *S. littoralis* (oral cannulation, 20 and 40 μg/larvae)

Triterpene type	*L. decemlineata*	*S. littoralis*				
	% Mortality[a]	ΔI 20 μg[b]	ΔB 20 μg[c]	ΔI 40 μg[b]	ΔB 40 μg[c]	pANCOVA2
Lupane						
1	26	104	104	101	113	
2	15	94	71	–	–	
3	0	137	170	69*	59*	0.064
4	0	100	103	–	–	
5	9	88	107	90	85	
6	–	100	99	89	91	
Oleanane						
7	15	100	102	79*	87	
8	0	106	101	–	–	
9	–	134	155	69*	64*	0.723
10	–	–	–	76*	64*	0.442
11	–	–	–	63*	115	
12	–	–	–	89	106	
13	0	103	85	–	–	
14	0	77	166	–	–	
D:B-*friedo*-Oleanane						
15	27	105	127	–	–	
Friedelane						
16	20	131	124	67*[d]	52*[d]	0.149[d]
17	nt	nt	nt	nt	nt	–
18	–	–	–	59*[d]	32*[d]	0.233[d]
19	–	–	–	73*[d]	56*[d]	0.366[d]
20	–	132	120	79*[d]	77*[d]	0.064[d]
Ursane						
21	32	127	142	57*	37*	0.051
22	0	108	110	60*	107	
D:B-*friedo*-ursane						
23	14	101	89	–	–	
24	0	–	–	–	–	
25	6	–	–	97	99	

nt Not tested

* $P < 0.05$, ANCOVA1 (initial larval weight as covariate)

[a] 72 h. Corrected according to Abbott (1925)

[b] Food consumption

[c] Biomass gains

[d] From Moiteiro et al. (2006)

Table 3 Cytotoxic effects of different triterpenoids on insect Sf9 and mammalian CHO cells

Triterpene type	Sf9[a] (ED_{50}) μg/ml	CHO[b]
Lupane		
1	>50	28.59 (9.04–90.28)
2	100	100
3	9.10 (3.08–27.24)	4.20 (0.59–28.63)
5	14.86 (3.08–71.65)	>100
6	$25 > ED_{50} > 10$	23.88(15.76–36.16)
Oleanane		
7	>100	>100
9	>100	>100
Friedelane[c]		
16	16.21 (11.26–23.33)	>100
17	79.56 (41.87–151.21)	>100
18	>100	>100
19	>100	>100
20	16.52 (3.89–70.16)	9.56 (5.71–16.35)
Ursane		
21	13.32 (9.87–17.96)	7.55 (5.89–9.67)
22	>100	>100

[a] Cells derived from *S. frugiperda* pupal ovarian tissue

[b] Mammalian Chinese hamster ovary cells

[c] From Moiteiro et al. (2006)

including triterpene such as oleanolic and β-glycyrrhetinic acids, induced ecdysteroid secretion in the lepidopteran *Lymantria dispar* (Caballero-George et al. 2004; Loeb et al. 1998), suggesting that these triterpenes could interfere with the insect's ecdysteroid levels.

Triterpenes such as oleanolic and ursolic acids have fluidity-modulating effects on liposomal membranes (Han et al. 1997). Semisynthetic derivatives of friedelin (**20**), including epifriedelinol acetate (**17**), have been described as exhibiting low-moderate cytotoxicity to Sf9 and CHO cells (Moiteiro et al. 2006) and also as inhibitors of human lymphocyte and cancer cell proliferation (Moiteiro et al. 2001, 2004). Other friedelane, oleanane, lupane, and ursane triterpenes have also been reported as tumoral cell growth inhibitors (Setzer and Setzer 2003; Chang et al. 2004; Fu et al. 2005; Mukherjee et al. 2006). Insect cell lines have been shown to respond to ecdysone by clumping, generating filamentous extensions and increased mortality (Hu et al. 2004), however, there are few reports on pentacyclic triterpenes acting on insect-derived cells.

Compounds, such as betulinic acid (**5**) and its acetate (**6**), showed cytotoxic activity to Sf9 cells without negative post-ingestive effects on *S. littoralis*, suggesting metabolic detoxification by this insect. Similarly, metabolic detoxification has been described for ecdysteroids in this insect (Webb et al. 1995). Furthermore, among the IGR compounds without short-term antifeedant effects on *S. littoralis,* the non-cytotoxic ones (**9, 18** and **19**) could be digestive toxins, while the cytotoxic ones (**3, 16** and **20**) could act unspecifically on cell membranes in addition to being digestive toxins. Therefore, the IGR effects observed here could be the result of a multifaceted biological action.

Oleanolic and ursolic acids have fluidity-modulating effects on liposomal membranes (Han et al. 1997). Asiatic acid (**26**) reduced the consumption and growth of the rice grasshopper *Oxya fuscovita,* and inhibited the digestive enzymes amylase and invertase (Sanjan and Partho 1993). Angiotensin blockers, including the triterpene oleanolic and β-glycyrrhetinic acids, induced ecdysteroid secretion in the lepidopteran *Lymantria dispar* (Caballero-George et al. 2004; Loeb et al. 1998), suggesting that these triterpenes could interfere with the insect's ecdysteroid levels.

Phytotoxic effects

Among all the triterpenes tested, **11**–**13**, **16**, **17** and **19** did not have significant effects on *L. sativa* germination at 24 h (Table 4), while **7**–**10**, **12**, **14**, **16**–**20** and **22** did not affect germination after 72 h. The rest of the tested compounds affected germination to varying degrees, β-amyrin acetate (**8**) and uvaol (**21**) being the most active at 24 h, followed by betulin (**3**), rhoiptelenone (**25**) >betulinic acid (**5**), oleanolic acid methyl ester (**11**; 1% <germination <15%, Table 4). After 72 h, β-amyrin acetate (**8**) and uvaol (**21**) were the most active compounds (germination <50%), followed by betulinic acid (**5**; germination <60%). All the compounds tested inhibited radicle elongation (Table 4). Uvaol (**21**) and its acetate (**22**) were the most active (growth <35%), followed by friedelinol (**18**), friedelin (**20**), oleanolic acid methyl ester (**11**), betulin (**3**), betulinic acid (**5**)

Table 4 Phytotoxic effects of several triterpenoids on *L. sativa*. Data is represented as average ± standard error (SE)

Triterpene type	Germination (% control)			Radicle length (% control)
	24 h	48 h	72 h	
Lupane				
1	46[a] ± 4	76[a] ± 5	88[a] ± 2	–
2	69[a] ± 7	77[a] ± 35	82[a] ± 2	54[b] ± 0.1
3	12[a] ± 7	61[a] ± 65	66[a] ± 5	45[b] ± 0.1
4	30[a] ± 87	52[a] ± 14	63[a] ± 16	57[b] ± 0.1
5	19[a] ± 2.7	44[a] ± 2	56[a] ± 3	46[b] ± 0.2
6	44[a] ± 37	45[a] ± 2	67[a] ± 2	50[b] ± 0.1
Oleanane				
7	64[a] ± 8	86[a] ± 3	90[a] ± 1	49[b] ± 0.2
8	6[a] ± 2	30[a] ± 4	43[a] ± 4	–
9	45[a] ± 6	65[a] ± 6	80[a] ± 3	50[b] ± 0.1
10	80[a] ± 4	100	100	46[b] ± 0.1
11	19[a] ± 2	100	100	40[b] ± 0.1
12	80 ± 9	100	100	55[b] ± 0.1
13	53[a] ± 8	63[a] ± 7	87[a] ± 2	55[b] ± 0.1
14	89 ± 7	98 ± 1	98 ± 1	56[b] ± 0.1
Friedelane[c]				
16	91 ± 3	96 ± 2	99 ± 1	44[b] ± 0.1
17	81 ± 3	100	100	68[b] ± 0.2
18	73[a] ± 7	94 ± 2	98 ± 1	36[b] ± 0.1
19	90 ± 4	99 ± 1	99 ± 1	50[b] ± 0.2
20	31[a] ± 3	76[a] ± 4	82[a] ± 4.64	38[b] ± 0.1
Ursane				
21	1a ± 1	15[a] ± 1	46[a] ± 2	32[b] ± 0.1
22	54[a] ± 8	100	100	34[b] ± 0.1
D:B-*friedo*-ursane				
23	–	–	–	–
24	–	–	–	–
25	15[a] ± 3	48[a] ± 6	62[a] ± 3	56[b] ± 0.2

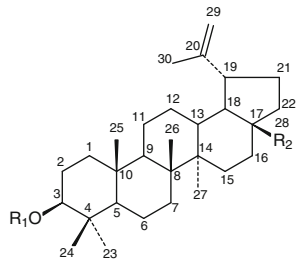
[a] $P < 0.05$, Kruskall-Wallis test

[b] $P < 0.05$, LSD test

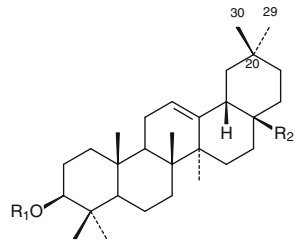
[c] From Moiteiro et al. (2006)

and β-amyrin (**7**; 35 <% growth <50). The lack of molecular selectivity observed indicates a non-specific phytotoxic effect for these triterpenes on *L. sativa*, suggesting their interference with the plant membrane rather than acting as chemical signals.

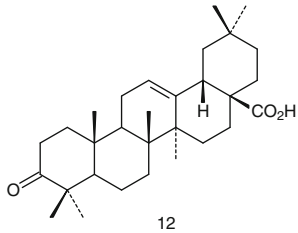
Previous reports have shown that pentacyclic triterpenes, including 3-*epi*-β-amyrin, β-amyrinone, 3-*epi*-lupeol, lupenone, taraxerol, taraxerone and friedelin (**20**), inhibited the root growth of *Echinochloa crusgalli* (Castañeda et al. 1992; Macías-Rubalcava et al. 2007). α-Amyrin inhibited the root growth of *Amaranthus hypochondriacus* and *E. crusgalli* (Anaya et al. 2003), while friedelinol (**18**) and friedelin (**20**) also inhibited *L. sativa* radicle elongation (Moiteiro et al. 2006). The phytotoxic effects of these types of molecules have been attributed to their membrane-modifying properties (Han et al. 1997; Duke and Oliva 2004).



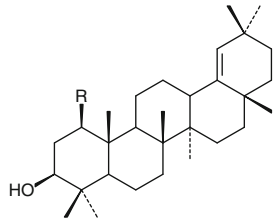
1 $R_1 = H$ $R_2 = Me$
2 $R_1 = Ac$ $R_2 = Me$
3 $R_1 = H$ $R_2 = CH_2OH$
4 $R_1 = Ac$ $R_2 = CH_2OAc$
5 $R_1 = H$ $R_2 = CO_2H$
6 $R_1 = Ac$ $R_2 = CO_2H$



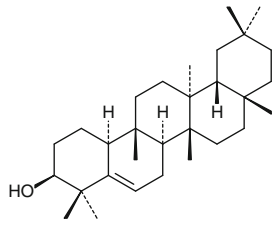
7 $R_1 = H$ $R_2 = Me$
8 $R_1 = Ac$ $R_2 = Me$
9 $R_1 = H$ $R_2 = CO_2H$
10 $R_1 = Ac$ $R_2 = CO_2H$
11 $R_1 = H$ $R_2 = CO_2Me$



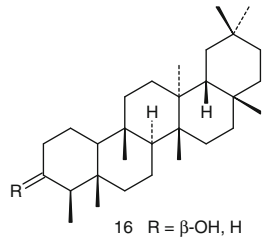
12



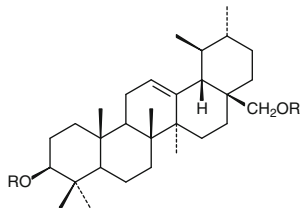
13 R = H
14 R = OH



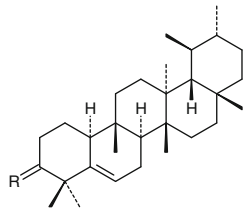
15



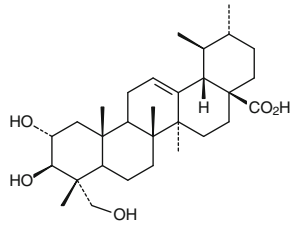
16 R = β-OH, H
17 R = β-OAc, H
18 R = α-OH, H
19 R = α-OAc, H
20 R = O



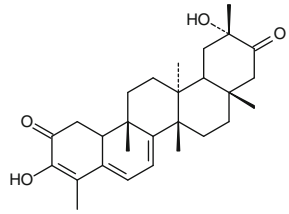
21 R = H
22 R = Ac



23 R = β-OH, H
24 R = β-OAc, H
25 R = O



26



27

Structure–activity study

The triterpenes have been grouped according to their structure in order to facilitate the structure–activity study.

a. *Lupane type triterpenes* (**1–6**). Their antifeedant effects on *L. decemlineata* depended on the presence of the acetate group at C-3 (**2** and **6**) with the exception of the diacetate **4**. Betulin (**3**) with hydroxyl groups at C-3 and C-28 showed IGR effects on *S. littoralis* larvae and had the strongest cytotoxic and phytotoxic action. Betulinic acid (**5**), with a C-28 carboxyl group, did not affect larval growth but showed selective cytotoxic effects to Sf9 and was the second most phytotoxic lupane derivative after betulin (**3**). Lupeol (**1**), with a C-28 methyl group, showed selective effects to CHO cells.
b. *Oleanane type* (**7–14**). The presence of a C-28 carboxyl group in oleanolic acid (**9**), or an acetoxy group at C-3 as in **8**, eliminated the CPB antifeedant activity observed for *β*-amyrin (**7**)**.** Acetylation of C-3 (**10**) and methylation of C-28 (**11**) in **9** resulted in antifeedant effects to *S. littoralis,* while a 3-oxo group was inactive (**12**). A carboxyl group at C-28, as in **9** and **10**, resulted in significant *S. littoralis* consumption and growth reduction. Methylation of the C-28 carboxyl (**11**) decreased the larval postingestive effects and increased *L. sativa* root growth reduction with respect to **9** and **10**. The acetate **8** exhibited a strong antigerminative effect.
c. *D:B*-friedo-*Oleanane*: Glutinol (**15**), with a rearranged oleanane framework and a C-3 hydroxyl group exhibited CPB antifeedant effects similar to other oleanane derivatives such as *β*-amyrin (**7**).
d. *Friedelane type* (**16-20**). Epifriedelinol (**16**) and friedelin (**20**) were active against *L. decemlineata*, being the former, with a *β*-hydroxyl group at C-3, more active than the latter**,** with an oxo group at this position. Acetylation of **16** resulted in an inactive compound epifriedelinol acetate (**17**). This triterpene class was the most toxic to *S. littoralis* larvae when orally injected. Substitution of the friedelin ketone at C-3 (**20**) by an α- or *β*-hydroxy group (**18** and **16**), or an α-acetoxy group (**19**), resulted in elevated larval postingestive activity (Moiteiro et al. 2006). Selective cytotoxic effects on Sf9 depended on a *β*-hydroxy group at C-3 (**16**) and disappeared with an α-hydroxyl (**18**), while an 3-oxo group (**20**) resulted in unspecific cytotoxicity. The presence of an oxo or a *β*-hydroxyl substituent at C-3 (**20** and **16**) led to the strongest inhibition of *L. sativa* germination and root growth. The configuration at C-3 is also important, thus a *β*-hydroxyl (**16**) led to stronger insecticidal and lower phytotoxic effects than an α-hydroxyl group (**18**; Moiteiro et al. 2006).
e. *Ursane type* (**21** and **22**). The strong activity of uvaol (**21**) decreased (antifeedant and postingestive effects on *S. littoralis*, antigerminative effect and root growth reduction) or disappeared (antifeedant effect to *L. decemlineata,* cytotoxicity) with the presence of acetoxy groups at C-3 and C-28 in uvaol diacetate (**22**).
f. *D:B*-friedo-*Ursane* type (**23-25**). Rhoiptelenol (**23**) derivatives showed a CPB-antifeedant activity pattern similar to that of the *β*-amyrin (**7**) type. This activity disappeared or decreased with the presence of an acetoxy or an oxo group at C-3 (**24** and **25**, respectively).

Jagadeesh et al. (1998) have described an increase in the antifeedant effect on *S. litura* of betulinic acid (**5**) derivatives, with different substituents at C-3. Huang et al. (1995) and Lugemwa et al. (1990) did not find *L. decemlineata* antifeedant effects for betulin (**3**), in contrast to several of its derivatives. Previous results have shown that 3-*O*-fatty acid ester derivatives of ursolic and oleanolic acids were stronger antifeedants to *S. litura* when compared with their parent acids (Mallavadhani et al. 2003). Additionally, decreasing the polarity of the C-3 substituents reduced their postingestive toxicity to *Sitophilus oryzae* in oleanane triterpene acids (Pungitore et al. 2005), thus emphasizing the importance of the substitution pattern at this carbon. Substitution of the friedelin (**20**) 3-oxo group by an α- or *β*-hydroxyl (**18** and **16**), or an acetate group (**19**), resulted in elevated larval postingestive activity (Moiteiro et al. 2006). Triterpenes with a *β*-hydroxyl group at C-3 (**16**) resulting in stronger insecticidal and lower phytotoxic effects than those with an α-hydroxyl (**18**; Moiteiro et al. 2006).

Additionally, Avilla et al. (2000) found that the C-20 hydroxylation in 20α-hidroxytingenone (**27**) increased the antifeedant effects of nortriterpene

quinone methides on the codling moth (*Cydia pomonella*). Conversion of the C-3, C-6 hydroxyl groups to the corresponding tosyl derivatives resulted in a significant decrease in the IGR activity of plant sterols such as peniocerol and macdougallin (Cespedes et al. 2005). The results of Mazoir et al. (2008) supported the importance of the C-3 substituent, also suggesting the involvement of the C-7 substituent groups, and indicated that a hydroxyl at C-3 is not essential for the IGR effects of lanostane triterpenes.

The presence of large amounts of triterpenoids in the latex and resins of numerous plants suggests a defensive role in these plants. Our results have demonstrated that uvaol (**21**), β-amyrin (**7**), glutinol (**15**), roipthelenol (**23**), roipthelenone (**25**), lupeol acetate (**2**), epifriedelinol (**16**), friedelin (**20**) and the acetate of betulinic acid (**6**) showed antifeedant effects to *L. decemlineata*, while uvaol (**21**), uvaol diacetate (**22**), oleanolic acid methyl ester (**12**) and oleanolic acid acetate (**10**) were antifeedants to *S. littoralis*. Betulin (**3**), oleanolic acid (**9**), oleanolic acid acetate (**10**), epifriedelinol (**16**), friedelinol (**18**), friedelinol acetate (**19**), friedelin (**20**) and uvaol (**21**) significantly reduced *S. littoralis* larval biomass gains, while betulinic acid (**5**), epifriedelinol (**16**) and epifriedelinol acetate (**17**) exhibited selective Sf9 cytotoxicity. Uvaol (**21**), uvaol diacetate (**22**), friedelinol (**18**), friedelin (**20**), oleanolic acid methyl ester (**11**), betulin (**3**), betulinic acid (**5**) and β-amyrin (**7**) had strong *L. sativa* root growth inhibition, supporting this hypothesis.

Materials and methods

Plant material

All plants were collected during the flowering season (May–June) from their natural habitats. Voucher specimens of the plant species from the Canary Islands (Spain) have been deposited at the herbarium of the Jardin de Aclimatación de La Orotava, Tenerife, Spain.

Pericallis lanata (L'Hér.) B. Nord. (ex *Senecio heritieri*; Asteraceae) was collected in Barranco del Río, Tenerife (voucher no. ORT 32001). *Pericallis steetzi* (Bolle) B. Nord (Asteraceae) was collected in Parque Nacional de Garajonay, Gomera (voucher no. ORT 33451). *Echium wildpretii* H. Pearson ex Hook fil. (Boraginaceae) was collected in Cumbres de Fasnia, Tenerife (2,100 m; voucher no. ORT 32531). *Lavandula luisieri* (Rozeira) Riv.-Mart. (Lamiaceae) was collected in Villa de Almadén de la Plata (Sevilla, Spain) as previously described (Sanz et al. 2004; González-Coloma et al. 2006).

Triterpenes

Triterpenes isolated during this study

Lupeol (**1**) and epifriedelinol (**16**) were obtained from *Pericallis lanata* and *P. steetzi*, respectively; betulin (**3**), β-amyrin (**7**) and oleanolic acid (**9**) from *Lavandula luisieri*, and glutinol (**15**) from *Echium wildpretii* (see below).

Other triterpene samples

Uvaol (**21**) was isolated by Bretón et al. (1969) from *Euphorbia paralias*, germanicol (**13**) and anagadiol (**14**) from *Salvia broussonetii* (González et al. 1971), and rhoiptelenol (**23**) and rhoiptelenone (**25**) from *Sideritis macrostachya* (Fraga et al. 2003). Friedelinol (**18**), friedelinol acetate (**19**) and friedelin (**20**) were provided by Dr. Cristina Moiteiro (INETI, Lisbon). Betulinic acid (**5**) was purchased from Sigma–Aldrich.

Extraction and isolation

Ground dried plant material (above ground plant tissues including stems, leaves and flowers) was extracted in ethanol in a Sohxlet apparatus. *L. luisieri* extract was obtained by ethanolic extraction of the hexane extract as described (Baldovini et al. 2005). These extracts were chromatographed using silica gel vacuum-liquid chromatography, silica gel column chromatography and molecular exclusion chromatography (Sephadex LH20), with hexane/EtOAc/CH_2Cl_2/MeOH gradients. Further HPLC chromatography of the selected fractions (silica and C-18 semipreparative columns) afforded lupeol (**1**, *Pericallis lanata*, 0.01%), betulin (**3**, *L. luisieri,* 25×10^{-6}%), β-amyrin (**7**, *L. luisieri* 0.001%), oleanolic acid (**9**, *L. luisieri,* 0.03% yield), epifriedelinol (**16**, *P. lanata,* 0.001%), glutinol (**15**, *E. wildpretii,* 0.001%) and

uvaol (**21,** *L. luisieri,* 6×10^{-5}%). Compound yields expressed as % of plant dry weight.

Insect bioassays

Spodoptera littoralis, Leptinotarsa decemlineata, Myzus persicae and *Diuraphis noxia* were reared on artificial diet (Poitut and Bues 1970), potato foliage (*Solanum tuberosum*), 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.

Feeding assays

These experiments were conducted with newly emerged sixth-instar *S. littoralis* larvae, adult *L. decemlineata* and apterous adult aphids. For the chewing insects (*S. littoralis* and *L. decemlineata*) percent feeding inhibition (% FI) was calculated as previously described (Reina et al. 2001). Compounds with an FI >70% were tested in a dose–response experiment to calculate their relative potency (EC_{50} values, the effective dose for 50% feeding reduction), which was determined from linear regression analysis (% FI on log dose). For the sucking insect (*M. persicae*), each treatment consisted of 20 boxes with 10 insects each as described in (Gutiérrez et al. 1997). For each compound we compared % T (% aphids on treated surface) and % C (% aphids on control surface; Wilkoxon signed rank test).

Oral cannulation

This experiment was performed with pre-weighed newly emerged *S. littoralis* L6-larvae. Each experiment consisted of 20 larvae orally dosed with 40 μg of the test compound (Reina et al. 2001). Covariance analysis (ANCOVA 1) of food consumption (ΔI) and biomass gains (ΔB) with initial larval weight 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 (ANCOVA 2), was performed for those compounds that significantly reduced ΔB to understand their post-ingestive mode of action (antifeedant and/or toxic).

Hemolymph injection

DMSO solutions of the test triterpenoids (10 μg each per insect) were injected through the metapimeron suture of the thorax of 20 adults *L. decemlineata* beetles (average weight 130 mg) using a Hamilton repeating dispensor fitted with Hamilton 50 μL syringe (50 gauge pointed needle). Toxicity symptoms and mortality were recorded up to 3 days after injection while maintaining beetles on their respective potato leaf foods (González-Coloma et al. 1998). The mortality percentage was analyzed with contingency table analysis, and corrected according to Abbot (1925).

Phytotoxicity tests

These experiments were conducted with *Lactuca sativa* var. Carrascoy seeds placed on paper disks (Whatman no. 1, 2.0 cm^2) treated with 50 μg/cm^2 of the test compound or solvent for the control. The disks were placed in lidded clear plastic boxes (2×2 cm^2) lined with 4 g of calibrated sand humidified with 200 μL of deionizer water and then placed in a plant growth chamber (25 + 1°C, >70% relative humidity with a photoperiod of 16:8 h L:D) for 6 days. A total of 100 seeds were used (20 seeds/box, 5 boxes). The germination was monitored daily and the radicle length measured at the end of the experiment (20 digitalized radicles randomly selected for each experiment) with the application Image J. A Kruskall-Wallis test and an analysis of variance (ANOVA) was performed on germination and radicle length data respectively.

Cytotoxicity

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

Acknowledgments This work has been supported by the grant CTQ2009-14629-C02-01, MICINN, Spain. We thank S. Carlin for language revision.

References

Abbot WS (1925) A method of computing the effectiveness of fan insecticide. J Econ Entomol 18:265–267

Abe M, Matsuda K (2000) Feeding deterrents from *Momordica charantia* leaves to cucurbitaceous feeding beetle species. Appl Entomol Zool 35:143–149

Almeida MMB, Arriaga AMC, Dos Santos AKL, Lemos TLG, Braz-Filho R, Curcino-Vieira IJ (2007) Occurrence and biological activity of quassinoids in the last decade. Quim Nova 30:935–951

Anaya AL, Mata R, González-Coloma A, Cruz-Ortega R, Guadaño A, Hernández-Bautista BE, Ríos G, Gómez-Pompa A (2003) Allelochemical potencial of *Callicarpa acuminata* (Verbenaceae). J Chem Ecol 29:2725–2740

Avilla J, Teixidó A, Velázquez C, Alvarenga N, Ferro E, Canela R (2000) Insecticidal activity of *Maytenus* species (Celastraceae). Nortriterpene quinone methides against codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae). J Agric Food Chem 48:88–92

Baldovini N, Lavoine-Hanneguelle S, Ferrando G, Dusart G, Lizzani-Cuvelier L (2005) Necrodane monoterpenoids from *Lavandula luisieri*. Phytochemistry 66:1651–1655

Bloomquist JR (2001) GABA and glutamate receptors as biochemical sites for insecticide action. In: Ishaaya I (ed) Biochemical sites of insecticide action and resistance. Springer, New York, pp 17–41

Bloomquist JR (2003) Chloride channels as tools for developing selective insecticides. Arch Insect Biochem Physiol 54:145–156

Bloomquist JR, Boina DR, Chow E, Carlier PR, Reina M, González-Coloma A (2008) Mode of action of the plant-derived silphinenes on insect and mammalian GABA(A) receptor/chloride channel complex. Pest Biochem Physiol 91:17–23

Bretón J, Delgado-Martín J, Fraga BM, González AG (1969) Triterpenos de la *Euphorbia paralias*. Anales Real Soc Españ Fis Quim 65b:61–67

Butterworth JH, Morgan ED (1968) Isolation of a substance that suppresses feeding in locusts. Chem Commun, pp 23–24

Caballero-George C, Vanderheyden PML, Okamoto Y, Masaki T, Mbwambo Z, Apers S, Gupta MP, Pieters L, Vauquelin G, Vlietinck A (2004) Evaluation of bioactive saponins and triterpenoidal aglycons for their binding properties on human endothelin ETA and angiotensin AT1 receptors. Phytother Res 18:729–736

Castañeda P, García MR, Hernández E, Torres BA, Anaya AL, Mata R (1992) Effects of some compounds isolated from *Calaeodendron mexicanum* Standl (Euphorbiaceae) on seeds and phytopathogenic fungi. J Chem Ecol 18: 1025–1037

Cespedes CL, Salazar JR, Martinez M, Aranda E (2005) Insect growth regulatory effects of some extracts and sterols from *Myrtillocactus geometrizans* (Cactaceae) against *Spodoptera frugiperda* and *Tenebrio molitor*. Phytochemistry 66:2481–2493

Chandravadana MV (1987) Identification of triterpenoid feeding deterrent of red pumpkin beetles (*Aulacophora foveicollis*) from *Momordica charantia*. J Chem Ecol 13:1689–1694

Chang CI, Kuo CC, Chang JY, Kuo YH (2004) Three new oleanane-type triterpenes from *Ludwigia octovalvis* with cytotoxic activity against two human cancer cell lines. J Nat Prod 67:91–93

D'Abrosca B, Florentino A, Monaco P, Pacifico S (2005) Radical-scavenging activities of new hydroxylated ursane triterpenes from cv. Annurca apples. Chem Biodiv 2: 953–958

Danelli MGM, Soares DC, Abreu HS, Pecanha LMT, Saraiva EM (2009) Leishmanicidal effect of LLD-3, a *nor*-triterpene isolated from *Lophanthera lactescens*. Phytochemistry 70:608–614

De Andrade SF, Comunello E, Noldin VF, Delle Monache F, Cechinel Filho V, Niero R (2008) Antiulcerogenic activity of fractions and 3, 15-dioxo-21α-hydroxy-friedelane isolated from Maytenus robusta (Celastraceae). Arch Pharm Res 31:41–46

Duke SO, Oliva A (2004) Mode of action of phytotoxic terpenoids. In: Macías F, Galindo JCG, Molinillo JMG, Cutler HG (eds) Allelopathy, chemistry and mode of action of allelochemicals. CRC Press, Boca Raton, pp 201–216

Dzubak P, Hajduch M, Vydra D, Hustova A, Vasnika M, Biedermann D, Lenka M, Urban M, Sarek J (2006) Pharmacological activities of natural triterpenoids and their therapeutic implications. Nat Prod Rep 23:394–411

Eigenbrode SD, Espelie KE, Shelton AM (1991) Behaviour of neonate diamondback moth larvae *Plutella xylostella* (L.) on leaves and on extracted leaf waxes of resistant and susceptible cabbages. J Chem Ecol 17:1690–1691

Fraga BM, Reina M, Luis JG, Rodriguez ML (2003) Rhoiptelenol and rhoiptelenone, two pentacyclic triterpenes from *Sideritis macrostachya* Z. Natursforch 58c:621–625

Fu L, Zhang S, Li N, Wang J, Zhao M, Sakai J, Hasegawa T, Mitsui T, Kataoka T, Oka S, Kiuchi M, Hirose K, Ando M (2005) Three new triterpenes from *Nerium oleander* and biological activity of the isolated compounds. J Nat Prod 68:198–206

Golawska S (2007) Deterrence and toxicity of plants saponins for the pea aphid *Acyrthosiphon pisum* Harris. J Chem Ecol 33:1598–1606

González AG, Bretón JL, Fraga BM (1971) Constituents of Labiatae.1 Anagadiol, a new triterpenes from *Salvia broussonetii* Benth. Chem Commun, pp 567–568

González-Coloma A, Gutiérrez C, Cabrera R, Reina M (1997) Silphinene derivatives: their effects and modes of action on Colorado potato beetle. J Agric Food Chem 45: 946–950

González-Coloma A, Guadaño A, Gutiérrez C, Cabrera R, de la Peña E, de la Fuente G, Reina M (1998) Antifeedant *Delphinium* diterpene alkaloids. Structure-activity relationships. J Agric Food Chem 46:286–290

González-Coloma A, Valencia F, Martín N, Hoffmann JJ, Hutter L, Marco JA, Reina M (2002a) Silphinene sesquiterpenes as model insect antifeedants. J Chem Ecol 28:117–129

González-Coloma A, Guadaño A, de Inés C, Martínez-Díaz R, Cortés D (2002b) Selective action of acetogenin mitochondrial complex I inhibitors. Z Naturforsch 57c:1028–1034

González-Coloma A, Martin-Benito D, Mohamed N, Garcia-Vallejo MC, Soria AC (2006) Antifeedant effects and chemical composition of essential oils from different populations of *Lavandula luisieri* L. Biochem Syst Ecol 34:609–616

Govindachari TR, Suresh G, Krishna-Kumari GN, Rajamannar T, Partho PD (1999) Nymania-3: A bioactive triterpenoid from *Dysoxylun malabaricum*. Fitoterapia 70:83–86

Gutiérrez C, Fereres A, Reina M, Cabrera R, González-Coloma A (1997) Behavioural and sub-lethal effects of structurally related conger terpenes on *Myzus persicae*. J Chem Ecol 23:1641–1650

Han SK, Ko YI, Park SJ, Jin IJ, Kim YM (1997) Oleanolic acid stabilize liposomal membranes. Lipids 32:769–773

Hu W, Cook BJ, Ampasala DR, Zheng S, Caputo G, Krell PJ, Retnakaran A, Arif BM, Feng Q (2004) Morphological and molecular effects of 20-hydroxyecdysone and its agonist tebufenozide on CF-203, a midgut-derived cell line from the spruce budworm, *Choristoneura fumiferana*. Arch Insect Biochem Physiol 55:68–78

Hua Y, Bentley MD, Cole BJW, Murray KD, Alford AR (1991) Triterpenes from the outer bark of *Betula nigra*. J Wood Chem Tech 11:503–516

Huang F, Chung BY, Bentley MD, Alford AR (1995) Colorado potato beetle antifeedants by simple modification of the birch bark triterpene betulin. J Agric Food Chem 43:2513–2516

Jagadeesh SG, Kripadanam GLD, Srimannarayana G (1998) Tobacco caterpillar antifeedant from the Gotti stem wood triterpene betulinic acid. J Agric Food Chem 46:2797–2799

Jayasinghe ULB, Fujimoto Y (1999) Insecticidal saponin from *Pometia eximia*. Fitoterapia 70:87–88

Katerere DR, Gray AI, Nash RJ, Waigh RD (2003) Antimicrobial activity of pentacyclic triterpenes isolated from African Combretaceae. Phytochemistry 63:81–88

Kubo I, Hanke FJ, Asaka Y, Matsumoto T, He CH, Clardy J (1990) Insect antifeedants from tropical plants. Structure of dumsin. Tetrahedron 46:1512–1522

Kuo RY, Qian K, Morris-Natschke SL, Lee KH (2009) Plant derived triterpenes and analogues as antitumor and anti-HIV agents. Nat Prod Rep 26:1321–1344

Laurent P, Dooms C, Braeckman JC, Daloze D, Habib-Jiwan JL, Rozenberg R, Termonia A, Pasteels JM (2003) Recycling plant wax constituents for chemical defense: hemi-biosynthesis of triterpene saponins from β-amyrin in a leaf beetle. Naturwissenschaften 90:524–527

Lee MK, Kim YC (2001) Five novel neuroprotective triterpene esters of *Ulmus davidiana* var. *japonica*. J Nat Prod 64:328–331

Ley SV, Abad-Somovilla A, Anderson JC, Ayats C, Bänteli R, Beckmann E, Boyer A, Brasca MG, Brice A, Broughton HB, Burke BJ, Cleator E, Craig D, Denholm AA, Denton RM, Durand-Reville T, Gobbi LB, Göbel M, Gray BL, Grossmann RB, Gutteridge CE, Hahn N, Harding SL, Jennens DC, Jennens L, Lovell PJ, Lovell HJ, de la Puente ML, Kolb HC, Koot WJ, Maslen SL, McCusker CF, Mattes A, Pape AR, Pinto A, Santafianos D, Scott JS, Smith SC, Somers AQ, Spilling CD, Stelzer F, Toogood PL, Turner RM, Veitch GE, Wood A, Zumbrunn C (2008) The synthesis of azadirachtin: a potent insect antifeedant. Chem Eur J 14:10683–10704

Liu J (1995) Pharmacology of oleanolic and ursolic acids. J Ethnopharm 49:57–68

Loeb MJ, De Loof A, Schoofs L, Isaac E (1998) Angiotensin II and angiotensin-converting enzyme as candidate compounds modulating the effects of testis ecdysiotropin in testes of the gypsy moth *Lymantria dispar* L. Gen Comput Endocrinol 112:232–239

Lugemwa PN, Huang FY, Bentley MD, Mendel MJ, Alford AR (1990) A *Heliothis zea* antifeedant from the abundant birch bark triterpene, betulin. J Agric Food Chem 38:493–496

Macías FA, Simonet AM, Esteban MD (1994) Potential allelophatic lupane triterpenes from bioactive fractions of *Melilotus messanensis*. Phytochemistry 36:1369–1379

Macías FA, Simonet AM, Esteban MD, Galindo JCG (1996) Triterpenoids from *Melilotus messanensis*: soyasapogenol G, the first natural carbonate derivative. Phytochemistry 41:1573–1577

Macías FA, Simonet AM, Galindo JCG (1997) Bioactive steroids and triterpenes from *Melilotus messanensis* and their allelopathic potential. J Chem Ecol 23:1781–1803

Macías-Rubalcava ML, Hernández-Bautista BE, Jiménez-Estrada M, Cruz-Ortega R, Anaya AL (2007) Pentacyclic triterpenes with selective bioactivity from *Sebastiana adenophora* leaves, Euphorbiaceae. J Chem Ecol 33: 147–156

Mallavadhani UV, Mahapatra A, Raja SS, Manjuka C (2003) Antifeedant activity of some pentacyclic triterpene acids and their fatty acid ester analogues. J Agric Food Chem 51:1952–1955

Marheineke K, Grunewald S, Christie W, Reilander H (1998) Lipid composition of *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (Tn) insect cells used for baculovirus infection. FEBS Lett 441:49–52

Matsuda K (1998) Feeding stimulants of leaf beetles. In: Jolivet P, Petitipierre E, Hsiao TH (eds) Biology of chrysomelidae. Kluwer Academic Publishers, Dordrecht, pp 41–56

Mazoir N, Benharref A, Bailén M, Reina M, González-Coloma A (2008) Bioactive triterpene derivatives from the latex of two *Euphorbia* species. Phytochemistry 69:1328–1338

Moiteiro C, Justino F, Tavares R, Marcelo-Curto MJ, Florencio MH, Nascimento MSJ, Pedro M, Cerqueira F, Pinto MM (2001) Synthetic *seco*-friedelane and friedelane derivatives as inhibitors of human lymphocyte proliferation and growth of human cancer cell lines in vitro. J Nat Prod 64:1273–1277

Moiteiro C, Justino F, Tavares R, Marcelo-Curto MJ, Nascimento MSJ, Pedro M, Pinto MM (2004) Hemisynthetic secofriedelane triterpenes with inhibitory activity against the growth of human cancer cell lines in vitro. J Nat Prod 67:1193–1196

Moiteiro C, Joao M, Curto OM, Mohamed N, Bailen M, Martínez-Díaz R, González-Coloma A (2006) Biovalorization of friedelane triterpenes derived from cork processing industry byproducts. J Agric Food Chem 54:3566–3571

Muceniece R, Saleniece K, Rumaks J, Krigere L, Dzirkale Z, Mezhapuke R, Zharkova O, Klusa V (2008) Betulin binds to γ-aminobutyric acid receptors and exert anticolvunsant action in mice. Pharm Biochem Behav 90:712–716

Mullin CA, Chyb S, Eichenseer H, Hollister B, Frazier JL (1994) Neuroreceptor mechanisms in insect gustation: a pharmacological approach. J Insect Physiol 40:913–931

Mullin CA, González-Coloma A, Gutiérrez C, Reina M, Eichenseer H, Hollister B, Chyb S (1997) Antifeedant effects of some novel terpenoids on Chrysomelidae beetles: comparison with alkaloids on an alkaloid-adapted and non adapted species. J Chem Ecol 23:1851–1865

Nakatani M (1999) Insect antifeeding limonoids from the chinaberry tree *Melia azedarach* Linn. and related compounds. In: Cooper R, Snyder JK (eds) Biology–chemistry interface: a tribute to Koji Nakanishi. Marcel Dekker, New York, pp 1–22

Nathan SS, Choi MY, Paik CH, Seo HY (2007) The food consumption, utilization, and detoxification enzyme activity of the rice leaffolder larvae after treatment with *Dysoxylum* triterpenes. Pest Biochem Physiol 88:260–267

Oleszek W (1993) Allelophatic potentials of alfalfa (*Medicago sativa*) saponins: their relation to antifungal and hemolytic activities. J Chem Ecol 19:1063–1074

Poitut S, Bues S (1970) Elevage de plusieursespeces de Lepidopteres Noctuidae sur milleu artificial simplifié. Ann Zool Ecol Anim 2:79–91

Polonsky J (1985) Quassinoid bitter principles II. In: Herz W, Grisebach H, Kirby GW, Ch Tamm (eds) Progress in the chemistry of organic natural products, vol 47. Springer, New York, pp 221–264

Pungitore CR, García M, Gianello JC, Sosa ME, Tonn CE (2005) Insecticidal and antifeedant effects of *Junellia aspera* (Verbenaceae) triterpenes and derivatives on *Sitophilus oryzae* (Coleoptera: Curculionidae). J Stored Prod Res 41:433–443

Qi SA, Wu DG, Chen L, Ma YB, Luo XD (2003) Insect antifeedant from *Munronia henryi*: structure of munroniamide. J Agric Food Chem 51:6949–6952

Reina M, González-Coloma A, Gutiérrez C, Cabrera R, Rodríguez ML, Fajardo V, Villarroel L (2001) Defensive chemistry of *Senecio miser* Hook. J Nat Prod 64:6–11

Sanjan KP, Partho PD (1993) Influence of asiatic acid, a triterpenoid from *Shorea robusta*, in the feeding and enzyme activity of *Oxya fuscovittata* (Insecta, Orthoptera). J Appl Entomol 115:506–510

Sanz J, Soria AC, García-Vallejo MC (2004) Analysis of volatile components of *Lavandula luisieri* L. by direct thermal desorption-gas chromatography-mass spectrometry. J Chromatography 1024:139–146

Saxena BB, Zhu L, Hao M, Kisilis E, Katdare M, Oktem O, Bomshteyn A, Rathnam P (2006) Boc-lysinate-betulonic acid: a potent, anti-prostate cancer agent. Bioorg Med Chem 14:6349–6358

Segal R, Pisanty S, Wormser R, Azaz E, Seia MN (2006) Anticariogenic activity of licorine and glycyrrhizine I. Inhibition of in vitro plaque formation by Streptococcus mutans. J Pharm Sc 74:79–81

Setzer W, Setzer MC (2003) Plant-derived triterpenoids as potential antineoplastic agents. Mini Rev Med Chem 3:540–556

Shankaranarayana KH, Ayyar KS, Rao GSK (1980) Insect growth inhibitor from the bark of *Santalum album*. Phytochemistry 19:1239–1240

Shen LR, Guo D, Yu YM, Yin BW, Zhao L, Shi QW, Wang YL, Huo CH (2009) Chemical constituents of plant from the genus *Xylocarpus*. Chem Biodiv 6:1293–1308

Shukla YN, Srivastava R, Tripathi AK, Prajapati V (2000) Characterization of an ursane triterpenoid from *Centella asiatica* with growth inhibitory activity against *Spilarctia obliqua*. Pharm Biol 38:262–267

Mukherjee R, Srivatava SK, Siddiqui MJA, Jaggi M, Singh AT, Vardhan A, Singh MK, Rajendran P, Jajoo HK, Burman AC, Kumar V, Rani D, Agarwal SK (2006) Novel betulinic acid derivatives A-ring condensed to a heterocyclic group. Pat. WO/2006/085334

Simmonds MSJ, Stevenson PC, Porter EA, Veitch N (2001) Insect antifeedant activity of three new tetranortriterpenois from *Trichilia pallida*. J Nat Prod 64:1117–1120

Suresh G, Gopalakrishnan G, Wesley SD, Singh NDP, Malathi R, Rajan SS (2002) Insect antifeedant activity of tetranortriterpenoids from the Rutales. A perusal of structural relations. J Agric Food Chem 50:4484–4490

Tamura Y, Hattori M, Konno K, Kono Y, Honda H, Ono H, Yoshida M (2004) Triterpenoid and caffeic acid derivatives in the leaves of ragweed, *Ambrosia artemisiifolia* L. (Asterales: Asteraceae), as feeding stimulants of Ophraella communa Le Sage (Coleoptera: Chrysomelidae). Chemoecology 14:113–118

Tapondjou LA, Lontsi D, Sondengam BL, Choi J, Lee KT, Jung HJ (2003) In vivo anti-conceptive and anti-inflammatory effects of the two triterpenes, ursolic acid and 23-hydroxyursolic acid, from *Cussonia bancoensis*. Arch Pharm Res 26:143–146

Tava A, Avato P (2006) Chemical and biological activity of triterpene saponins from *Medicago* species. Nat Prod Commun 1:1159–1180

Taylor WG, Fields PG, Sutherland DH (2004) Insecticidal components from field pea extracts: soyasaponins and lysolecithins. J Agric Food Chem 52:7484–7490

Webb J, Fowls R, Rees HH (1995) Enzymes of ecdysteroid transformation and inactivation in the midgut of the cotton leafworm *Spodoptera littoralis*: Properties and development profiles. Biochem J 312:561–568

Wen Y, Sun H, Liu J, Wu G, Zhang L, Wu X, Ni P (2005) Pentacyclic triterpenes. Part 1. The first example of naturally occurring pentacyclic triterpenes as a new class of inhibitors of glycogen phosphorylases. Bioorg Med Chem Lett 15:4944–4948

Wu J, Zhang S, Bruhn T, Xiao Q, Diung H, Bringmann G (2008) Xylogranatins F-R: Antifeedant from the Chinese mangrove, *Xylocarpus granatum*, a new biogenetic pathway to tetranortriterpenoids. Chem Eur J 14:1114–1129

Yasui H (2002) Identification of antifeedant in bitter gourd leaves and their effects on feeding behaviour of several lepidopteran species. JARQ 36:25–30

Yuan WH, Yi YH, Xue M, Zhang HW, La MP (2008) Two antifungal active triterpene glycosides from sea cucumber *Holothuria (Microthele) axiloga*. Chin J Nat Med 6: 105–108

Zhu M, Philipson JD, Greengrass PM, Bowery NG (1996) Chemical and biological investigation of the root bark of *Clerodendrum mandarinorum*. Planta Med 62:393–396

Zhu YM, Shen JK, Wang HK, Cosentino LM, Lee KH (2001) Synthesis and anti-HIV activity of oleanolic acid derivatives. Bioorg Med Chem Lett 11:3115–3118