Antifeedant/Insecticidal Terpenes from Asteraceae and Labiatae Species Native to Argentinean Semi-arid Lands

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To validate the potential as added-value resources of Asteraceae and Labiatae species of Argentinean semi-arid lands, we have selected 13 of their major terpenoids belonging to several chemical classes and tested their insect antifeedant and toxic activity on the herbivorous insects *Spodoptera littoralis* and *Leptinotarsa decemlineata*. The antifeedant effects of the test compounds were structure- and species-dependent. The most active antifeedant to *L. decemlineata* was the eudesmane sesquiterpene γ-costic acid (**13**), followed by the labdane diterpene 2α,3α-dihydroxycativic acid (**8**), the clerodane diterpenes 6-acetylteucjaponin B (**5**), bacchotricuneatin A (**1**), bartemidiolide (**7**), butanolide (**4**), and the sesquiterpenes ilicic acid (**11**) and tessaric acid (**10**) (eudesmane and eremophilane type, respectively). *S. littoralis* was only affected by the clerodanes and showed the strongest response to salviarin (**3**) and **5**, followed by hawtriwaic acid (**6**) and 12-*epi*-bacchotricuneatin A (**2**). Orally injected *S. littoralis* larvae were negatively affected by **5**. Most of the diterpenes had selective cytotoxic effects to insect-derived Sf9 cells with the clerodane **1** being the most active, followed by the eudesmane costic acid (**12**), the only cytotoxic sesquiterpene. None of these compounds was cytotoxic to mammalian CHO cells.

Key words: Terpenes, Antifeedant, *Leptinotarsa decemlineata*, *Spodoptera littoralis*

Introduction

Plants that produce significant yields of relatively high valued products, such as pharmaceuticals, biologically active materials, and essential oils, and have low water requirements are likely new crop candidates for arid lands (Thompson, 1990). Plant species of the families Asteraceae and Labiatae are known for their content in diterpenes and sesquiterpenes. Sesquiterpenes display extensive structure variety and different skeletal types and have been reported to serve as toxic or feeding deterrents to herbivore insects (Fraga, 2004). Among the diterpenes, the clerodanes are a large chemical group and a rich source of natural insect antifeedants and attractants (Gebbinck *et al.,* 2002; Nishida *et al.,* 2004).

As part of our ongoing assessment of plant species native to the central-western semi-arid region of Argentina, we have reported on Labiatae and Asteraceae species containing antifeedant and toxic diterpenes and sesquiterpenes against two stored-products pests (Pungitore *et al.,* 2004; Cifuente *et al.,* 2002; García *et al.,* 2003a, b; Enriz *et al.* 2000; Sosa *et al.,* 1994).

To further validate the added-value of these plants based on their content in active phytochemicals, we have selected 13 of their major terpenoids belonging to several chemical classes (Fig. 1). Some have reported antifeedant action on the stored-product pests *Tenebrio molitor* (**1**–**5**, **7**, **10**, **11**; Sosa *et al.,* 1994; García *et al.,* 2003a; Cifuente *et al.,* 2002) and *Tribolium castaneum* (**6**, **10**–**13**; Juan *et al.,* 2004; García *et al.,* 2003b). We have investigated their antifeedant and toxic effects against the herbivorous insect models *Spodoptera littoralis* (Boisduval) and *Leptinotarsa decemlineata* (Say). We have also tested the selective cytotoxicity of these compounds on insect Sf9 cells derived from *S. frugiperda* pupal ovarian tissue and mammalian chinese hamster ovary (CHO) cells.

Materials and Methods

General experimental procedures

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was from Sigma-Aldrich.

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Cell viability was measured in an SLT Lab Instruments (Salzburg, Austria), microplate reader.

Compounds

Bacchotricuneatin A (**1**) (Fig. 1) was isolated from the aerial parts of *Baccharis spicata* (Lam.) Beill. (Gallardo *et al.,* 1996). 12-*Epi*-bacchotricuneatin A (**2**) and hawtriwaic acid (**6**) were isolated from *Laennecia sophiifolia* (Kunth) G. L. Nesom. (Simirgiotis *et al.,* 2000). Salviarin (**3**) was isolated from *Salvia reflexa* Hornem. (Nieto *et al.,* 1996). Butanolide (**4**) was isolated from the aerial parts of *B. triangularis* Haumann. (Gianello and Giordano, 1989). 6-Acetylteucjaponin B (**5**) was isolated from *Teucrium nudicaule* H. (Gallardo *et al.,* 1996) and bartemidiolide (**7**) from *B. artemisioides* H. et A. (Tonn *et al.,* 1988). The *ent*-labdane **8** (2α,3α-dihydroxycativic acid) was isolated from *B. petiolata* D. C. (Gianello *et al.,* 1990). Solidagenone (**9**) was isolated from *Solidago chilensis* Meyen (Asteraceae) (Gutierrez *et al.,* 1981). Tessaric acid (**10**) was isolated from *Tessaria absinthioides* H. et A. (Kurina *et al.,* 1997). Ilicic acid (**11**) was isolated from the aerial parts of *Flourensia oolepis* Blake and its derivatives costic acid (**12**) and γ-costic acid (**13**) were obtained by chemical transformations (Donadel *et al.,* 1998).

Insect bioassays

Laboratory colonies of *S. littoralis* and *L. decemlineata* were reared on artificial diet and potato foliage, respectively, and maintained at (22 + 1) °C, relative humidity > 70% with a photoperiod of 16 h:8 h (L:D) in a growth chamber.

Choice feeding assays

These experiments were conducted with newly emerged sixth-instar *S. littoralis* larvae and adult *L. decemlineata*. *Capsicum annuum* or *Solanum tuberosum* leaf disks (1 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 cm diameter). Three insects were placed in each dish 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 50–75% of the control disks. Percentage feeding inhibition (%FI) was calculated as described by Reina *et al.* (2001). Compounds with an FI > 70% were tested in a dose-response experiment (dose series between 50.00 and 0.08 $\mu g/cm^2$) to calculate their relative potency (EC_{50} values, the effective dose for 50% feeding reduction) which was determined from linear regression analysis (STATGRAPHICS Plus) (%FI on log dose).

Oral cannulation

This experiment was performed with preweighed newly molted *S. littoralis* L6-larvae. Each experiment consisted of 20 larvae orally dosed with 20 mg of the test compound in 4 μl of DMSO (treatment) or solvent alone (control) as described by Reina *et al.* (2001). At the end of the experiments (72 h), larval consumption and growth were calculated on a dry weight basis. An analysis of covariance (ANCOVA1) on biomass gains with initial biomass as covariate (covariate $p > 0.05$) showed that initial insect weights were similar among all treatments. A second analysis (ANCOVA2) was performed on biomass gains with food consumption as covariate to test for post-ingestive effects.

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.,* 2002b). 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 (LD_{50} values, the effective dose to give 50% cell viability) which was determined from linear regression analysis (% cell viability on log dose).

Results and Discussion

The antifeedant effects of the test compounds (Fig. 1) were structure- and species-dependent (Table I). Overall, *L. decemlineata* (CPB) was sensitive to all the chemical classes and responded to a larger number of compounds than *S. littoralis* (61% and 31%, respectively), according to their different feeding adaptations (oligophagous *vs.* polyphagous). However, *S. littoralis* only responded to the clerodane diterpenes and had a 10-times stronger response to the active compounds than CPB (EC_{50} values ranging between 0.5–11.0

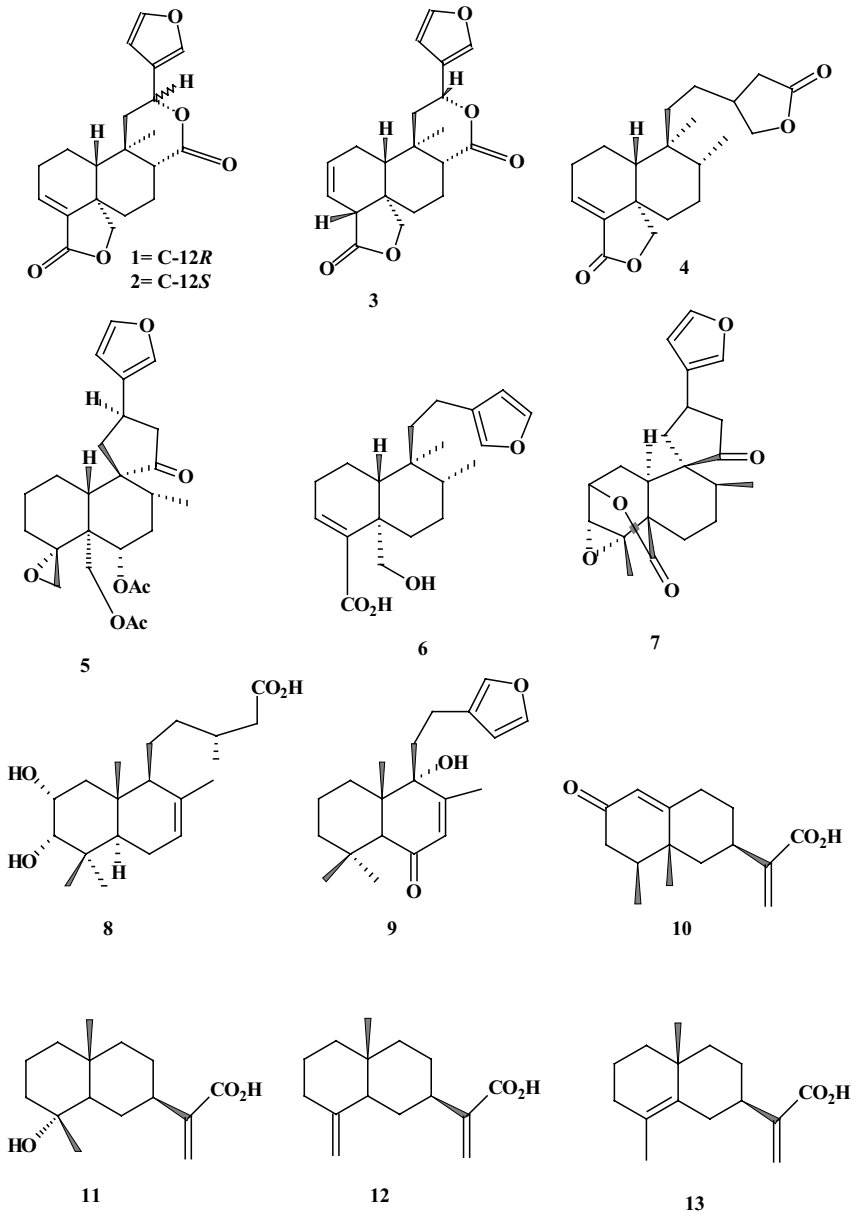

Fig. 1. Bacchotricuneatin A (**1**), 12-*epi*-bacchotricuneatin A (**2**), salviarin (**3**), butanolide (**4**), 6-acetylteucjaponin B (**5**), hawtriwaic acid (**6**), bartemidiolide (**7**), 2α,3α-dihydroxycativic acid (**8**), solidagenone (**9**), tessaric acid (**10**), ilicic acid (**11**), costic acid (**12**), γ-costic acid (**13**).

and 0.03–1.5 μg/cm^2 for CPB and *S. littoralis*, respectively), as previously shown for *nor*-diterpene alkaloids (González-Coloma *et al.,* 2004). The most active CPB antifeedant was compound **13** (EC_{50} = 0.5 μg/cm^2), followed by **8**, **5** (EC_{50} < 2 μg/cm^2), **1**, **7** (EC_{50} < 4 μg/cm^2), **11** (EC_{50} < 7 μg/cm^2), **4** and **10** (EC_{50} < 11 μg/cm^2). *S. littoralis* showed the strongest response to **3** and **5** (EC_{50} < 0.05 μg/cm^2), followed by **6** (EC_{50} < 0.5 μg/cm^2) and **2** (EC_{50} < 2 μg/cm^2).

The antifeedant response of *L. decemlineata* to the clerodanes showed a different pattern from that of *S. littoralis,* with the exception of the epoxide **5**. Both insect species showed a C-12- stereodependent antifeedant response to **1** (*R*) and **2** (*S*) with opposite patterns. This stereo-dependence of the antifeedant action could also explain the activity of **3** on *S. littoralis*. The lack of activity of **4** on the lepidopteran emphasizes the importance of the side chain, while the strong activity of **5** further

Table I. Antifeedant effects of the test compounds on adult *L. decemlineata* and *S. littoralis* L6 larvae. Consumption (ΔI) and biomass gain (ΔB) of orally injected *S. littoralis* L6 larvae, expressed as percentage of the control. Cytotoxic effects on *S. frugiperda* Sf9 and mammalian CHO cells.

Compound	Chemical class[a]	EC_{50} [μg/cm^2][b]		*S. littoralis*		ED_{50} [μg/cm^2][b]	
		L. decemlineata	*S. littoralis*	ΔB	ΔI	Sf9	CHO
1	CD	3.5 (1.9, 6.7)	≅ 50	113	104	3.57 (2.87, 4.45)[c]	> 100
2		≅ 50	1.5 (0.4, 5.7)	113	103	> 100	> 100
3		> 50	0.03 (0.01, 0.15)	97	96	64.71 (40.14, 104.33)	> 100
4		10.3 (5.7, 18.6)	> 50	94	102	39.95 (20.37, 78.36)	> 100
5		1.9 (0.7, 4.9)	0.04 (0.01, 0.01)	64*	100	26.06 (13.72, 49.50)	> 100
6		50	0.47 (0.46, 0.48)	87	87	27.29 (19.33, 38.62)	> 100
7		3.2 (0.8, 11.9)	≅ 50	108	113	33.14 (21.71, 50.59)	> 100
8	LD	1.6 (0.4, 6.4)	> 50	95	99	72.36 (32.30, 133.25)	> 100
9		> 50	> 50	82	105	64.01 (45.41, 90.24)	> 100
10	ERS	10.7 (3.6, 32.5)	> 50	99	95	> 100	> 100
11	EUS	6.7 (2.6, 17.3)	≅ 50	103	108	> 100	> 100
12		> 50	> 50	106	100	11.78 (9.14, 15.18)	> 100
13		0.5 (0.1, 2.8)	> 50	92	92	> 100	> 100

[a] CD, clerodane diterpenes; LD, labdane diterpenes; ERS, eremophilane sesquiterpene; EUS, eudesmane sesquiterpenes.
[b] Effective antifeedant and cytotoxic doses (EC_{50} and ED_{50}).
[c] 95% Confidence limits.
* Significantly different from the control, $P < 0.05$, LSD test.

confirms the importance of the 4,18-epoxide for the clerodane diterpenes (Simmonds *et al.,* 1989; Enriz *et al.,* 1994, 2000; González-Coloma *et al.,* 2000; Bruno *et al.,* 2000).

There have been reports on the antifeedant effects of salviarin (**3**) and 6-acetylteucjaponin B (**5**) on the lepidopteran *S. littoralis* (Simmonds *et al.,* 1996; Coll and Tandron, 2004). However, most of the reports on the antifeedant effects of the compounds tested here have been on the coleopterans *T. molitor* (**1**–**5**, **7**, **10**, **11**; Sosa *et al.,* 1994; García *et al.,* 2003a; Cifuente *et al.,* 2002) and *T. castaneum* (**6**, **10**–**13**; Juan *et al.,* 2004; García *et al.,* 2003b). The diterpenes **1** and **5** were the most active antifeedants to *T. molitor*, followed by the sesquiterpenes **10** and **11**. *T castaneum* responded to diterpene **6** and eudesmanes **11** and **12**, with **13** being inactive. From a total of seven compounds active on *Tenebrio* spp., three were also active on *L. decemlineata* (**1**, **10**, **11**), one on *S. littoralis* (**6**), one on both (**5**) and one did not overlap (**12**). Furthermore, *T. molitor* showed a stereo-dependent antifeedant response to **1** (*R*) and **2** (*S*) similar to *L. decemlineata* (Cifuente *et al.,* 2002) and did not respond to **3** (Sosa *et al.,* 1994). A GABA-mediated antifeedant effect of terpenes has been proposed for chrysomelids, aphids and lepidopterans (González-Coloma *et al.,* 2002a; Mullin *et al.,* 1994, 1997; Passreiter and Isman, 1997; Reina *et al.,* 2002). There is an overall closer parallelism between the behavioral response of *Tenebrio* spp. and *L. decemlineata*, with one response in common (**5**) with *S. littoralis*, supporting a similar neuroreceptor-mediated taste regulation for these insects, with the two coleopterans tuned to similar structures. Furthermore, a correlation of behavioral and electrophysiological responses of *S. littoralis* has been shown for antifeedant *neo*-clerodanes (Simmonds *et al.,* 1996).

Compound **5** reduced biomass gains (DB) without decreasing food consumption (ΔI) of orally injected *S. littoralis* larvae (ANCOVA1 $p = 0.03$ for DB and $p > 0.05$ for ΔI). Treatment effects of **5** on ΔB did not disappear with covariance adjustment (ANCOVA2 $p = 0.01$), indicating that this compound acts as post-ingestive toxin without delayed antifeedant effects. Previous experiments showed that *neo*-clerodane diterpenoids with a 4,18- epoxy fragment in their molecule had antifeedant post-ingestive effects without further toxicity on *S. littoralis* and increased *S. litura* larval mortality according to their antifeedant effects (González-Coloma *et al.,* 2000; Kumari *et al.,* 2003).

Similar post-ingestive toxic effects of *nor*-diterpene alkaloids on *S. littoralis* growth have been attributed to their interference with neurochemical mechanisms (González-Coloma *et al.,* 2004). The *neo*-clerodane diterpene salvinorin has been

shown to be a highly selective K-opioid receptor agonist in the human brain (Yan and Roth, 2004). However, more research is needed on the identification of the neurochemical effects of *neo*-clerodane diterpenes on insect neuroreceptors.

Most diterpenes and one sesquiterpene had selective cytotoxic effects on insect-derived Sf9 cells (none of these compounds was cytotoxic to mammalian CHO cells) (Table I). The selectivity between insect and mammal cells might be related to membrane factors. Similarly, several furanoid labdane diterpenes showed selective cytotoxic effects to Sf9 and mosquito C6/36 cells vs. mammalian Vero cells (Kitakoop *et al.,* 2001). This cytotoxicity indicates a mode of action other than neurotoxic. Compound **1** was the most active ($ED_{50} < 4$), followed by **12** ($ED_{50} < 12$), **5**, **6** ($ED_{50} < 28$), **4**, **7** ($ED_{50} < 40$), **3**, **8** and **9** ($ED_{50} < 75$) (Table I). The lack of activity of **2** indicates the stereo-dependency of this effect when compared to **1**, similar to the stereo-dependent antifeedant action of these compounds on *L. decemlineata*. Furthermore, the lack of cytotoxicity of **13**, with a C4, C5 double bond in the A-ring also demonstrates an elevated molecular selectivity of action when compared to **12**. Among these cytotoxic compounds, **5** also had an antifeedant effect and was toxic to *S. littoralis*; therefore its insecticidal effects could be the result of neurotoxicity and/or cytotoxicity. The lack of insect toxicity of the other cytotoxic compounds could be the result of metabolic detoxification or excretion. Mice hepatic P450 enzymes bioactivate furane-*neo*-clerodane diterpenes such as teucrin A (Lekehal *et al.,* 1996), indicating that these compounds are potential P450 oxidases substrates.

A large number of clerodane and labdane diterpenes are cytotoxic to mammalian tumoral cell lines (Shen *et al.,* 2004; Oberlies *et al.,* 2002; Prakash *et al.,* 2002; Hayashi *et al.,* 2002; Ahsan *et al.,* 2003; Scio *et al.,* 2003; among others) and their effects might be partially attributed to DNA-damaging (De Carvalho *et al.,* 1998), induction of apoptosis (Dimas *et al.,* 2001) or membrane modifying effects (Asili *et al.,* 2004). Among the few known cytotoxic eudesmane sesquiterpenes, ilicic acid (**11**) has been reported to have weak cytotoxicity on mammalian tumoral cell lines (Xiao *et al.,* 2003). The selective cytotoxicity of the diterpenes studied here suggests membrane-dependent-modifying effects rather than a more generalized cytotoxic action.

In summary, Argentinean Asteraceae and Labiatae species contain terpene antifeedants that act on a broad spectrum of insects with divergent feeding adaptations, with 6-acetylteucjaponin B (**5**), isolated from *T. nudicaule*, being active on all the species tested. These compounds also have selective cytotoxic effects to insect-derived Sf9 cells with the clerodanes diterpenes (CDs) being active on all the species tested. Therefore, plants producing CDs (*Teucrium*, *Baccharis* and *Salvia* spp.) are potential added-value crops for Argentinean semiarid lands. However, more research is needed in order to establish molecular and metabolomic libraries of local populations of the productive species prior to their selection for multiplication and field-adaptation.

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Ahsan W., Islam S. K. N., Gray A. I., and Stimson W. H. (2003), Cytotoxic diterpenes from *Scoparia dulcis*. J. Nat. Prod. **66**, 958–961.

Asili J., Lambert M., Ziegler H. L., Staerk D., Sairafianpour M., Witt M., Asghari G., Ibrahimi I. S., and Jaroszewski J. W. (2004), Labdanes and isopimaranes from *Platycladus orientalis* and their effects on erythrocyte membrane and on *Plasmodium falciparum* growth in the erythrocyte host cells. J. Nat. Prod. **67**, 631–637.

Bruno M., Roselli S., Pibiri I., Piozzi F., and Simmonds M. S. J. (2000), Hydrogenation derivatives of *neo*-clerodanes and their antifeedant activity. Heterocycles **53**, 599–612.

Cifuente D., Borkowski E. J., Sosa M. E., Gianello J. C., Giordano O. S., and Tonn C. E. (2002), New clerodane diterpenes from *Baccharis sagittalis:* Insect-antifeedant activity. Phytochemistry **61**, 899–905.

Coll J. and Tandron Y. (2004), Neo-clerodane diterpenes from *Teucrium fruticans*. Phytochemistry **65**, 387–392.

De Carvalho P. R., Furlan M., Young M. C. M., Kingston D. G. I., and Bolzani V. da S. (1998), Acetylated DNA-damaging clerodane diterpenes from *Casearia sylvestris*. Phytochemistry **49**, 1659–1662.

Dimas K., Demetzos C., Vaos V., Ioannidis P., and Trangas T. (2001), Labdane type diterpenes down-regulate the expression of *c-Myc* protein, but not of *Bcl-2*, in human leukemia T-cells undergoing apoptosis. Leukemia Res. **25**, 449–454.

Donadel O. J., García E. E., Guerreiro E., and Tonn C. E. (1998), Easy preparation of bioactives eudesman-12(5β)-olide derivatives from ilicic acid. An. Asoc. Quím. Argent. **86**, 90–93.

Enriz R. D., Baldoni H., Jáuregui E., Sosa M. E., Tonn C. E., and Giordano O. S. (1994), Structure activity relationships of clerodane diterpenoids acting as antifeedant agents. J. Agric. Food Chem. **42**, 2958–2963.

Enriz R. D., Baldoni H., Zamora M. A., Giordano O. S., Luco J. M., and Gordaliza M. J. (2000), Structure-antifeedant activity relationship of clerodane diterpenoids. Comparative study with withanolides and azadirachtin. J. Agric. Food. Chem. **48**, 1384–1392.

Fraga B. M. (2004), Natural sesquiterpenoids. Nat. Prod. Rep. **21**, 669–693.

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

García M., Donadel O., Sosa M. E., and Tonn C. E., (2003a), Allelochemical effects of eudesmane and eremofilane sesquiterpenes on *Tribolium castaneum* larvae. J. Chem. Ecol. **29**, 175–189.

García M., Sosa M. E., Donadel O., Giordano O. S., and Tonn C. E., (2003b), Effects of some sesquiterpenes on the stored-product insect *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Rev. Soc. Entomol. Argent. **62**, 17–26.

Gebbinck E. A. K., Jansen B. J. M., and de Groot A. (2002), Insect antifeedant activity of clerodane diterpenes and related model compounds. Phytochemistry **61**, 737–770.

Gianello J. C. and Giordano O. S. (1989), Chemical components from *Baccharis triangularis*. Anal. Asoc. Quím. Argent. **77**, 353–355.

Gianello J. C., Pestchanker M. J., Tonn C. E., Guo M., and Giordano O. S. (1990), 2α,3α-Dihydroxycativic acid, a new clerodane diterpenoid form *Baccharis petiolata* D.C. Phytochemistry **29**, 656–659.

González-Coloma A., Gutierrez C., Del Corral J. M., Gordaliza M., De La Puente M. L., and Sanfeliciano A. (2000), Structure- and species-dependent insecticidal effects of *neo*-clerodane diterpenes. J. Agric. Food Chem. **48**, 3677–3681.

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

González-Coloma A., Guadaño A., De Inés C., Martinez-Díaz R. C., and Cortes D. (2002b), Selective action of acetogenin mitochondrial Complex I inhibitors. Z. Naturforsch **57c**, 1028–1034.

González-Coloma A., Reina M., Medinaveitia A., Guadaño A., Santana O., Martínez-Díaz R., Ruiz-Mesía L., Alva A., Grandez M., Díaz R., Gavín J. A., and De la Fuente G. (2004), Structural diversity and defensive properties of norditerpenoid alkaloids. J. Chem. Ecol. **30**, 1393–1408.

Gutierrez A. B., Oberti J. C., and Juliani H. R. (1981), Constituyentes de *Solidago chilensis* (Compuestas). Anal. Asoc. Quím. Argent. **69**, 27–31.

Hayashi K., Nakanishi Y., Bastow K. F., Cragg G., Nozaki H., and Lee K.-H. (2002), Antitumor agents. Part 212: Bucidarasins A–C, three new cytotoxic clerodane diterpenes from *Bucida buceras*. Bioorg. Med. Chem. Lett. **12**, 345–348.

Juan H. V., Saad J. R., López V. M.A., Borkowski E. J., Sosa M. E., Tonn C. E., and Giordano O. S. (2004), Bioactivity of clerodane diterpenes from *Baccharis flabellata* Hook & Arn. var. *flabellata* against *Tribolium castaneum* Herbst. Biocell **28**, 370. In: Abstracts from the XXII Annual Scientific Meeting of Cuyo Biology Society. Argentina.

Kitakoop P., Wanasith S., Watts P., Kramyu J., Tantichaoren M., and Thebtaranonth Y. (2001), Potent antiviral potamogetonyde and potamogetonol, new furanoid labdane diterpenes from *Poatmogeton malaianus*. J. Nat. Prod. **64**, 385–388.

Kumari G. N. K., Balachandran J., Aravind S., and Ganesh M. R. (2003), Antifeedant and growth inhibitory effects of some *neo*-clerodane diterpenoids isolated from *Clerodendron* species (Verbenaceae) on *Earias vitella* and *Spodoptera litura*. J. Agric. Food Chem. **51**, 1555–1559.

Kurina S. M. B., Donadel O. J., Rossomando P. C., Tonn C. E., and Guerreiro E. (1997), Sesquiterpenes from *Tessaria absinthioides*. Phytochemistry **44**, 897–900.

Lekehal M., Pessayre D., Lereal J. M., Moulis C., Fouraste I., and Fau D. (1996), Hepatotoxicity of the herbal medicine germander: metabolic activation of its furano diterpenoids by cytochrome P450 3A depletes cytoskeleton-associated protein thiols and forms plasma membrane blebs in rat hepatocytes. Hepatology **24**, 212–218.

Mossman T. (1983), Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Meth. **65**, 55–63.

Mullin C. A., Chyb S., Eichenseer H., Hollister B., and Frazier J. L. (1994), Neuroreceptor mechanisms in in-

sect gustation: a pharmacological approach. J. Insect Physiol. **40**, 913–931.

Mullin C. A., González-Coloma A., Gutiérrez C., Reina M., Eichenseer H., Hollister B., and Chyb S. (1997), Antifeedant effects of some novel terpenoids on Chrysomelidae beetles: Comparisons with alkaloids on an alkaloid-adapted and a non-adapted species. J. Chem. Ecol. **23**, 1851–1866.

Nieto M., Gallardo V. O., Rossomando P. C., and Tonn C. E. (1996), 8-Hydroxysalviarin and 7,8-didehydrorhyacophiline, two new diterpenes from *Salvia reflexa* Hornem. J. Nat. Prod. **59**, 880–882.

Nishida R., Kawai K., Amano T. and Kuwahara Y. (2004), Pharmacophagous feeding stimulant activity of *neo*-clerodane diterpenoids for the turnip sawfly, *Athalia rosae ruficornis*. Biochem. Syst. Ecol. **32**, 15–25.

Oberlies N. H., Burgess J. P., Navarro H. A., Pinos R. E., Fairchild C. R., Peterson R. W., Soejarto D. D., Farnsworth N. R., and Kinghorn A. D. (2002), Novel bioactive clerodane diterpenoids from the leaves and twigs of *Caessaria sylvestris*. J. Nat. Prod. **65**, 95–99.

Passreiter C. M. and Isman M. B. (1997), Antifeedant bioactivity of sesquiterpene lactones from *Neurolaena lobata* and their antagonism to gamma-aminobutyric acid. Biochem. Syst. Ecol. **25**, 371–377.

Prakash C. V. S., Hoch J. M., and Kingston D. G. I. (2002), Structure and stereochemistry of new cytotoxic clerodane diterpenoids from the bark of *Caesaria lucida* from the Madagascar rainforest. J. Nat. Prod. **65**, 100–107.

Pungitore C. R., Juri Ayub M., Garcia M., Borkowski E. J., Sosa M. E., Ciuffo G., Giordano O. S., and Tonn C. E. (2004), Iridoids as allelochemicals and DNA polymerase inhibitors. J. Nat. Prod. **67**, 357–361.

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

Reina M., Nold M., Santana O., Orihuela J. C., and González-Coloma A. (2002), C-5 substituted antifeedant silphinene sesquiterpenes. J. Nat. Prod. **65**, 448–453.

Scio E., Ribeiro A., Alves T. M. A., Romanha A. J., Dias de Souza Filho J., Cordell G. A., and Zani C. (2003), Diterpenes from *Alomia myriadenia* (Asteraceae) with cytotoxic and trypanocidal activity. Phytochemistry **64**, 1125–1131.

Shen Y.-C., Wang C.-H., Cheng Y.-B., Wang L.-T., Guh J.-H., Chien C.-T., and Khalil A. T. (2004), New cytotoxic clerodane diterpenoids from the leaves and twigs of *Caesaria membranacea*. J. Nat. Prod. **67**, 316–321.

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

Simmonds M. S. J., Blaney W. M., Ley S. V., Savona G., Bruno M., and Rodriguez B. (1989), The antifeedant activity of clerodane diterpenoids from *Teucrium*. Phytochemistry **28**, 1069–1071.

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

Sosa M. E., Tonn C. E., and Giordano O. S. (1994), Insect antifeedant activity of clerodanes diterpenoids. J. Nat. Prod. **57**, 1262–1265.

Thompson A. E. (1990), Arid-land industrial crops. In: Advances in New Crops (Janick J. and Simon J. E., eds.). Timber Press, Portland, Oregon, pp. 232–241.

Tonn C. E., Giordano O. S., Frolow F., Besalle R., and Lavie D. (1988), The structure of bartemidiolide a clerodane-type diterpene from *Baccharis artemisioides* H. et A. Phytochemistry **27**, 489–491.

Xiao Y., Zheng Q., Zhang Q., Sun H., Guéritte F., and Zhao Y. (2003), Eudesmane derivatives from *Laggera pterodonta*. Fitoterapia **74**, 459–463.

Yan F. and Roth B. L. (2004), Salvinorin A: a novel and highly selective k-opioid receptor agonist. Life Sci. **75**, 2615–2619.