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LARVAL GROWTH INHIBITORS FROM SPECIES OF *PARTHENIUM* (ASTERACEAE)

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Key Word Index—*Parthenium*; Asteraceae; guayule; allelochemics; methylated flavonoids; triterpenes; sesquiterpene lactones.

Abstract—Natural products isolated from various species of *Parthenium* were fed (in artificial diets) to the herbivorous insects *Heliothis zea* and *Spodoptera exigua* to assess the ability of the compounds to inhibit larval growth. Sesquiterpene esters and triterpenes from guayule (*P. argentatum*) were relatively non-inhibitory over a range of concentrations reflecting those in the plant. A quercetagetin-based methylated flavonoid from guayule was equitoxic to quercetin, whereas a kaempferol-based methylated flavonoid was stimulatory to both insect species. Sesquiterpene lactones from *Parthenium* were consistently inhibitory to *H. zea*; those oxygenated at C-14 and/or C-15 (parthenolides) were more inhibitory than their unsubstituted ambrosanolide analogues. At a dietary concentration of 3.0 mM/kg fr. wt, tetraneurin-A (a parthenolide) reduced larval growth of *H. zea* by 88% relative to controls in a chronic feeding bioassay.

INTRODUCTION

Guayule (*Parthenium argentatum* Gray), a perennial shrub native to the arid regions of central Mexico, produces natural rubber in its stems (up to 20% by weight) equal in quality to that of the rubber tree *Hevea brasiliensis*. At present, several countries are involved in research aimed at commercial cultivation of guayule in arid lands (e.g. U.S.A., Australia, India, Israel). Guayule, related species of *Parthenium* (Asteraceae), and F_1 hybrids thereof, contain a diverse array of natural products that are potential inhibitors of larval growth of pestiferous insects. These compounds include several structural types of sesquiterpene lactones, sesquiterpene esters, triterpenes, and methylated flavonoids. This last group of compounds are characteristic of desert flora of the New World [1, 2]. Among the 17 recognized species in the genus, *P. argentatum* is unusual from a chemical viewpoint in that it is one of only two species which lack sesquiterpene lactones altogether [1], instead producing sesquiterpene esters. However, related species in the subgenus *Parthenichaeta* produce varying, often large, quantities of sesquiterpene lactones, and F_1 hybrids of guayule and these related species (e.g. *P. schottii*) are known to contain the lactones [3].

In the present report, we have investigated the efficacy of several natural products from species of *Parthenium* as inhibitors of early larval growth of two species of polyphagous pest insects, the bollworm *Heliothis zea* and the beet armyworm *Spodoptera exigua*, using a standard laboratory chronic feeding bioassay. In particular, we have compared the inhibitory effects of a series of sesquiterpene lactones, differing both in skeletal type and degree of substitution, on *H. zea*. There is increasing evidence that sesquiterpene lactones are effective physiological and behavioural inhibitors against insects [4–6], suggesting that these compounds may play an important role in plant defense against herbivory by insects [7].

RESULTS AND DISCUSSION

From our present results, it appears that only the sesquiterpene lactones, and possibly catechol-type flavonoids, are significantly inhibitory at dietary concentrations to which an herbivorous insect feeding on species of *Parthenium* would normally be exposed (Table 1). With respect to the methylated flavonoids tested in the present report, our results are in accord with earlier findings that indicate that whereas flavonoids possessing catechol substitution in the B ring are quite inhibitory to *Heliothis* larvae, related flavonoids lacking this substitution pattern (e.g. derivatives of kaempferol) are non-inhibitory or in some cases even stimulatory [8, 9]. Penduletin (6-hydroxykaempferol 3,6,7-trimethyl ether, **2**) enhanced larval growth of both *H. zea* and *S. exigua* at doses above 2 mM/kg. Methylation itself does not appear to increase toxicity; in the present study the ED_{50}'s for quercetagetin 3,7-dimethyl ether (**1**) were 4.8 and 2.4 mM/kg for *H. zea* and S. exigua respectively. However, concurrent bioassays with quercetin yielded ED_{50}'s of 4.9 and 2.5 mM/kg for these species respectively, indicating that the two compounds are equitoxic. Although we know of no quantitative data on the flavonoid content of species of *Parthenium*, it is not uncommon for as many as a dozen flavonoids to occur in foliage of these plants [1, 2], and our qualitative observations (TLC) suggest that several of these flavonoids are major secondary constituents of the foliage and resin thereof.

The sesquiterpene esters of guayule, guayulins A and B were found to be relatively non-inhibitory in our bioassays. It is noteworthy that guayulin-A (**3**) is a very potent elicitor of allergenic dermatitis in mammals: 0.5 μg is sufficient to produce marked erythemas in the guinea pig dermal bioassay [10]. In contrast, *H. zea* larvae appear

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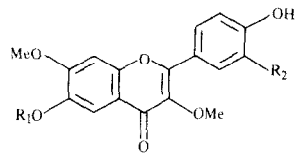
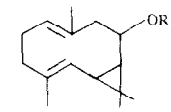
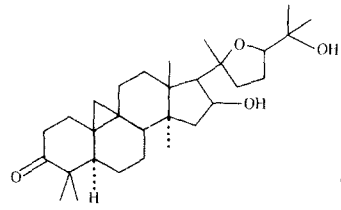


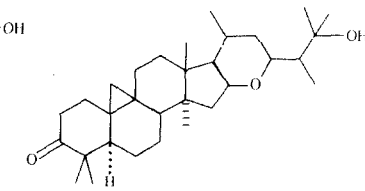
	R_1	R_2	
1	H	OH	Quercetagetin 3,7-DME
2	Me	H	6-OH-Kaempferol 3,6,7-TME



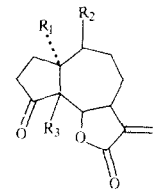
	R	
3	cinnamoyl	Guayulin - A
4	*p*-anisoyl	Guayulin - B

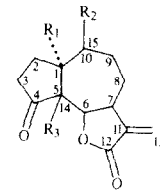


5 Argentatin - A

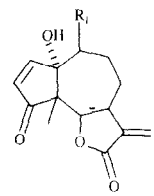


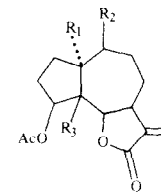
6 Argentatin - B



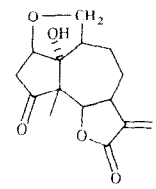


	R_1	R_2	R_3	
7	OH	Me	Me	Coronopilin
8	OH	CH_2OAc	Me	Tetraneurin - A
9	OH	Me	CH_2OAc	Tetraneurin - B
10	OH	Me	CH_2OiBut	Isochiapin - B
11	H	Me	CH_2OAc	Ligulatin - B

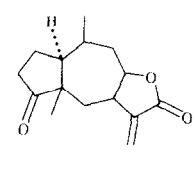




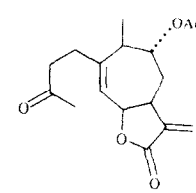
	R		R_1	R_2	R_3	
12	Me	Parthenin				
13	CH_2OAc	Conchosin - B				
14			OH	CH_2OH	Me	Tetraneurin - E
15			H	Me	CH_2OAc	Ligulatin - C



16 Conchosin - A



17 Confertin



18 Acetylivalbatin

capable of consuming over 1 mg of this compound with no apparent adverse effects. Similarly, the triterpenes of guayule, argentatins A and B, were relatively non-inhibitory with respect to *H. zea* larval growth. However, the leaf and stem resin of guayule is rich in both sesquiterpene esters and triterpenes, so it remains possible that these compounds could be important in plant defense against piercing and sucking insects.

All twelve of the sesquiterpene lactones tested were found to be significantly inhibitory to *H. zea* larvae (Table 2). The xanthanolide, ivalbatin acetate (**18**) was the least inhibitory, whereas the parthenolide, tetraneurin-A

Table 1. Efficacy of some *Parthenium* allelochemics as inhibitors of larval growth of *Heliothis zea* and *Spodoptera exigua*

Compound	Type*	Nat. conc. range (mM/kg)	ED_{50} mM/kg)† *H. zea*	*S. exigua*
1 6-Hydroxykaempferol TME	MFL	no data	⩾6.0	⩾6.0
2 Quercetagetin DME	MFL	no data	4.8	2.4
3 Guayulin-A	SQE	1.0–3.0	⩾4.0	⩾3.0
4 Guayulin-B	SQE	0.5–2.5	⩾4.0	⩾3.0
5 Argentatin-A	TTP	1.0–3.0	4.9	—
6 Argentatin-B	TTP	1.0–2.5	6.6	—
7 Coronopilin	SQL	0.5–2.0	3.7	—
17 Confertin	SQL	1.0–3.5	2.8	—
11 Ligulatin-B	SQL	0.1–1.0	2.1	—
8 Tetraneurin-A	SQL	0.5–2.5	1.5	1.5

*MFL-methylated flavonoid; SQE-sesquiterpene ester; TTP = triterpene; SQL = sesquiterpene lactone.

†ED_{50} is the dietary concentration which reduces larval growth by 50% relative to controls.

Table 2. Inhibition of larval growth of *H. zea* by sesquiterpene lactones and triterpenes of *Parthenium* species

Compound	Type*	Source	Larval growth (% of control)
18 Acetylivalbatin	X	*P. fruticosum* v. *trilobatum* Rollins	58 ± 12
7 Coronopilin	A	*P. schottii* Greenman	51 ± 7
17 Confertin	A	*P. schottii*	47 ± 9
12 Parthenin	A	*P. hysterophorus* L.	41 ± 6
16 Conchosin-A	P	*P. confertum* Gray	29 ± 4
13 Conchosin-B	P	*P. confertum*	25 ± 4
10 Isochiapin-B	P	*P. fruticosum* Less.	27 ± 4
11 Ligulatin-B	P	*P. schottii*	27 ± 4
15 Ligulatin-C	P	*P. incanum* H. B. K.	17 ± 3
8 Tetraneurin-A	P	*P. fruticosum*	12 ± 2
9 Tetraneurin-B	P	*P. schottii*	27 ± 4
14 Tetraneurin-E	P	*P. fruticosum* v. *trilobatum*	30 ± 4
5 Argentatin-A	T	*P. argentatum* Gray	69 ± 10
6 Argentatin-B	T	*P. argentatum*	79 ± 10
Triterpene-A	T	*P. tomentosum* DC.	88 ± 13
Triterpene-B	T	*P. tomentosum*	78 ± 19
Triterpene-C	T	*P. tomentosum*	84 ± 12

*X = xanthanolide; A = ambrosanolide; P = parthenolide; T = triterpene. Values are expressed as the mean ± s.e.m. Structures of the triterpenes from *P. tomentosum* are currently under investigation by Drs. A. Romo de Vivar and A. Ortega, UNAM, Mexico City. All compounds were tested at a dietary concentration of 3.0 mM/kg (fr. wt).

(**8**) was the most active. Tetraneurin-A (**8**) was also found to be equally inhibitory to *S. exigua* larvae. Dose-response relationships for four of the sesquiterpene lactones fed to *H. zea* were relatively linear over the dose range tested (Fig. 1). Closer examination of the results in Table 2 support the conclusion that the parthenolides (pseudoguianolides oxygenated at the C-14 and/or C-15 position) tend to be more inhibitory than their parent ambrosanolides. For example, tetraneurin-A (**8**) is more inhibitory than coronopilin (**7**), and conchosin-B (**13**) is more inhibitory than parthenin (**12**); in both cases the only difference between these pairs of compounds is the acetylation at the C-15 position in the former compounds. Similarly, ligulatin-B (**11**) is more inhibitory than confertin (**17**), the major difference being acetylation of the C-14 methyl group. Confertin is the only sesquiterpene lactone amongst those tested which is lactonized to the C-8 position; the others are all lactonized to the C-6 position. The exact positioning of the lactone ring does not appear relevant to its activity against *H. zea*, because confertin (**17**) is essentially equitoxic to coronopilin (**7**) (Table 2, Fig. 1).

The degree of substitution of the parthenolides does not appear to be a major factor in structure–activity

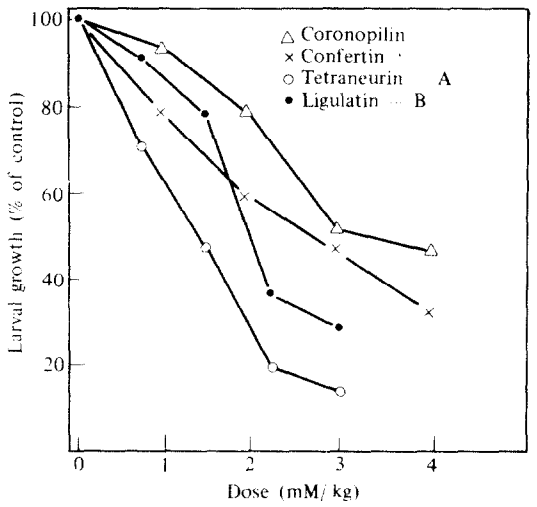


Fig. 1. Relationship between larval growth of *H. zea* and dietary concentrations of sesquiterpene lactones added to artificial diets.

relationships: isochiapin-B (**10**), which contains an *iso*-butyl function at C-14 is equitoxic to tetraneurin-B (**9**) and ligulatin-B (**11**), both of which are acetylated at C-14. Why tetraneurin-A (**8**) should be significantly more inhibitory than other parthenolides with similar or equal degrees of substitution remains unclear.

Inhibition of larval growth of *H. zea* by sesquiterpene lactones in the diet is limited to, or at least more pronounced in, the earliest (1st and 2nd) larval instars. When parthenin (**12**), coronopilin (**7**), tetraneurin-A (**8**) and ligulatin-B (**11**) were fed to 3rd instar larvae at 3 mM/kg, only ligulatin-B (**11**) was slightly inhibitory to growth (42% reduction in growth relative to controls). All four of the compounds were weak feeding inhibitors (10–30% reduction in consumption rates, relative to controls). The question of whether inhibition of early instar larvae is attributable to inhibition of feeding, or toxicity, is currently under investigation.

A recent report indicated that sesquiterpene lactones from species of *Melampodium* are very inhibitory to larval growth of the fall armyworm *Spodoptera frugiperda* [6]. In this example, melampodinin-A was more inhibitory than melampodin-A, the two compounds differing only by acetylation of the former in two positions. Curiously, another sesquiterpene lactone of the same skeletal type, enhydrin, was non-inhibitory, even though this latter compound possesses the unsaturated lactone ring which is thought to be responsible for biological activity against many organisms [11]. In the present study, we found that all lactones tested were inhibitory to *H. zea*. The bioassay we have used does not delineate behavioural response (inhibition of feeding) from physiological response (post-ingestive toxicity). However, available evidence from the literature suggests that inhibition of larval growth is not always correlated with feeding inhibition as determined in separate bioassays [6].

Tropical arborescent species of *Parthenium* often contain large quantities of sesquiterpene lactones. *P. schottii* was recently examined in our laboratory and found to contain up to 1.1% dry wt of sesquiterpene lactones in foliage, and up to 5.8% dry wt in inflorescences [3]. On a fresh weight basis, the foliar concentrations are approximately 8 mM/kg, with confertin (**17**), coronopilin (**7**) and tetraneurin-B (**9**) making up almost 90% of the total lactone content. Thus it seems quite likely that sesquiterpene lactones constitute an effective defense against insect herbivory in this species. The precise role of sesquiterpene lactones in herbivore deterrence, and the fate of these compounds following ingestion by insects is currently under investigation.

EXPERIMENTAL

Eggs and pupae of *H. zea* were provided by Shell Development Co., Modesto, California. Pupae of *S. exigua* were provided by Dr. R. Meyer, Dept. of Entomology, University of California, Davis. Compounds not available in our laboratory were kindly provided by Drs. A. Ortega (Mexico City), W. W. Schloman (Akron) and G. H. N. Towers (Vancouver).

Artificial diets used in this study were based on a commercial diet (no. 9763, BioServ Inc., Frenchtown, New Jersey). Experimental diets were prepared by dissolving pure compounds in a small vol. (e.g. 2 ml) of Me_2CO or CH_2Cl_2 which was subsequently coated onto the dry constituents of the diet. When the carrier had completely evaporated, the diet was prepared following the manufacturer's directions. Control diets were similarly treated with carrier alone. On average, the diets contained about 1 mg of pure assayed compound per g (fr. wt) of diet, and that under ideal circumstances, larvae would eat about 0.5 g of diet during the course of the bioassay.

Bioassays were performed by placing 20 neonate larvae individually in 1 oz plastic cups with *ca* 0.5 g fr. wt of diet; larvae were reared for 10 days at 27 ± 1°, *ca* 90% RH and 15:9 LD. Larvae were provided with fresh diet *ad libitum* during the course of the expt. Dose-response curves were prepared by rearing larvae on diets of at least three (usually four) dietary concns of a particular compound; ED_{50}'s were determined by linear regression. All bioassays were performed in duplicate except in cases (e.g. some sesquiterpene lactones) where pure compounds were in limited supply.

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REFERENCES

1. Rodriguez, E. (1977) *Biochem. Syst. Ecol.* **5**, 207.
2. Mears, J. A. (1980) *Biochem. Syst. Ecol.* **8**, 361.
3. Marchand, B., Behl, M. H. and Rodriguez, E. (1983) *J. Chromatogr.* **265**, 97.
4. Jones, S. B. Jr., Burnett, W. C. Jr., Coile, N. C., Mabry, T. J. and M. F. Betkouski (1979) *Oecologia* **39**, 71.
5. Picman, A. K., Elliott, R. H. and Towers, G. H. N. (1981) *Can. J. Zool.* **59**, 285.
6. Smith, C. M., Kester, K. M. and Fischer, K. H. (1983) *Biochem. Syst. Ecol.* (in press).
7. Mabry, T. J. and Gill, J. E. (1979) *Herbivores. Their Interaction with Secondary Plant Metabolites* (Rosenthal, G. A. and Janzen, D. H., eds.) p. 502. Academic Press, New York.
8. Elliger, C. A., Chan, B. G. and Waiss, A. C. Jr. (1980) *Naturwissenschaften* **67**, 358.

9. Isman, M. B. and Duffey, S. S. (1982) *Entomol. Exp. Appl.* **31**, 370.
10. Rodriguez, E., Reynolds, G. W. and Thompson, J. A. (1981) *Science* **211**, 1444.
11. Rodriguez, E., Towers, G. H. N. and Mitchell, J. C. (1976) *Phytochemistry* **15**, 1573.