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Antifeedant and Growth Inhibitory Effects of Some *neo*-Clerodane Diterpenoids Isolated from *Clerodendron* Species (Verbenaceae) on *Earias vitella* and *Spodoptera litura*

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Antifeedant and growth inhibitory effects of various *neo*-clerodane diterpenoids having a furofuran moiety, isolated from *Clerodendron* spp., were studied using *Earias vitella* and *Spodoptera litura*. The compounds clerodendrin B, 3-epicaryoptin, 15-hydroxyepicaryoptin, and clerodin were effective antifeedants at 10 μg/cm^3 (30 μg/g) of diet against *E. vitella* and at 10 μg/cm^2 of leaf against *S. litura*. All of the tested compounds, namely, clerodendrin B, 3-epicaryoptin, clerodendrin C, 15-hydroxyepicaryoptin, clerodendrin B acetate, and clerodin, showed good insect growth inhibitory activity even at lower concentrations.

KEYWORDS: Diterpenoids; *Earias vitella*; *Spodoptera litura*; antifeedant; insect growth inhibition (IGI)

INTRODUCTION

Worldwide attention is focused on the use of natural pest-controlling agents that are biodegradable and environmentally benign. In view of the ecotoxicity of synthetic insecticides and resistance developed by the insects, antifeedants offer considerable promise as components of emerging integrated pest management (IPM) due to their capacity to reduce feeding by insects (*1*).

Neem-based formulations, containing the most potent natural antifeedant compound,azadirachtin A, are now being employed for insect control as a complementary strategy in IPM. Plant extracts of the genus *Clerodendron* are used as armyworm antifeedants in East Africa (*2*). *neo*-Clerodane diterpenoids from *Clerodendron inerme* are simpler in their chemical structure compared to the complex azadirachtins, but they show some selective structural similarity in their furofuran moieties (*3*).

Most of the antifeedant studies of clerodanes have been conducted on *Spodoptera* spp. (common cutworms) (*4*). To our knowledge, the antifeedant activity of *neo*-clerodane diterpenoids on the spotted bollworm *Earias vitella* and systematic insect growth inhibitory studies of these compounds are not available. Herein we report antifeedant and insect growth inhibitory activity of these compounds against the spotted bollworm *E. vitella* and the tobacco cutworm *Spodoptera litura*. *C. inerme*, a wild as well as a hedge plant in India, is reported to contain three *neo*-clerodanes, clerodendrin B (**1**), 3-epicaryoptin (**2**), and clerodendrin C (**3**). In our present investigation an additional constituent, 2-acetoxyclerodendrin B (**4**) [which was earlier reported as a synthetic derivative of clerodendrin B (*5*)], and 15-hydroxyepicaryoptin (**5**) were isolated and assayed for antifeedant activity and insect growth inhibition (IGI) studies. Clerodin (**6**), the parent compound of this class, has been isolated from the aerial parts of *Clerodendron infortunatum*.

MATERIALS AND METHODS

Plant Material. *C. inerme* was collected from Loyola College, Chennai, India, and *C. infortunatum* from the Kolli hills, Tamilnadu, India, in October 2000; they were identified by Dr. T. S. Lokeshwari, Centre for Biotechnology, SPIC Science Foundation, Chennai.

Chemicals and Solvents. All chemicals used for semisynthetic diet were commercially available. Agar, ascorbic acid, sorbic acid, choline chloride, formaldehyde, vitamins, yeast, Wesson salt mixture, and streptomycin were purchased from Sigma Chemical Co. *n*-Hexane, ethyl acetate, silica gel 70−325 mesh (for column chromatography), and precoated TLC plates (silica gel 60 F_{254}) were purchased from Merck.

Azadirachtin A was a generous gift from Prof. T. R. Govindachari. It was isolated by the procedure prescribed in Govindachari et al. (*6*).

Apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded at 200 and 75 MHz, respectively, on a Bruker 200 MHz spectrometer. Chemical shifts (parts per million) are relative to tetramethylsilane as an internal reference. $CDCl_3$ from Aldrich Chemical Co. was used as solvent.

Isolation of *neo*-Clerodanes. *neo*-Clerodane diterpenoids (**1**−**5**, **Figure 1**) were isolated from the *n*-hexane extract of the aerial parts (leaves and stems) of *C. inerme* (see **Scheme 1**). The shade-dried, crushed aerial parts (2.5 kg) were percolated with *n*-hexane (10 L, three times) for 24 h. The resulting extract was then concentrated under vacuum to obtain a residue (23.1 g). The residue was chromatographed over a column of silica gel (70−325 mesh) and eluted with *n*-hexane/ethyl acetate combinations of increasing polarity. A diterpenoid-rich fraction obtained (elution solvent *n*-hexane/ethyl acetate 40:60) was pooled together and separated by reverse phase HPLC using a Shimadzu LC-8A HPLC system, linked to CR 4A data processor, and the peaks were detected at 215 nm. A Shimpack RP-18 preparative column (25 cm × 20 mm i.d., 15 μm) was used with acetonitrile/water 70:30 as mobile phase. For each run, 300 mg of sample was dissolved in 1.5

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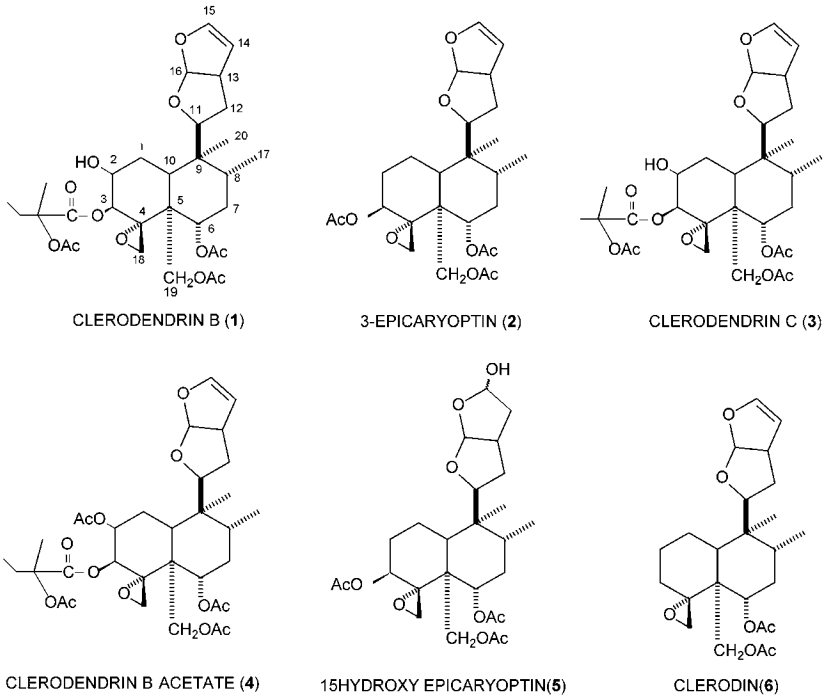
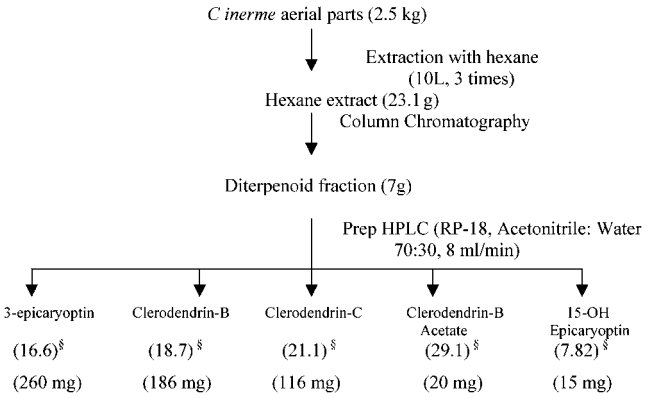


Figure 1. *neo*-Clerodanes isolated from *Clerodendron* spp.

Scheme 1



§ - Indicates HPLC retention time in minutes.

mL of 2% chloroform in methanol and was injected. The solvent flow rate was maintained at 8 mL/min.

Compounds **1**−**4** were identified by comparison of the spectral data with the literature. The structure of compound **5** was ascertained by NMR spectroscopic means as follows.

The ^{1}H NMR spectrum of **5** was similar to that of 3-epicaryoptin with the exception that the peaks at 4.8 and 6.5 ppm (corresponding to the olefinic protons at C-14 and 15) were missing. They were shifted to 1.5 and 5.7 ppm, respectively, in **5**, indicating the nonexistence of vinylic ether in the furofuran moiety. Also, the H-16 doublet was shifted to 5.8 ppm.

^{13}C NMR also revealed the absence of a peak at 146 ppm (corresponding to vinyl ether C-15). The position of the C-15 signal at 98 ppm indicated a hydroxyl substitution. An additional methylene at 39 ppm was assigned to C-14. Thus, the structure was confirmed as 15-hydroxyepicaryoptin (**5**): ^{1}H NMR δ 5.4 dd (1H, H_3), 4.7 dd (1H, H_6), 4.0 dd (1H, H_{11}), 3.5 m (1H, H_{13}), 5.7 d (1H, H_{15}), 5.8 d (1H, H_{16}), 0.8 d (3H, H_{17}), 2.5 d (1H, H_{18a}), 2.8 d (1H, H_{18b}), 4.3 d (1H, H_{19a}), 4.7 d (1H, H_{19b}), 0.9 s (3H, H_{20}); ^{13}C NMR δ 30.8 (C_1), 31.2 (C_2), 67.1(C_3), 65 (C_4), 46.2 (C_5), 71.1(C_6), 33.0 (C_7), 36.0 (C_8), 40.0 (C_9), 45.9 (C_{10}), 84.4 (C_{11}), 33.0 (C_{12}), 47.8 (C_{13}), 39.8 (C_{14}), 98 (C_{15}), 109.5 (C_{16}), 16.2 (C_{17}), 42.5 (C_{18}), 61.2 (C_{19}), 13.9 (C_{20}), 21.1, 20.9, 20.7 (OCOMe), 171.1, 170.0, 169.6 (OCOMe).

Spectroscopic and other physical data of compounds **1**−**4** are given in references *6* and *7*.

Clerodin (**6**) was isolated from *C. infortunatum* by the following procedure. The shade dried, crushed aerial parts (2.5 kg) were exhaustively extracted with *n*-hexane (3 × 5 L). The extract was then concentrated in vacuo to ~250 mL. This resulting solution on cooling gave colorless needles (2.5 g) and was filtered. The crystals were identified as clerodin (**6**) by comparison with an authentic sample supplied by Prof. T. R. Govindachari.

Insect Rearing and Bioassay. *E. vitella.* For rearing and bioassay, modified Klein medium was used, replacing alfalfa meal with bhendi [*Abelmoschus esculentus* (L) Moench] seed powder (*8*). The diet contained 700 mL of sterile water, 15 g of agar, 30 g of yeast, 100 g of bhendi seed meal, 2 g of sorbic acid, 2 g of choline chloride, 4 g of ascorbic acid, 2.5 g of *p*-hydroxybenzoic acid methyl ester, 7 g of Wesson's salt mixture, 2 mL of vitamins (ABDEC), 2.5 mL of formaldehyde, and 0.1 unit of streptomycin. For the bioassay, compounds were dissolved in acetone and mixed with bhendi meal 12 h prior to the preparation of diet for complete evaporation of solvent. After gelling, the diet was applied to a diet of volume 8 cm^3. This resulting diet cube (weight = 3 g) was placed in a 21 cm^3 plastic container, five freshly molted third-instar larvae were added, and the container was covered tightly with a muslin cloth. Bhendi meal treated with acetone alone served as control. After 24 h, diet cubes were taken out and weighed to determine the amount of diet consumed in treated versus control containers.

S. litura. The test insect *S. litura* L. was reared in the laboratory on *Ricinus communis* (castor) leaves at 25 ± 2 °C. Bioassay was conducted by no-choice method. Fresh castor leaf disks (180 cm^2) were cut and kept in Petri dishes. The individual compounds were dissolved in acetone and painted on the abaxial side using a pipet. Controls were treated with acetone alone and air-dried. Freshly molted third-instar larvae were taken from the culture and starved for 4 h prior to testing. Five larvae were placed in each Petri dish containing a leaf disk with a small piece of wet cotton to prevent desiccation. Five replicates were maintained. The setup was undisturbed for 24 h, after which time the leaf disks were removed for analysis. The disks were placed on a ΔT leaf area meter to determine leaf area consumed in treated versus control. The antifeedant activity percentage was calculated with the following equation:

$$\% \text{ AF} = 100 - (\text{treated/control}) \times 100$$

Table 1. Antifeedant Activity of Diterpenoids against *E. vitella* and *S. litura*[a]

	antifeedant activity (%)			
	S. litura		*E. vitella*	
compound	10 μg/cm^2	5 μg/cm^2	10 μg/cm^3	5 μg/cm^3
clerodendrin B (**1**)	84.9 ± 3.6	76.5 ± 4.2	82.14 ± 0.01	74.85 ± 0.01
3-epicaryoptin (**2**)	82.6 ± 4.2	73.4 ± 4.2	79.76 ± 0.01	73.0 ± 0.01
clerodendrin C (**3**)	65.0 ± 2.8	56.0 ± 3.9	41.54 ± 0.01	31.0 ± 0.01
clerodendrin B acetate (**4**)	75.0 ± 3.7	65.8 ± 2.7	69.0 ± 0.02	51.0 ± 0.01
15-hydroxyepicaryoptin (**5**)	78.0 ± 2.9	74.0 ± 3.2	71.84 ± 0.01	62.15 ± 0.1
clerodin (**6**)	80.0 ± 7.3	72.0 ± 3.7	72.13 ± 0.01	62.87 ± 0.01
azadirachtin A (**7**)[b]	79.2 ± 4.7		79.2 ± 4.7	

[a] Values are mean ± SD. [b] Treatment at 0.5 μg/cm^2 for *S. litura* and at 0.5 μg/cm^3 for *E. vitella*.

Table 2. Larval Growth Parameters of *E. vitella* Fed Diterpenoids at 5 or 10 μg/cm^3 of Diet[a]

	total larval duration (days)		larval mortality (%)	
compound	10 μg/cm^3	5 μg/cm^3	10 μg/cm^3	5 μg/cm^3
1	11.2 ± 0.4c	10.6 ± 0.48de	20	15
2	10.3 ± 0.4b	10.0 ± 0.63cd	12	8
3	8.2 ± 0.4a	8.4 ± 0.48b	8	4
4	9.7 ± 0.4b	9.2 ± 0.48c	4	4
5	10.2 ± 0.4b	9.8 ± 0.97cd	8	8
6	9.9 ± 0.48b	9.4 ± 0.48c	8	4
control	8.2 ± 0.48a	8.2 ± 0.48a	4	4
azadirachtin A (**7**)[b]	11.0 ± 0.66c		68	

[a] Values are mean ± SD. Values followed by a common letter within a column are not significantly different at $p < 0.05$. Twenty-five insects (five replicates with five individuals each) were used. [b] Treatment at 0.5 μg/cm^3.

Insect Growth Inhibition. Freshly molted third-instar larvae were left to feed on the diet incorporated with compounds till molting. After molting, the larvae were transferred to fresh diet (untreated) and reared to pupation. Larval duration (in days), mortality (percent), pupal duration (days), pupal weight (milligrams), adult emergence (percent), longevity (days), and fecundity (numbers) were recorded.

Statistical Analysis: The data were subjected to analysis of variance (ANOVA) in a completely randomized block design. The means were subjected to Duncan's (*9*) multiple-range test (DMRT) to prove their significance.

RESULTS AND DISCUSSION

Six *neo*-clerodane diterpenoids isolated from *Clerodendron* spp. were tested for their efficacy as insect antifeedant against *E. vitella* and *S. litura*. The most potent insect antifeedant, azadirachtin A, was used as an active control for comparison. Initially the activities of these compounds were tested at 1 μg/cm^2 for *S. litura* and at 1 μg/cm^3 for *E. vitella*, for which the results were not encouraging. However, 100% antifeedancy was reported for clerodin (**6**) and epicaryoptin (**2**) at 50 and 200 ppm, respectively, against *S. littoralis* (*10*). Hence, the dosages of the compounds for our study were fixed at 5 and 10 μg/cm^3 (approximately 15 and 30 μg/g of diet) for *E. vitella* and at 5

Table 3. Pupal Parameters of *E. vitella* Fed Diterpenoids at 5 or 10 μg/cm^3 of Diet[a]

	pupal duration (days)		pupal weight (mg)		pupal mortality (%)	
compound	10 μg/cm^3	5 μg/cm^3	10 μg/cm^3	5 μg/cm^3	10 μg/cm^3	5 μg/cm^3
1	12.2 ± 0.7e	10 ± 0.63c	50.4 ± 3.44a	55 ± 1.41a	42.5	40
2	10.2 ± 0.7d	8.8 ± 0.4b	53.2 ± 3.05ab	57.4 ± 2.9ab	40	30
3	7.8 ± 0.73b	7.2 ± 0.4a	66.2 ± 2.31e	69 ± 5.1c	22.5	20
4	8.8 ± 0.49bc	7.0 ± 0.63a	64.2 ± 2.03de	71.2 ± 2.9c	32	24
5	9.6 ± 0.48cd	9.0 ± 0.63b	57.2 ± 4.70bc	58.0 ± 2.6ab	34	28
6	8 ± 0.63b	7.2 ± 0.40a	58.0 ± 4.4bc	60.4 ± 3.3b	32	28.5
control	6.4 ± 0.21a	6.4 ± 0.21a	88.2 ± 4.01f	88.2 ± 4.01d	8	8
azadirachtin A (**7**)[b]	8.4 ± 1.0d		59.2 ± 3.24ab		37.5	

[a] Values are mean ± SD. Values followed by a common letter within a column are not significantly different at $p < 0.05$. Twenty-five insects (five replicates with five individuals each) were used. [b] Treatment at 0.5 μg/cm^3.

Table 4. Adult Emergence, Longevity, and Reproduction of *E. vitella* Fed Diterpenoids at 5 or 10 μg/cm^3 of Diet[a]

	adult emergence (%)		adult deformity (%)		adult longevity (days)				adult fecundity/female (numbers)	
					male		female			
compound	10 μg/cm^3	5 μg/cm^3	10 μg/cm^3	5 μg/cm^3	10 μg/cm^3	5 μg/cm^3	10 μg/cm^3	5 μg/cm^3	10 μg/cm^3	5 μg/cm^3
1	44.0	52.5	16.0	8.0	4.6 ± 0.48a	5.4 ± 0.48a	4.8 ± 0.78a	5.8 ± 0.74a	35.8 ± 4.3a	47.2 ± 4.0a
2	52.0	60.4	8.0	8.0	5.6 ± 0.48b	5.8 ± 0.4a	5.8 ± 0.40b	6.2 ± 0.40ab	37.0 ± 2.4a,b	55.6 ± 4.0b
3	70.5	76.0	8.0	4.0	6.8 ± 0.40c	7.0 ± 0.63b	7.2 ± 0.4c	7.6 ± 0.48d	55.8 ± 4.6c	59.4 ± 3.3b
4	64.0	72.5	4.0	4.0	6.4 ± 0.48c	6.8 ± 0.4b	7.2 ± 0.40c	7.4 ± 0.48cd	58.0 ± 3.6c	66.6 ± 3.2c
5	62.0	68.0	4.0	4.0	6.2 ± 0.40bc	6.6 ± 0.48b	6.6 ± 0.48c	6.8 ± 0.40bc	54.2 ± 3.6c	60.0 ± 5.6bc
6	60.0	68.6	8.0	4.0	6.4 ± 0.48c	6.6 ± 0.48b	6.6 ± 0.48c	6.8 ± 0.40bc	41.0 ± 2.6b	55.2 ± 3.4b
control	88.0	88.0	4.0	4.0	8.2 ± 0.4d	8.2 ± 0.4c	8.6 ± 0.48d	8.6 ± 0.48e	120.6 ± 6.8d	120.6 ± 6.8d
azadirachtin A (**7**)[b]	37		25		6.6 ± 0.48b		6.8 ± 0.48bc		58.0 ± 6.57b	

[a] Values are mean ± SD. Values followed by a common letter within a column are not significantly different at $p < 0.05$. Twenty-five insects (five replicates with five individuals each) were used. [b] Treatment at 0.5 μg/cm^3.

Table 5. Larval Growth Parameters of *S. litura* Fed Diterpenoids at 5 or 10 μg/cm^2 of Leaf[a]

	total larval duration (days)		larval mortality (%)	
compound	10 μg/cm^2	5 μg/cm^2	10 μg/cm^2	5 μg/cm^2
1	15.8 ± 0.97d	14.5 ± 0.48b	44.0	32.0
2	14.5 ± 1.26b–d	14.0 ± 1.26b	36.0	24.0
3	12.5 ± 1.0a	12.5 ± 0.8a	14.0	12.5
4	13.1 ± 1.4ab	12.6 ± 0.48a	24.0	20.0
5	14.2 ± 0.97b–d	13.5 ± 0.48ab	32.0	20.0
6	14.8 ± 0.74cd	14.3 ± 1.01b	35.2	24.5
control	12.4 ± 0.48a	12.4 ± 0.48a	0.0	0.0
azadirachtin (**7**)[b]	13.9 ± 0.80a–c		78	

[a] Values are mean ± SD. Values followed by a common letter within a column are not significantly different at $p < 0.05$. Twenty-five insects (Five replicates with five individuals each) were used. [b] Treatment at 0.5 μg/cm^2.

and 10 μg/cm^2 of leaf area for *S. litura*, respectively. Among the diterpenoids tested (**Table 1**), the most effective was clerodendrin B (**1**) and the least effective was clerodendrin C (**3**). The order of decreasing activity was **1** > **2** > **6** > **5** > **4** > **3**. The differences in susceptibility of the two insects to the diterpenoids were only marginal, *S. litura* being slightly more susceptible than *E. vitella*. Significant difference in antifeedant activity between two concentrations of each compound was noticed irrespective of the insect used, but this does not indicate a linear relationship. Although azadirachtin A shows good antifeedancy at 0.5 μg/cm^2 for S. *litura* and at 0.5 μg/cm^3 for *E. vitella*, compounds **1** and **2** equal the activity at 5 and 10 μg concentrations.

Compound **1** significantly increased the total larval duration (**Tables 2** and **5**), although there were no significant increases in the duration of individual instars. This showed that the above compounds had slow and sustainable effects on larval duration. The least effect was observed with **3**. All of the tested compounds showed significant effect on larval mortality of *S. litura*, indicating that the insect was comparatively more susceptible to these compounds. Interestingly, the efficacy order was the same as in the case of antifeedant activity tests.

Compared to the larval mortality, the tested compounds showed significant effect on pupal mortality (**Tables 3** and **6**). The most potent was **1**, and the least being **3**, in both insects. The lethal effect was greater in *E. vitella*, for which a negative linear relationship was observed between pupal duration and pupal weight. Compound **1** showed maximum dose-related response toward total larval duration, pupal duration, and adult emergence, whereas compound **2** exhibited maximum dose-related response toward pupal mortality, adult emergence, and fecundity.

Compound **3** showed the minimum dose-related response to all parameters.

Perusal of the data on adult stage (**Tables 4** and **7**) indicated many fascinating observations. The tested compounds showed considerable reduction on adult emergence. Adults derived from azadirachtin A-treated third-instar larvae showed improper eclosion in both insects, whereas shrivelled wings and reduced abdominal growth were associated with diterpenoid-fed ones. Reduced longevity was seen with females compared to males, regardless of compounds and doses. Fecundity of females of both insects was reduced greatly. However, all laid eggs were fertile.

Many workers have reported quantitative structure−activity relationship (QSAR) studies for clerodane diterpenoids. Munakata et al. reported higher activity for 14−15-saturated compounds compared to unsaturated compounds (*7*), whereas, Bruno et al. reported that saturation of the 14−15 double bond

Table 6. Pupal Parameters of *S. litura* Fed Diterpenoids at 5 or 10 μg/cm^2 of Leaf[a]

	pupal duration (days)		pupal weight (mg)		pupal mortality (%)	
compound	10 μg/cm^2	5 μg/cm^2	10 μg/cm^2	5 μg/cm^2	10 μg/cm^2	5 μg/cm^2
1	13.0 ± 1.0c	12.0 ± 0.7c	220.2 ± 16.0a	240 ± 10.98a	37.0	29.4
2	11.8 ± 0.74bc	11.0 ± 0.54bc	250.2 ± 14.7b	261.6 ± 8.47b	30.0	26.5
3	10.6 ± 0.48b	10.0 ± 0.63ab	282.2 ± 11.65de	289.8 ± 7.93de	22.7	20.2
4	11.0 ± v0.8b	10.2 ± 0.74ab	273.2 ± 13.9cd	284.4 ± 9.52cd	27.0	22.7
5	11.6 ± 1.2bc	10.8 ± 0.5b	258.8 ± 17.4bc	272.2 ± 11.5bc	27.6	22.5
6	11.6 ± 0.8bc	10.9 ± 0.66bc	250.2 ± 15.6b	270.2 ± 12.3bc	27.0	27.0
control	9.2 ± 0.74a	9.2 ± 0.74a	302.4 ± 5.9e	302.4 ± 5.95e	0.0	0.0
azadirachtin (**7**)[b]	11.0 ± 0.89bc		247.2 ± 11.0a		28.0	

[a] Values are mean ± SD. Values followed by a common letter within a column are not significantly different at $p < 0.05$. Twenty-five insects (five replicates with five individuals each) were used. [b] Treatment with A at 0.5 μg/cm^2.

Table 7. Adult Emergence, Longevity, and Reproduction of *S. litura* Fed Diterpenoids at 5 or 10 μg/cm^2 of Leaf[a]

	adult emergence (%)		adult deformity (%)		adult longevity (days)				adult fecundity/female (numbers)	
					male		female			
compound	10 μg/cm^2	5 μg/cm^2	10 μg/cm^2	5 μg/cm^2	10 μg/cm^2	5 μg/cm^2	10 μg/cm^2	5 μg/cm^2	10 μg/cm^2	5 μg/cm^2
1	46.4	59.2	16.8	12.0	6.2 ± 0.4a	6.8 ± 0.74a	5.2 ± 0.75a	6.4 ± 0.48a	114.2 ± 4.16a	127.6 ± 9.95ab
2	56.8	64.8	12.8	8.8	6.4 ± 0.48a	7.4 ± 0.48ab	6.0 ± 0.63a	7.2 ± 0.74bc	130 ± 11.5ab	141.2 ± 14.8ab
3	69.6	76.0	8.0	4.0	8.5 ± 0.44d	9.2 ± 0.4de	8.2 ± 0.74c	8.9 ± 0.4e	155.8 ± 13.9c	178.4 ± 13.39b
4	64.0	72.0	8.8	4.8	8.5 ± 0.44d	9.0 ± 0.63de	8.0 ± 0.63bc	8.2 ± 0.4de	154.0 ± 11.2c	168.0 ± 15.4ab
5	62.4	70.4	9.6	7.2	7.5 ± 0.44bc	8.0 ± 0.63bc	7.0 ± 0.63b	7.4 ± 0.48b–d	142.0 ± 11.9bc	158.0 ± 9.94a
6	63.2	64.0	9.6	8.0	8.0 ± 0.63cd	8.4 ± 0.48cd	7.0 ± 0.8bc	8.0 ± 0.63c–e	131.6 ± 11.3ab	143.8 ± 18.6ab
control	100	100	0.0	0.0	9.4 ± 0.48e	9.4 ± 0.48e	10 ± 0.63d	10 ± 0.63f	248 ± 21.0d	248 ± 21.0c
azadirachtin A (**7**)[b]	42.8		28.5		7.2 ± 0.4ab		7.0 ± 0.63ab		145.0 ± 15.6ab	

[a] Values are mean ± SD. Values followed by a common letter within a column are not significantly different at $p < 0.05$. Twenty-five insects (five replicates with five individuals each) were used. [b] Treatment at 0.5 μg/cm^2.

and introduction of an acyloxy function at C-1 or C-7 decreased the activity (*11*). Our observations also indicated a decrease in activity with 14−15 saturation and introduction of an acyloxy function at C-3 in compound **6** when compared to **5**. The change in carbon chain length at C-19 in Jodrellins had no effect on the antifeedancy, whereas we observed a considerable decrease in activity from **1** to **3**, although they differ by only one carbon at C-3. Acetylation of the hydroxyl at C-2 in **1** also reduced the activity to a considerable extent (**1** and **4**). Considering the different observations by different group of workers, it appears that the QSAR studies for *neo*-clerodanes need further research.

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LITERATURE CITED

(1) Alford, A. R. Deployment strategies for antifeedants in management of Colorado potato beetle. In *Potato Pest Management*: *A Global Perspective*; Zehnder, G. W., Jansson, R. K., Powelson, M. L., Raman, K. V., Eds.; American Phytopathological Society: St. Paul, MN, 1994.

(2) Cooper, R.; Solomon, P. H.; Kubo, I.; Nakanishi, K.; Shoolery, J. N.; Occolowitz, J. N. Myricoside, an African Armyworm Antifeedant: Separation by Droplet Countercurrent Chromatography. *J. Am. Chem. Soc.* **1980**, *102*, 7953.

(3) Morgan, E. D.; Wilson, I. D. Insect Hormones and Insect Chemical Ecology. In *Comprehensive Natural Products Chemistry*; Derek, B., Koji, N., Eds.; Elsevier Press: Amsterdam, The Netherlands, 1999; Vol. 8.

(4) Ben Jannet, H.; Harshallah-Shikri, F.; Mighri, Z.; Simmonds, M. S. J.; Blaney, W. M. Responses of *Spodoptera litorralis* to Tunisian plant extracts and to neo-clerodanes from *Ajuga pseudoiva* leaves. *Fitoterapia* **2000**, *71* (2), 105−112.

(5) Jagan Mohan Rao, A.; Pereira, J.; Gurudutt, K. N. Neo-Clerodane diterpenes from Clerodendron Inerme. *Phytochemistry* **1993**, *34* (2), 572−574.

(6) Govindachari, T. R.; Gopalakrishnan, G.; Suresh, G. Isolation of various azadirachtins from neem oil by preparative high performance chromatography. *J. Liq. Chromatogr. Relat. Tech.* **1996**, *19*, 1729−1734.

(7) Hosozawa, S.; Kato, N.; Munakata, K. Diterpenoids from Clerodendron Calamitosum. *Phytochemistry* **1974**, *13*, 308−309.

(8) Klein, M.; Levski, S.; Keren, S. Development of artificial diets for laboratory rearing of larvae of the spiny bollworm, *Earias vitella*. *Entomol. Exp. Appl.* **1983**, *34*, 121−122.

(9) Duncan, D. B. Multiple range and multiple F tests. *Biometrics* **1955**, *11*, 1−42.

(10) Hosozawa, S.; Kato, N.; Munakata, K.; Chen, Y.-L. Antifeedant active substances for insect in Plant. *Agric. Biol. Chem.* **1974**, *38*, 1045−1048.

(11) Bruno, M.; Piozzi, F.; Roselli, S. Natural and hemisynthetic neoclerodane diterpenoids from *Scutellaria* and their antifeedant activity. *Nat. Prod. Rep.* **2002**, *19*, 357−378.

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