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Antifeedant Effects of the Limonoids from *Entandrophragma candolei* (Meliaceae) on the Gram Pod Borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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The biological activity of the limonoids prieurianin and epoxyprieurianin isolated from *Entandrophragma candolei* (Harms) (Meliaceae) and their respective acetates was assessed using the gram pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). The compounds exhibited strong antifeedant activity in a diet choice bioassay with epoxyprieurianin acetate being most effective with 48.3 ppm deterring feeding by 50% (DI_{50}) and prieurianin the least effective (DI_{50} = 91.4 ppm). The effect on growth of larvae was concomitant with the reduced feeding by neonate and third instar larvae. In nutritional assays, all the compounds reduced growth and consumption when fed to larvae without any effect on efficiency of conversion of ingested food (ECI), suggesting antifeedant activity alone. No toxicity was observed nor was there any significant affect on nutritional indices following topical application, further suggesting that prieurianin-type limonoids act specifically as feeding deterrents.

KEYWORDS: ***Entandrophragma candolei*; prieurianin; epoxyprieurianin; prieurianin acetate; epoxyprieurianin acetate; antifeedants; *Helicoverpa armigera***

INTRODUCTION

The Meliaceae family of plants has shown great potential for pest management in terms of secondary plant chemistry or the presence of allelochemicals in its various genera (*1*). These allelochemicals have considerable potential as antifeedants or biopesticides (*2*). The idea of using nontoxic feeding deterrents as crop protectants has attracted much attention during the past decade, but many antifeedants also show some post ingestive physiological activity (*3*). Azadirachtin from *Azadirachta indica* is the most potent insect feeding deterrent and natural insect growth regulator isolated to date. It occurs at concentrations of 0.1 to 0.9% in the seed kernel and it has been established that 30 to 60 g azadirachtin per hectare is sufficient to combat and repel key pests of various crops (*4*). As an insecticide, azadirachtin-based products control more than 400 species of insects including those in the insect orders Lepidoptera, Coleoptera, Homoptera, Diptera, Heteroptera, Caelifera, and Thysanoptera, etc. It is also well-known now that the biopesticide potential of neem in particular, and the family Meliaceae in general, is due to the presence of characteristic limonoid-type compounds. Many oxidative products in various species impart an edge to Meliaceae plants as their biological activity seems to relate to current concepts of the evolution of the limonoids (*5*).

In searching for antifeedants of natural origin we decided to investigate Meliaceous plants, apart from *Melia* species, and accordingly selected *Entandrophragma candolei* (Harms) for investigation against a lepidopteran pest. Extracts from the bark of this tree are known to inhibit feeding in stored grain pests (*6*). In an earlier preliminary study of prieurianin isolated from *Nymania capensis*, antifeedant activity was demonstrated in a leaf-disc choice assay against tobacco budworms and Mexican bean beetles (*7*).

MATERIALS AND METHODS

Chemistry. The bark of the tree *Entandrophragma candolei* (Harms) was collected in the primeval forest in Zaire near Kisangani in 1990, shade-dried, and kept in stock for further processing and chemical investigations. Prof. F. Szafranski, University of Kisangani, identified the trees (*8*). The shade-dried bark was pulverized into a powder and subsequently subjected to sequential solvent extraction. A choloroform extract (1.5 g) of the material, found to possess activity against insects, was subjected to column chromatography on silica gel using a benzene/acetone gradient mobile system. The chromatography was monitored by TLC (C_6H_6/Me_2CO, 4:1 solvent system) and yielded 380 mg of prieurianin and 910 mg of epoxyprieurianin. To produce analytical samples, final purification was performed using a semi-prep HPLC column (30 cm × 0.8 cm) filled with 10-μm Nucleosil C-18 RP. The mobile phase was a mixture of acetonitrile, methanol, and water (58:15:27). The main peaks were collected and evaporated to dryness, and finally the residue was subjected to spectral investigation, the results of which compared well with that of Gullo et al. (*9*) and Lukacova et al. (*10*).

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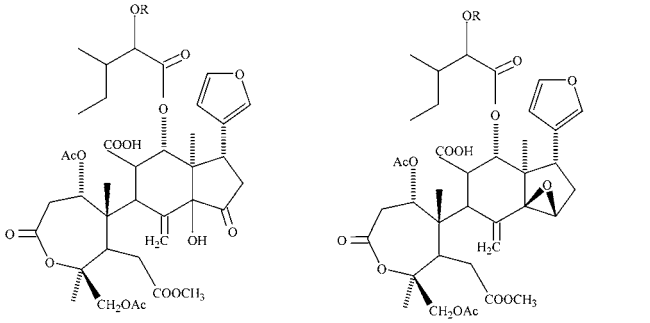
Prieurianin data revealed the following. mp 214−218 °C. $[\alpha]^{20}_D$ = −13.3 ($CHCl_3$, c = 1.22). IR (Nujol film): 3364, 2964, 1742, 1207, 1027 cm^{-1}. HRMS $[M]^+$: 762.30998 for $C_{38}H_{50}O_{16}$; calculated, 762.30998. MS 70 eV, *m/z* (rel int. %): 762 (0.1), 419 (5), 297 (20), 287 (22), 241 (32), 227(25), 185 (28), 167 (35), 149 (200, 135 (20), 121 (40), 95 (25), 43 (100). ^{13}CNMR (125 MHz, $CDCl_3$): 11.5 q (C5′), 13.0 q (C28), 15.2 q (C6′), 20.7 q (Ac), 21.2 q (Ac), 32.9 q (C18), 38.0 q (C19), 53.4 q ($COOCH_3$), 73.9 d (C2′), 110.5 d (C22), 122.9 s (C20), 137.8 s (C8), 140.6 d (C21), 143.1 d (C23), 160 d (OCOH), 206.0 s (C15). ^{1}HNMR (500 MHz, $CDCl_3$): 0.76 t (H5′), 0.84 d (H6′), 1.25 s (H28), 2.05 s (Ac), 2.11 s (Ac), 3.45 brs (H2′), 6.24 d (H22), 7.19 s (H21), 7.38 s (H23).

Similarly, 14β,15β-epoxy-prieurianin gave the following. $[\alpha]^{20}_D$ = 16° ($CHCl_3$, c = 1.55). IR (Nujol film) 3507, 2965, 1736, 1233, 1231 cm^{-1}. HRMS $[M]^+$ 746.31522; calculated for $C_{38}H_{50}O_{15}$, 746.31497. MS 70 eV, *m/z* (rel int. %): 746 (0.1), 509 (0.8), 283 (20), 241 (38), 223 (40), 209 (35), 183 (30), 167 (30), 135 (20), 107 (40), 43 (100). ^{13}CNMR ($CDCl_3$) 11.5 q (C5′), 13.7 q (C28), 15.2 q (C6′), 20.3 q (2* Ac), 27.1 q (C18), 37.8 q (C19), 52.14 q ($COOCH_3$), 59.5 d (C15), 65.6 t (C29), 75.0 (C2′), 111.1 d (C22), 121.0 s (C20), 124 t (C30), 134 s (C8), 140.5 d (C21), 142.9 d (C23). ^{1}HNMR (500 MHz, $CDCl_3$,): 0.79 t (H5′), 0.85 d (H6′), 0.95 s (H28), 1.49 s (H18,19), 2,05 s (Ac), 1.61 brs (H2′), 3.65 s ($COOCH_3$), 4.25 d (H29), 4.59 d (H29), 5.28 s (H30), 5.50 brs (H11, 30), 5.78 d (H12), 6.14 d (H22), 7.09 s (H21), 7.32 s (H23), 7.09 s (OCHO).

Acetylation of Prieurianin. Prieurianin (100 mg, 0.131 mmol) dissolved in dry pyridine (2 mL) was treated with acetic anhydride (0.3 mL). The reaction mixture was left at room temperature for 3 h. Subsequently the volatile contents were removed using a rotary evaporator. The residue was purified by chromatography on silica gel in a benzene/acetone gradient system. Subsequently fractions were collected and evaporated to dryness to obtain 2′-acetyl-prieurianin in 95% yield.

IR (Nujol film): 3349, 2963, 1746, 1439, 1374, 1233 cm^{-1}. HRMS $[M]^+$ 804.32065 for $C_{40}H_{52}O_{17}$; calculated, 804.32045. MS 70 eV, *m/z* (rel int. %): 804 (0.1), 744 (5), 461 (12), 419 (11), 241 (30), 244 (25), 167 (25), 157 (45), 129 (50), 121 (25), 69 (24), 43 (100). ^{13}CNMR (125 MHz, $CDCl_3$): 11.4 q (C5′), 13.0 q (C28), 38.7 q (C19), 110.4 d (C21), 122.7 (C20), 130.8 t (C30), 137.7 s (C8), 141 d (21), 143.1 d (C23), 206 (C15). ^{1}HNMR (500 MHz, $CDCl_3$): 0.76 t (H5′), 0.82 d (H6′), 0.99 s (H28), 1.25 6 H s (H18, H19), 2.05 s (Ac), 2.11 6H s (Ac), 3.69 s ($COCH_3$), 4.60 d (H2′), 6.29 d (H22), 7.36 s (H21), 7.38 s (H23).



Acetylation of 14β,15β-Epoxy-Prieurianin. 14β,15β-epoxy-prieurianin (100 mg, 0.134 mmol) dissolved in dry pyridine (2 mL) was treated with acetic anhydride (0.3 mL). The reaction mixture was left at room temperature for 3 h. Subsequently the volatile contents were removed using a rotary evaporator. The residue was purified by chromatography on silica gel in a benzene/acetone gradient system. Proper fractions were collected and evaporated to dryness to obtain the 2′- acetyl-14β,15β-epoxyprieurianin in 95% yield.

IR (Nujol film): 2966, 1742, 1374, 1233 cm^{-1}. HRMS $[M]^+$ 788.32564 for $C_{40}H_{52}O_{16}$; calculated, 788.32556. MS 70 eV, *m/z* (rel int. %): 788 (0.1), 728 (0.8), 509 (10), 475 (15), 415 (20), 305 (20), 283 (25), 241 (30), 223 (30), 157 (45), 129 (45), 69 (25), 43 (100). ^{13}CNMR (125 MHz, $CDCl_3$): 11.4 q (C5′), 13.7 q (C28), 15.4 q (C6′), 20.6 q (Ac), 20.7 q (Ac), 21.1 q (Ac), 33.9 q (C18), 38.2 q (C19), 52.0 q ($COCH_3$), 59.6 d (C15), 66.7 t (C29), 111.2 d (C22), 121.8 d (C20), 123.9 t (C30), 134.9 s (C8), 140.9 d ((C21), 142.8 d (C23), 160.7 s (CHO). ^{1}HNMR (500 MHz, $CDCl_3$): 0.76 t (H5′), 0.82 d (H6′), 1.25 m (H4′), 2.05 s (Ac), 2.11 s (Ac), 1.15 s (Ac), 3.63 s ($COOCH_3$), 4.25 d (C29), 4.60 d (H29), 4.80 d (H2′), 5.32 brs (H30), 5.52 brs 2H (H11.H30), 5.80 d (H12), 6.20 s (H22), 7.35 s (H21), 7.40 s (H23), 8.85 s (CHO).

Acetylation of the isolated compounds was done to assess whether further potentiation in activity could be achieved. Azadirachtin, a well-known biopesticide (>95% purity by HPLC), was used for comparison and isolated from seeds of Indian neem, *Azadirachta indica*, by the modified method of Nakanishi (*11*). The results of the spectral investigation of isolated azadirachtin were identical to reported data (*12*) based on ^{1}HNMR and ^{13}CNMR spectroscopic analyses.

Insects. The gram pod borers, *Helicoverpa armigera*, were obtained from a laboratory culture maintained on an artificial diet prepared in the laboratory (*13*). The culture was maintained at 27 ± 1 °C and a 16:8 LD photoperiod. Generally neonate, and third and fourth stage larvae were used in various experiments.

Growth Evaluation. Prieurianin and epoxyprieurianin isolated from *E. candolei* and their acetyl derivatives in acetone were individually mixed with the dry portion of the artificial diet to give final concentrations ranging from 5 to 50 ppm fresh weight. The carrier solvent was evaporated; control diet was treated with carrier alone.

Upon hatching, two 24-h-old neonate larvae were placed on a 1-g fresh weight diet in an individual solo cup (30 mL) as described earlier (*14*). The cups were kept in a plastic tray lined with moistened filter paper to maintain humidity. The experiments were carried out in a growth chamber at 27 ± 2 °C and 16:8 LD photoperiod. Larval growth was assessed as a percentage of the controls after 7 d based on larval weight. Larval mortality, if any, was also recorded. Forty larvae were used for each concentration. The concentration inhibiting 50% growth relative to controls (EC_{50}) was determined by regression analysis. Azadirachtin was used for comparison in the same fashion at a dietary range of 0.1 to 0.5 ppm.

Early third instar (average weight 20 ± 3 mg) and fourth instar (average weight 100 ± 6 mg) were also used to determine EC_{50} values in artificial diet for these stadia as mentioned above. However, dietary concentrations ranged from 50 to 100 ppm, except for azadirachtin where the range was from 0.2 to 1.4 ppm. Compounds were also applied topically to larvae at a dose range of 2−10 μg/larva, except for azadirachtin where the dose range was 0.02−0.08 μg/larva.

Choice Feeding Assay. Antifeedant activity was determined by a modified diet choice test (*15*). Two small preweighed cubes of artificial diet containing various concentrations (25−100 ppm) of all the test compounds individually and two preweighed control diet cubes were placed in alternating positions in 9-cm-diam. Petri dishes. A single 2- to10-h-old third instar larva, pre-starved for 4 h, was placed in the center of each dish. Individual larvae were used to avoid cannibalism that is prevalent in this species. There were 10 replicates per concentration. Consumption by larvae from each diet cube was recorded after 6 h; the short duration evaluation gives specific assessment of behavioral response. A feeding deterrence index (DI) was calculated as $(C - T)/(C + T) \times 100$, where C is consumption of control diet and T is the consumption of treated diet (*13*).

Nutritional Analysis. To distinguish behavioral effects from toxicity mediated effects, the insects were subjected to nutritional analysis by providing diets treated with prieurianin, epoxyprieurianin, and their respective acetates. The experiment was carried out using 2- to 4-h-old fourth instar larvae. In this experiment 30 larvae per treatment were provided with the compounds at 50 and 100 ppm in diet. Relative growth per unit weight of the insect at the outset of experiment (RGRi) and relative consumption rate at the outset of experiment (RCRi) were calculated on a dry weight basis after 3 d of feeding. An index of food conversion efficiency (ECI) was calculated as described earlier (*13*). In another set of experiments the compounds were applied topically to larvae at 5 and 10 μg/larva dose. Larvae were treated on the dorsal terga with the dose in 0.5 μL of acetone using a fine 25-μL syringe (7105 series syringe, Hamilton Co., Reno, NV) attached to a repeating

Table 1. Effective Concentrations (ppm) of the Compounds from *Entandrophragma candolei* Inhibiting Growth of *H. armigera* Neonates in a Dietary Assay ($n = 40$)[a]

compound	EC_{50} ppm (95% CI)	EC_{95} ppm (95% CI)	slope
prieurianin	18.8d (15.7–22.7)	169.2d (132.8–268.5)	1.73 ± 0.3
prieurianin acetate	11.5c (9.5–13.9)	106.7c (69.8–122.8)	1.58 ± 0.2
epoxyprieurianin	3.2b (2.1–4.9)	30.6b (20.5–45.8)	1.68 ± 0.4
epoxyprieurianin acetate	2.6b (1.6–4.4)	30.8b (19.9–47.1)	1.60 ± 0.3
azadirachtin[b]	0.26a (0.15–0.36)	1.46a (0.43–4.13)	2.2 ± 0.6

[a] Means within a column followed by the same letter are not significantly different $P > 0.05$ based on Tukeys test. [b] Azadirachtin was used as standard for comparison.

dispenser (PB-600, Hamilton Co.). Controls were treated with acetone alone. In the case of azadirachtin treatments, care was taken to avoid any contact with the mouthparts of the larvae during topical application. The larvae were then allowed to feed on an untreated diet.

RESULTS AND DISCUSSION

The EC_{50} values (concentration inhibiting larval growth by 50% relative to controls) found for prieurianin, epoxyprieurianin, and their acetyl derivatives after 7 days of feeding are shown in **Table 1**. Epoxyprieurianin was more active ($EC_{50} = 3.2$ ppm) than prieurianin ($EC_{50} = 18.8$ ppm), and similarly the epoxy derivative of its acetate also showed increased activity (Table 1) against neonate *H. armigera* larvae. Epoxyprieurianin and epoxyprieurianin acetate, however, were significantly similar in their activity. It was obvious from the experiment that there was a significant decrease in diet consumption during the course of development of the larvae. A dose-dependent decrease in consumption of diet from 10.5 to 50.2% within 7 d in comparison to that of controls was recorded. This illustrates that the prieurianin-type limonoids act effectively as antifeedant compounds, which subsequently deplete the growth of larvae. No lethal effect to the gram pod borer, *H. armigera*, was observed at the levels of evaluation in any experiment. Azadirachtin, however, remained the most potent with $EC_{50} = 0.26$ ppm against the same stage larvae. Our study indicates that the acetylation of both compounds increases the efficacy (**Table 1**), however more significantly in the case of prieurianin. Obviously two conclusions could be drawn: first epoxy compounds are more efficacious, and second acetylation enhances the activity of these limonoids. However, azadirachtin, which remains the most potent active botanical biopesticidal compound, is about 12- and 70-fold more active than epoxyprieurianin and prieurianin, respectively. This is in contrast to the observations recorded for prieurianin against Mexican bean beetles, *Epilachna varivestis*, (*7*) where activity has been considered as close to that of azadirachtin. This apparently is because the conclusions were based on a wide range of efficacy obtained in a choice bioassy that in fact points to a moderate appetite loss. A previous review (*5*) of limonoid bioactivity revealed that the majority of limonoids tested showed some level of antifeedant activity. Accordingly, deterrence observed in the present study compares favorably with that of other limonoids (*16, 17*). Prieurianin and epoxyprieurianin are ring B opened limonoids known to inhibit growth of the murine P-388 lymphocytic leukemia cell line after they were isolated from root bark of *Guarea guidona* plant (*18*). However, their high antifeedant activity has been recorded against stored grain insect pests (*6*) after they were isolated from *Entandrophragma candolei*.

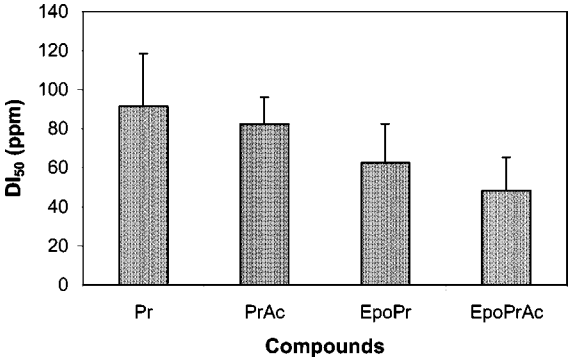


Figure 1. Feeding inhibition of 50% (DI_{50} ± SE) induced by various compounds in third instar *H. armigera* larvae in a chocie feeding assay ($n = 40$ per treatment) [Pr = prieurianin; PrAc = prieurianin acetate; EpoPr = epoxyprieurianin and EpoPrAc = epoxyprieurianin acetate].

Table 2. Effective Concentrations (ppm) of the Compounds from *Entandrophragma candolei* Inhibiting Growth of *H. armigera* Third Instar Larvae in a Dietary Assay ($n = 40$)

compound	EC_{50} (ppm)	95% confidence interval	slope value
prieurianin	92.2	74.1–114.8	1.83 ± 0.1
prieurianin acetate	67.5	43.1–72.6	1.28 ± 0.2
epoxyprieurianin	55.7	46.3–67.0	1.56 ± 0.3
epoxyprieurianin acetate	45.7	35.7–60.2	2.17 ± 0.1
azadirachtin	0.4	0.23–1.2	2.12 ± 0.5

All four compounds, after topical application to third instar larvae, showed no significant change in the weight gain of the larvae and consumption of food did not deviate from the control consumption. Even at the highest dose of 10 μg/larva, the weight gain was more than 130 mg and was similar to that of the control larvae. However, when same-stage larvae were subjected to diet choice assay, there was a significant decrease in feeding, and a gradual increase in efficacy was observed from prieurianin to epoxyprieurianin acetate (**Figure 1**). The most effective deterrence was observed in epoxyprieurianin acetate ($DI_{50} = 48.3$ ppm) and prieurianin was least in its activity ($DI_{50} = 91.4$ ppm). Even in artificial diet assay with third instar larvae, there was significant decrease in growth due to reduced feeding, and EC_{50} value for prieurianin acetate was 62.7 ppm followed by prieurianin, 92.2 ppm. Thus, epoxy derivatives were more potent, though less active, than azadirachtin (**Table 2**). Results obtained from the treatment to third instar larvae, therefore, support the above results, showing epoxy limonoid to be more effective than prieurianin, with their acetates being correspondingly more efficacious.

Once the antifeedant activity of prieurianin-type limonoids was established, experiments were carried out to investigate whether feeding deterrence was physiological toxicity mediated or due to specific behavioral affects. The results from dietary utilization experiments on fourth instar larvae (**Table 3**) revealed that relative growth and consumption rates were reduced after oral administration with no concomitant reduction in ECI at any level of treatment for all four limonoids. At the highest dose used (100 ppm) there was substantial inhibition in consumption: prieurianin inducing 57.7, prieurianin acetate 67.5, epoxy prieurianin 69.1, and apoxyprieurianin acetate 72.8% of reduction in consumption. Following topical treatment there was no significant change in any of the parameters evaluated (**Table**

Table 3. Feeding (RCRi[a]), Growth (RGRi[b]), and Efficiency of Conversion of Ingested Food (ECI) by Fourth Instar *H. armigera* Larvae ($n = 30$) Fed an Artificial Diet Containing Compounds Isolated from *Entandrophragma candolei* and Their Corresponding Acetates[c]

	Nutritional Index (mean + SE)		
ppm	RGRi (mg/mg/d)	RCRi (mg/mg/d)	ECI (%)
	prieurianin		
0	0.80 ± 0.03a	3.85 ± 0.9a	20.3 ± 2.7a
50	0.41 ± 0.02b	2.04 ± 0.2b	20.0 ± 2.1a
100	0.32 ± 0.02c	1.63 ± 0.3c	19.8 ± 1.3a
	prieurianin acetate		
0	0.84 ± 0.09a	4.03 ± 1.0a	20.4 ± 2.8a
50	0.38 ± 0.02b	1.78 ± 0.5b	21.5 ± 3.1a
100	0.27 ± 0.01c	1.31 ± 0.3bc	20.8 ± 2.7a
	epoxyprieurianin		
0	0.94 ± 0.08a	3.43 ± 0.9a	27.4 ± 3.1a
50	0.36 ± 0.02b	1.38 ± 0.2b	26.2 ± 1.8a
100	0.28 ± 0.04c	1.06 ± 0.1bc	26.4 ± 2.0a
	epoxyprieurianin acetate		
0	0.83 ± 0.10a	3.97 ± 0.7a	20.9 ± 2.7a
50	0.35 ± 0.04b	1.74 ± 0.1b	20.1 ± 2.3a
100	0.20 ± 0.03c	1.08 ± 0.1c	19.0 ± 2.9a

[a] RCRi, relative consumption rate per unit weight. [b] RGRi, relative growth rate per unit weight. [c] Means within a column in each row followed by the same letter are not significantly different $P > 0.05$ based on Tukeys test.

Table 4. Feeding (RCRi[a]), Growth (RGRi[b]), and Efficiency of Conversion of Ingested Food (ECI) by Fourth Instar *H. armigera* Larvae ($n = 30$) after Topical Application of Isolated Compounds from *Entandrophragma candolei* and Their Corresponding Acetates[c]

	Nutritional Index (mean + SE)		
μg/larva	RGRi (mg/mg/d)	RCRi (mg/mg/d)	ECI (%)
	prieurianin		
0	0.80 ± 0.03a	3.85 ± 0.9a	20.3 ± 2.7a
5	0.83 ± 0.04a	3.74 ± 0.2a	22.1 ± 2.7a
10	0.82 ± 0.04a	3.79 ± 0.3a	21.6 ± 1.8a
	prieurianin acetate		
0	0.81 ± 0.09a	3.93 ± 1.0a	20.6 ± 2.4a
5	0.88 ± 0.02a	3.98 ± 0.9a	22.0 ± 3.6a
10	0.81 ± 0.01a	3.96 ± 0.7a	20.5 ± 2.3a
	epoxyprieurianin		
0	0.90 ± 0.08a	3.41 ± 0.6a	26.2 ± 3.0a
5	0.88 ± 0.02a	3.48 ± 0.2a	25.2 ± 1.8a
10	0.87 ± 0.04a	3.36 ± 0.3a	25.9 ± 2.0a
	epoxyprieurianin acetate		
0	0.83 ± 0.10a	3.84 ± 0.7a	21.9 ± 2.9a
5	0.85 ± 0.04a	3.74 ± 0.1a	22.1 ± 2.8a
10	0.80 ± 0.03a	3.78 ± 0.1a	21.3 ± 2.5a

[a] RCRi, relative consumption rate per unit weight. [b] RGRi, relative growth rate per unit weight. [c] Means within a column in each row followed by the same letter are not significantly different $P > 0.05$ based on Tukeys test.

4). Therefore, dietary utilization experiments show that compounds incorporated into an artificial diet reduce the growth of larvae by 60−75% within 3 d with very significant effect on the relative consumption rate. Index of dietary utilization (ECI), however, did not drop significantly (**Table 3**). ECI is an overall measure of an insect's ability to utilize the food that it ingests for growth. A drop in ECI indicates more food is being metabolized for energy and less is being converted to body mass, i.e., growth of insect. As there was no affect on ECI, therefore, prieurianin-type limonoids do not induce a chronic toxicity. Obviously these results do implicate antifeedant affect and, therefore, primary mode of action of prieurianin-type limonoids. The nutritional experiments in which treatment was given topically also support the conclusion drawn, as after topical application of these compounds none of the parameters were significantly different (**Table 4**). There was no reduction in consumption and, therefore, no subsequent affect on growth or ECI, which clearly demonstrates that these limonoids affect the feeding stimulus only when fed orally to insect larvae. Other limonoids such as limonin, epilimonol, nomilin, meliantriol, melianol, numbinene, salannin, trichillins, pedonin, and toonacilins, etc. also act as antifeedants rather than toxins to various insect species (*19*−*21*).

Azadirachtin also, when incorporated into the artificial diet of fourth instar larvae of *H. armigera*, significantly reduced the consumption (up to 76% reduction) and relative growth rate (up to 80% reduction) of larvae compared to that of controls. Efficiency of conversion of ingested food into biomass was not significantly reduced. In fact, in the case of azadirachtin, consumption rate and growth rate decrease with increasing azadirachtin concentration as would be expected from the involvement of chemoreceptors (*22*, *23*). Azadirachtin has a marked antifeedant effect on most insect species, which is regulated through the chemoreceptors located in mouthparts. However, the degree of deterrence varies from species to species depending upon the deterrent chemoreceptor sensitivity to the compound, so that incorporation of azadirachtin into the diet will involve a multiple effect of starvation, growth inhibition, and growth regulation. Of course, this has correlation with the mode of treatment (*19*). Prieuranin-type limonoids seem to follow the pattern that might involve the chemoreceptors and must be stimulating specific deterrent receptors to induce an antifeedant effect.

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