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INSECT ANTIFEEDANT AND GROWTH-REGULATING ACTIVITIES OF SALANNIN AND OTHER C-SECO LIMONOIDS FROM NEEM OIL IN RELATION TO AZADIRACHTIN

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Abstract—The antifeedant and insect growth-regulating activities of salannin, nimbin, and 6-deacetylnimbin, in comparison with azadirachtin-A, have been studied against *Spodoptera litura*, *Pericallia ricini*, and *Oxya fuscovittata*. Salannin deterred feeding, delayed molt by increasing larval duration, caused larval and pupal mortalities, and decreased pupal weights in the two lepidopterans. Salannin also caused molt delays and nymphal mortalities in *Oxya fuscovittata*. The role of salannin and other compounds in conferring bioactivity, along with azadirachtin-A, to neem oil/neem seed extracts is emphasized.

Key Words—Antifeedant activity, insect growth-regulating activity, salannin, nimbin, 6-deacetylnimbin, azadirachtin-A.

INTRODUCTION

Neem oil and neem seed kernel extracts from *Azadirachta indica* A. Juss (Meliaceae) have been used in India since ancient times for the protection of plants from insect attack. The wisdom of this traditional practice was validated by the isolation, by Butterworth and Morgan (1968), of azadirachtin, which was stated to be a feeding deterrent against the desert locust at a concentration of 40 μg/liter. This remarkable observation aroused the interest of chemists and biologists all over the world during the past two decades, and as a result more than 100

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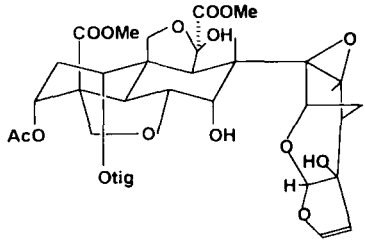
compounds have been isolated from all parts of the tree (Siddiqui et al., 1989; Devkumar and Sukh Dev, 1993; Kraus, 1995). Azadirachtin has been shown to be a potent antifeedant at 10–100 ppm and an ecdysis inhibitor at 1–10 ppm (Warthen, 1989; Govindachari, 1992; Champagne et al., 1992; Hansen et al., 1994; Schmutterer, 1995) in over 200 species of insects. Since neem seeds and neem oil are readily available, there is every promise that formulations based on them will be useful for pest control without harmful effects to the ecosystem. Indeed, a large number of formulations have been produced and marketed during the past few years, which have been standardized in terms of azadirachtin content.

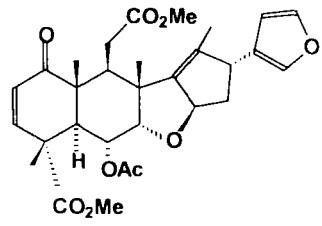
A recent study (Govindachari et al., 1995a) reported the isolation of the major triterpenoids in neem oil by preparative high-performance liquid chromatography and quantified their abundance by analytical HPLC. Salannin (1.4%), nimbin (0.5%), deacetylnimbin (0.4%), azadiradione (0.2%) and epoxyazadiradione (0.13%) are the major constituents in neem oil, while azadirachtin-A was present to the extent of only 0.03%. Interestingly, neem oil contains other azadirachtins, such as azadirachtins-B, -D, -H, and -I, which together constitute, on a conservative estimate, at least 0.2% of the oil. These azadirachtins possess the same order of activity as azadirachtin-A (Govindachari et al., 1994). Neem kernel extracts also contain all these compounds and azadirachtin-A alone is present, on average, at ca. 0.3%.

It is generally believed that the bioactivity of neem preparations is due to the azadirachtin content in them. According to Isman et al. (1990) "even though other limonoids from neem and related meliaceae have demonstrated bioactivity against insects, none of these are within two orders of magnitude as active as azadirachtin and thus their contribution to bioactivity of neem oils may be largely discounted."

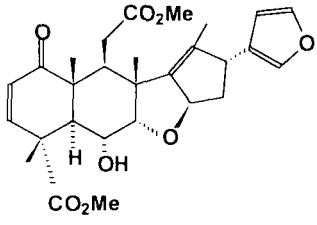
There are only a few entomological studies on the constituents of neem other than azadirachtin-A. Salannin, for instance, has been shown to be an antifeedant, as active as azadirachtin-A against *Epilachna varivestis* (Schwinger et al., 1984, Kraus et al., 1987) and more active against *Pieris brassicae* (Luo Lin-er et al., 1995). However, it had no insect growth regulatory (IGR) activity (Rembold, 1989, Simmonds et al., 1990). Azadiradione and epoxy azadiradione are feeding deterrents to a lesser extent than azadirachtin-A (Govindachari et al., 1995b). It may be noted that the major limonoids, except azadirachtin-A, have been tested for bioactivity, especially for IGR activity, only against a few insect species. While azadirachtin content in neem oil correlated well with behavior-disrupting and growth-regulating activity of *Peridroma saucia* (Isman et al., 1990), no such correlation was found with neem kernel extracts from different sources against *Epilachna varivestis* and *Aedes aegyptii* (Ermel et al., 1984). Subsequently, Ermel et al. (1987), based on 66 neem kernel extracts, claimed strong correlation of EC_{50} values of all samples with absolute amounts



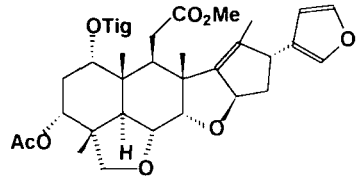
Azadirachtin A



Nimbin



6-deacetylnimbin



Salannin

FIG. 1. Limonoids tested in this study.

of azadirachtin. However, they have indicated that "because of the lack of sufficient data, this finding is statistically significant only for the Benin extract, but an obvious tendency existed with other extracts".

The present study was undertaken to assess the relative contribution of these major limonoids, viz., salannin, nimbin, deacetylnimbin, and also azadirachtin-A (Fig. 1) (for comparison) in terms of insect antifeedant and growth regulating activities.

METHODS AND MATERIALS

Salannin, deacetylnimbin, nimbin, and azadirachtin-A were isolated by a direct preparative HPLC method, their purities confirmed by analytical HPLC (Govindachari et al., 1995a), and their identities established by comparison with authentic samples (NMR and mass spectral data).

The test insects, *Spodoptera litura* and *Pericallia ricini*, were reared on the leaves of *Ricinus communis*. *Oxya fuscovitatta* was reared on the leaves of *Coix lachryma*. A dual-choice bioassay was performed (Govindachari et al., 1995b). For *S. litura* and *P. ricini*, circular disks (180 cm^2) were cut from the

leaves of *R. communis*, with the median vein as the marker between the two equal halves. One milliliter of acetone alone or the test compound in acetone (at 50, 10, 5, and 1 $\mu g/cm^2$ area of leaf) was spread with the help of a fine pipet on the right half (90 cm^2 treated), leaving the left half untreated. For each compound and concentration, five replicates were used. After air-drying, each leaf disk was placed inside separate Petri dishes and five freshly molted third instars (from the same egg batch, approximately similar in weight and size) were placed at the center of the leaf. After 24 hr, the insects were removed and the uneaten area in the treated half was measured using a ΔT area measurement meter. For *O. fuscovittata*, leaf strips (60 × 4 cm) were cut with the median vein acting as a marker between two equal halves and assayed as indicated above. Percent feeding index (PFI) was calculated (c.f. Luco et al., 1994) using the formula

$$\text{PFI} = \frac{\text{area fed in treated}}{\text{area fed in treated} + \text{area fed in solvent control}} \times 100$$

where treated is the area treated with the solution of compound in 1 ml acetone and solvent control is the area treated with 1 ml acetone.

For the insect growth regulation study, leaf disks (180 cm^2 of *R. communis* leaves for *S. litura* and *P. ricini* and 240 cm^2 of *C. lachryma* leaves for *O. fuscuvittata*) were spread with 2 ml of a solution of the test compound in acetone, to have 0.5 μg of test compound/cm^2 leaf area. Leaves treated with acetone alone were kept as control. The leaves were air-dried and placed in separate Petri dishes. Five replicates were maintained for each compound. Five freshly emerged third instars of *S. litura/P. ricini* were introduced into each Petri dish and forced to feed until they molted into the next instar. From the fourth instar, the larvae were provided with normal diet (*R. communis* leaves) until the end of the experiment. In the case of *O. fuscovittata*, five fifth instar females were introduced into the cage along with the treated *C. lachryma* leaves and the nymphs were fed till they molted to the adults. Durations of *S. litura* and *P. ricini* instars, mortality of larvae, numbers of pupae, and pupal weights were recorded. For *O. fuscovittata*, nymphal duration and mortality were recorded. The data were subjected to one-way ANOVA and Student Neumann-Keul means were presented.

RESULTS

Among the insects tested, *Oxya fuscovittata* was the most sensitive in terms of antifeedancy to all four compounds, even at concentrations of 1–10 $\mu g/cm^2$ leaf area, while *Spodoptera litura* was the least sensitive. As an antifeedant, azadirachtin was the most effective compound followed by salannin, nimbin,

TABLE 1. PERCENT FEEDING INDICES OF *Spodoptera litura*, *Pericallia ricini*, AND *Oxya fuscovittata* FED ON C-SECO LIMONOIDS FROM *Azadirachta indica*[a]

Compound	Insect species	Percent feeding index			
		1 $\mu g/cm^{2}$[b]	5 $\mu g/cm^{2}$[b]	10 $\mu g/cm^{2}$[b]	50 $\mu g/cm^{2}$[b]
Azadirachtin-A	*S. litura*[c]	27.50(4.0)	17.96(6.6)	16.50(2.8)	11.50(4.8)
	P. ricini[c]	20.80(3.5)	15.60(3.1)	12.50(3.5)	10.00(3.1)
	O. fuscovittata[d]	25.80(3.1)	18.40(3.2)	12.80(2.1)	10.60(2.7)
Salannin	*S. litura*[c]	36.50(7.5)	32.00(4.5)	30.20(5.2)	27.40(4.2)
	P. ricini[c]	29.30(5.4)	28.50(3.0)	22.50(2.2)	14.40(3.3)
	O. fuscovittata[d]	28.80(3.0)	20.50(4.8)	15.40(3.3)	12.40(2.8)
Nimbin	*S. litura*[c]	37.10(4.1)	35.50(2.0)	34.20(5.8)	26.00(3.6)
	P. ricini[c]	32.50(2.5)	30.40(3.3)	30.20(5.0)	16.60(3.0)
	O. fuscovittata[d]	26.20(4.8)	23.30(3.9)	18.20(4.0)	11.80(5.4)
6-Deacetyl nimbin	*S. litura*[c]	35.40(2.1)	35.00(3.6)	33.10(3.6)	30.50(4.4)
	P. ricini[c]	35.50(1.8)	34.00(2.6)	29.80(5.6)	20.20(3.1)
	O. fuscovittata[d]	32.40(2.6)	25.95(2.5)	20.40(1.8)	15.50(4.2)

[a]Values presented are Student-Neumann-Keul means; values in parentheses indicate ± SD.
[b]Concentration/cm^2 area of *R. communis* leaf for *S. litura* and *P. ricini*; *C. lachryma* for *O. fuscovittata*.
[c]Third instar.
[d]Fifth instar.

and deacetylnimbin in order of decreasing activity. Azadirachtin was twice as active as salannin against *S. litura* and *Pericallia ricini*; salannin, nimbin, and azadirachtin were equally active against *O. fuscovittata* (Table 1).

The insect growth-regulatory activities of the above four compounds were assessed in terms of larval duration, pupal duration, larval and pupal mortalities, and pupal weights. A significant increase in larval durations was noted in *S. litura* and *P. ricini* when fed on each of the four compounds (Table 2). The effect was pronounced in the fifth instars of *S. litura* and the third and fourth instars of *P. ricini*. No significant differences were found among salannin, nimbin, and deacetylnimbin, clearly indicating equal effectiveness in increasing the larval durations of *S. litura* and *P. ricini*. A marginal, but significant, increase in pupal duration was noted in *P. ricini* when fed on all four limonoids, while no such change was noticed with *S. litura*. In the case of *O. fuscovittata*, azadirachtin and salannin significantly increased duration of the fifth instars (Table 3).

Larval mortality was comparatively higher in azadirachtin-fed *S. litura* and *P. ricini*, while pupal mortality was as high in salannin- and nimbin-fed individuals as in azadirachtin-fed individuals (Table 2). In the case of *O. fuscovit-*

TABLE 2. DURATION OF DEVELOPMENT, PUPAL MORTALITY, AND PUPAL WEIGHT OF *Spodoptera litura* and *Pericallia ricini* FED ON C-SECO LIMONOIDS FROM *Azadirachta indica*[a]

Insect species	Compound	Duration of individual instars (days)					Mortality (%)		Pupal weight (mg)
		3	4	5	6	pupal dur.	L	P	
Spodoptera litura	Control	3.46 ± 0.5a	3.60 ± 0.45a	4.50 ± 0.82a	3.36 ± 0.78a	8.31 ± 0.46a	1.0	0	438.0 ± 10.6a
	Azadirachtin A	4.20 ± 0.86b	4.50 ± 0.62b	6.8 ± 0.85b	3.80 ± 0.41b	8.45 ± 0.52a	23.8	16	318.2 ± 12.5b
	Salannin	4.10 ± 0.35b	4.50 ± 0.5b	6.50 ± 0.5bd	3.90 ± 0.5b	8.40 ± 0.3a	13.6	12	360.0 ± 13.3c
	Nimbin	3.90 ± 0.46b	4.20 ± 0.68b	6.30 ± 0.4cd	3.50 ± 0.3a	8.50 ± 0.65a	9.1	12	385.4 ± 10.2d
	6-deacetyl nimbin	3.90 ± 0.55b	4.20 ± 0.51b	6.00 ± 0.6c	3.45 ± 0.4a	8.40 ± 0.55a	8.7	8	382.4 ± 9.5d
Pericallia ricini	Control	4.50 ± 0.70a	7.50 ± 0.2a	4.50 ± 0.35a		6.90 ± 0.38a	0	4	476.4 ± 10.5a
	Azadirachtin A	7.00 ± 0.85b	8.60 ± 0.6bc	5.70 ± 0.65c		7.50 ± 0.30b	28.5	16	344.0 ± 15.5b
	Salannin	6.80 ± 0.9b	8.50 ± 0.4c	5.20 ± 0.5b		7.40 ± 0.45b	14.3	16	381.0 ± 9.9c
	Nimbin	6.80 ± 0.6b	8.80 ± 0.5b	5.50 ± 0.6bc		7.10 ± 0.8a	18.2	12	398.8 ± 12.5d
	6-deacetyl nimbin	6.90 ± 0.72b	8.50 ± 0.5c	5.30 ± 0.35		7.00 ± 0.8a	9.5	16	412.3 ± 14.3e

[a]Values presented are Student-Neumann-Keul means; values in parentheses indicate ± SD. Experiment initiated with 25 individuals (five replicates with five individuals each). Concentration of compounds used = 0.5 $\mu g/cm^2$ area of *R. communis* leaf. Values followed by the same letter are statistically not significant at $P < 0.05$. L = larvae; P = pupae.

TABLE 3. DEVELOPMENTAL DURATION OF FINAL INSTAR OF *Oxya fuscovittata* FED ON SOME C-SECO LIMONOIDS[a]

Compound	Duration of development (days)	Mortality (%)
Control	6.2(0.55)a	1
Azadirachtin-A	8.4(0.46)b	20
Salannin	8.0(0.25)c	16.6
Nimbin	7.8(0.42)c	6.6
6-deacetyl nimbin	7.9(0.50)c	6.6

[a]Experiment initiated with 30 individuals (six replicates with five individuals each). Values presented are Student-Newmann-Keul means; values in parentheses indicate ± SD; concentration of compounds used = 0.5 $\mu g/cm^2$ area of *Coix lachryma* leaf.

tata, fifth instar mortality was much higher in azadirachtin- and salannin-fed individuals as compared to that observed for nimbin- and deacetylnimbin-fed individuals (Table 3). Significant reduction of pupal weight was characteristic of azadirachtin-fed individuals. Salannin, although less effective, brought about significant pupal weight reductions compared to nimbin, deacetyl nimbin, and the control.

DISCUSSION

In our bioassay experiments salannin, nimbin, and deacetylnimbin were half as active as azadirachtin as antifeedants. However, in growth-regulatory activity against *S. litura*, *P. ricini*, and *O. fuscovittata*, salannin is comparable to azadirachtin-A. Thus, salannin deters feeding, delays molt by increasing larval duration, causes larval and pupal mortality, decreases pupal weights in *S. litura* and *P. ricini*, and causes molt delay and nymphal mortalities in *O. fuscovitatta*. It was reported that salannin had no growth regulatory activity against *E. varivestis* (Rembold, 1989), *Spodoptera frugiperda*, *Spodoptera littoralis* (Simmonds et al., 1990), and *Heliothis virescens* (Simmonds et al., 1990, Klocke, 1987). In the present communication, salannin has been shown to be an effective insect growth regulator against some insects. Therefore, the absence of IGR activity of salannin against certain insects cannot be generalized for all insect species. In our bioassay experiments, nimbin and deacetylnimbin do not show any appreciable growth-regulating activity against *S. litura*, *P. ricini*, and *O. fuscovittata*.

Salannin, which has good antifeedant and growth-regulating activity, is present in neem oil in at least three to four times the concentration of azadi-

rachtin. Nimbin, deacetylnimbin, azadiradione, and epoxy azadiradione, although less active than azadirachtin, are also present in considerable quantities (Govindachari et al., 1995a). Therefore, while azadirachtin is undoubtedly a remarkable antifeedant and growth-regulating compound, the role of other limonoids (especially salannin) in the bioactivity of formulations should not be ignored or underestimated. Further experiments are in progress for the study of antifeedant and growth-regulating activities of salannin and other major terpenoids in neem against other insect pests.

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