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INSECT ANTIFEEDANT ACTIVITY OF FOUR PRIEURIANIN-TYPE LIMONOIDS

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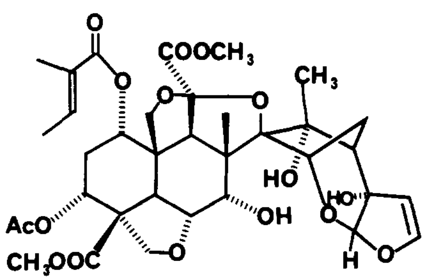
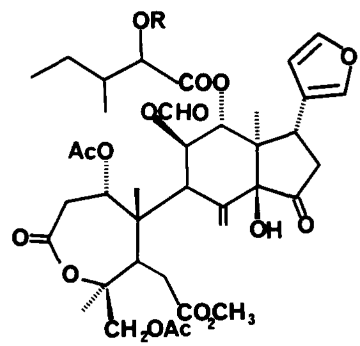
Plants of the Meliaceae family have been used in folk medicine (1) and to control insect pests (2, 3). Extracts obtained from seeds and leaves of a Meliaceae species, *Azadirachta indica* A. Juss, commonly occurring in many tropical areas, have been shown to give good crop protection against a broad range of economically important insect pests (4). The bioactive materials isolated from the neem tree are primarily azadirachtin (**1**)(5, 6) and a number of less active limonoids (7). The mode of action of these compounds is still not clearly understood, but the gross, measurable effects are inhibition of feeding, growth, and ecdysis (8). Other Meliaceae species have also yielded limonoid antifeedants (9), less active, on the whole, than azadirachtin.

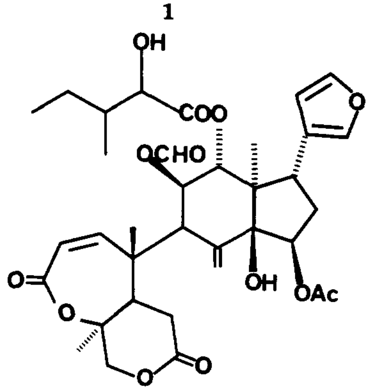
In the present paper we wish to report antifeedant activities of four structurally interrelated limoids: prieurianin acetate (**2**), prieurianin (**3**), rohituka-7 (**4**) and rohitukin (**5**), all belonging to the prieurianin group of the A and B-ring opened limonoids.



1



2 R=Ac
3 R=H



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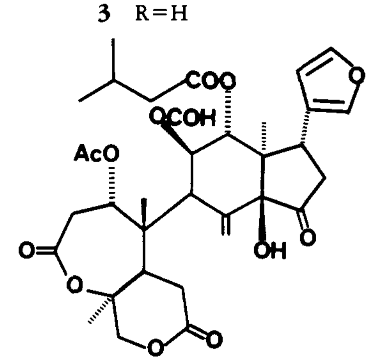

5

Previously in this group, 14,15-epoxyprieurianin from root bark of *Guarea guidona* L. Sleumer was found to inhibit growth of the murine P-388 lymphocytic leukemia cell line (10), hispidines from *Trichilia hispida* Pennig. were cytotoxic (11), while three com-

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pounds from the stem bark of *Trichilia roka* (Forsk.) Chiov. exhibited antifeedant properties against the Japanese pest insect, *Ajrotis sejetum* Denis (12).

EXPERIMENTAL

MATERIALS:—Prieurianin (**3**) was isolated from the bark and stem wood of *Nymania capensis* (13). The acetate (**2**) was obtained by treatment with Ac_2O and pyridine (mp 183°, $[\alpha]^{25}_D=56.4°$) (14). Rohitukin (**5**) and rohituka-7 (**4**) were obtained from seeds of *Aphanamixis polystacha* (15).

BIOASSAY:—Circular leaf discs 3 cm in diameter were punched out of the first true leaves of bean (subtrate for Mexican bean beetle and Southern armyworm) or cotton (subtrate for Tobacco budworm) and their upper surface was painted with 35 μl/disc of test emulsion containing the active ingredient, MeOH 5% (v/v), Me_2CO 5% (v/v), Triton CS-7 (surfactant from Rohm and Haas Co., Philadelphia) 0.1% (v/v), and H_2O 89.9% (v/v). The check discs received blank emulsion containing all ingredients with the exception of the test compound. The treated discs were individually placed in Gelman petri dishes (5 cm diameter) containing 4.7-cm diameter moist Gelman Filter pad (1.5 ml H_2O/pad). The dishes and the filter pads were obtained from Fisher Scientific Co.

After the emulsion evaporated to dryness, the leaf discs were infested with third instar test larval insects (one insect/dish). All treatments were replicated five times. The percent feeding was determined visually 2 and 6 days after treatment.

To eliminate the most serious source of error—mortality due to factors unrelated to the antifeedant activity—we routinely repeated tests in which not all larvae survived the first 48 h.

This bioassay is rapid, manageable, and reproducible and, therefore, suitable for testing large numbers of natural products and to guide the isolation work. It is not, however, suitable for testing acutely toxic substances.

RESULTS AND DISCUSSIONS

Many natural products (several limonoids, quassinoids, and sesquiterpene lactones) exhibiting good antifeedant properties during the first 48 h of the test subsequently loose activity due to factors that we do not always under-

TABLE 1. Activity of Four Prieurianin-Type Limonoids

Limonoid	Insect	19.8 μg/cm²		6 μg/cm²		1.5 μg/cm²	
		2 days	6 days	2 days	6 days	2 days	6days
Azadirachtin (**1**)	tbw[a]	++[b]	++	++	++	++	++
	saw	+++	+++	++	++	++	+
	mbb	+++	+++	+++	+++	+	++
Prieurianin acetate (**2**) . .	tbw	+++	+++	++	++	+	+
	saw	++	++	+	−	−	−
	mbb	+++	+++	+++	+++	++	++
Prieurianin (**3**)	tbw	++	++	+	++	+	−
	saw	+	−	−	−	−	−
	mbb	++	++	+	+	−	−
Rohitiuka-7 (**4**)	tbw	++	++	−	−	−	−
	saw	−	−	−	−	−	−
	mbb	−	−	−	−	−	−
Rohitiukin (**5**)	tbw	−	+	−	−	−	−
	saw	−	−	−	−	−	−
	mbb	−	−	−	−	−	−
Check	tbw	−	−	−	−	−	−
	saw	−	−	−	−	−	−
	mbb	−	−	−	−	−	−

[a]tbw=tobacco budworm, saw=southern armyworm, mbb=mexican bean beetle.

[b]+++ corresponds to 90-100% feeding control

++ corresponds to 60-90% feeding control

+ corresponds to 30-60% feeding control

− corresponds to 0-30% feeding control

% feeding control=100 [1-(%feeding/%feeding by stock)] stand[2]. Such compounds, although of certain theoretical interest, could not be applied to control insects. Prieurianin and its acetate belong to those few natural products that induce permanent "appetite loss" at the application level close to that of azadirachtin, the most potent antifeedant from biological sources (Table 1).

The apparent superiority of azadirachtin in the 6-day test at 1.5 $\mu g/cm^2$ on the lepidopterous larvae is due to 100% ecdysis inhibition—a hormonal disorder[3] (16, 17), which other limonoids do not seem to generate at this application level. One of its symptoms, sealing of the functional mouth parts by an extra head capsule, has an obvious effect on the ability of insects to feed.

Furthermore, our results indicate great activity variations with minor structural changes within the same structural framework. Particularly surprising is the inactivity of rohitukin (**5**), which is, indeed, very closely related to prieurianin acetate (**2**).

ACKNOWLEGMENTS

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