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INSECTICIDAL DEFENSES OF PIPERACEAE FROM THE NEOTROPICS

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Abstract—Insecticidal and growth-reducing properties of extracts of 14 species of American neotropical Piperaceae were investigated by inclusion in diets of a polyphagous lepidopteran, the European corn borer, *Ostrinia nubilalis*. Nutritional indices suggested most extracts acted by postdigestive toxicity. *Piper aduncum*, *P. tuberculatum*, and *P. decurrens* were among the most active species and were subjected to bioassay-guided isolation of the active components. Dillapiol was isolated from the active fraction of *P. aduncum*, piperlonguminine was isolated from *P. tuberculatum*, and a novel neolignan from *P. decurrens*. The results support other studies on Asian and African *Piper* species, which suggest that lignans and isobutyl amides are the active defence compounds in this family.

Key Words—Piperaceae, botanical insecticide, isobutyl amides, lignans, dillapiol, conocarpan, piperlonguminine, European corn borer.

INTRODUCTION

The role of plants from the pepper family (Piperaceae) in traditional pharmacopeia is well established in tropical areas where these vines and shrubs are

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particularly abundant (Atal et al., 1975; Schultes and Raffauff, 1990). Throughout the world, *Piper* plants have traditionally also been used against pests. For example, the Amazonian species, *Piper rotundistipulum*, is used locally as an insecticide and a fish poison (Schultes and Raffauf, 1990). *P. guineense* and *P. nigrum* are used as insecticides and molluskicides in several parts of Africa (Su and Horwat, 1981; Ivbijaro and Bolaji, 1990). The Indian species *P. longum*, *P. betle*, *P. peepuloides*, and *P. cubeba* have demonstrated insecticidal activity against mosquitos and flies (Srivastava, 1970; Miyakado et al., 1989) and were shown to repel grain pests (Kokate et al., 1980). The leaves of *P. futokatsura* from Taiwan and Japan are known as a feeding deterrent to the larvae of *Spodoptera litura* (Matsui and Munskata, 1975), and the leaves of *P. umbellatum*, *P. hispidum*, *P. auritum*, and plants reported only as *Piper* spp., which are native to Central America and the Northwest Amazonian basin, are used by indigenous peoples to prevent malaria and to remove head lice (Schultes, 1975, 1980).

Our knowledge about the compounds responsible for all of these insecticidal activities is limited. However, it is known that amides are frequently found in the Piperaceae (Greger, 1988), in particular olefinic or alkyl isobutylamides such as piperine, piperettine, trichostachine, peepuloidin, piplartin, and trichonine (Atal et al., 1975). They are toxic to fruit flies, azduki bean weevils, cockroaches, and several other insect species (Gbewonyo et al., 1993; Miyakado et al., 1983, 1989; Su and Horwat, 1981), and they act as neurotoxins (Gbewonyo et al., 1993).

Phenylpropanoids are another group of potentially active chemicals of common occurrence in *Piper* spp. They include monolignols, such as apiol, dillapiol, myristicin, eugenol, and safrol, and dimers of phenylpropanoids, such as the lignans sesamin, cubebin, yangambin or diaeudesmin. They often constitute a large part of the essential oil of the plant, as for instance, 85% safrol in the oil of the leaves of *P. auritum* (Castro and Poveda, 1983). However, the toxicity of these substances, with a few exceptions such as safrol, which has long been used as a natural insecticide (Coats, 1994), has not been established.

On the other hand, several of these compounds are known to synergize natural and synthetic insecticides. Sesamin and sesamolin, for instance, synergize the activity of natural and synthetic pyrethrins (Singh et al., 1976), and their synthetic analog, piperonyl butoxide, is currently included in most commercial preparations of pyrethrum (Worthing and Walker, 1993). Dillapiol, related to piperonyl butoxide, synergizes not only pyrethrins but also several carbamates and organochlorates (Handa and Dewan, 1974; Mukerjee et al., 1979; Parmar and Tomar, 1983). In addition, lignans and their monomers are known to repel or act as antifeedants to several grain pests (Harmatha and Nawrot, 1988; Nawrot and Harmatha, 1994; Pelter, 1986; Tomar et al., 1979). Kobusin and dillapiol reduce the growth of larvae of insect pests (MacRae and

Towers, 1984), including the larvae of a major pest of maize in North America, the European corn borer, *Ostrinia nubilalis* (Bernard et al., 1990).

These observations, together with our interest in the bioactivities of lignans in insects (Bernard et al., 1989, 1990), prompted us to investigate the activities of a group of *Piper* spp. from the neotropics (Costa Rica), a group largely unstudied from the point of view of their chemical ecology, against our model insect, the European corn borer. One African (*P. guineense*) and one Asian species (*P. betle*) were included for comparison with previous studies. We also report here the structure of a neolignan isolated for the first time from this family and the occurrence of piperlonguminine as the component responsible for the insecticidal activity of another newly investigated *Piper* species.

METHODS AND MATERIALS

Instrumentation

Melting points were determined on a Kofler hot stage and are uncorrected. ^{1}H NMR spectra were obtained on a Bruker 500 MHz spectrometer, and chemical shifts (δ) are reported in parts per million downfield from TMS. The number of protons, multiplicity (s = singlet, d = doublet, q = quartet, quint = quintet, m = multiplet, non = nonet), coupling constant, and assignment are presented in parentheses.

Mass spectrometry was carried out by a VG analytical 7070E spectrometer. UV visible and IR absorption spectra were both recorded on Perkin-Elmer spectrophotometers.

Plant Material

Plants collected in Costa Rica by C. Bernard, L. Poveda, and C. Hasbun in February 1992 included: *Piper aduncum* L., *P. auritum* Kunth, *P. aequale* Vahl, *P. hispidum* Sw., and *P. reticulatum* L. (E.A.R.T.H. station in Guapiles); *P. decurrens* C.DC, *P. marginatum* Jacq., and *P. pseudo-lindenii* C.DC. (C.A.T.I.E. in Cartago); and *P. tuberculatum* Jacq. in the city of Colon. *P. lanceiaifolium* Kunth was collected by J.T. Arnason, L. Poveda, and P. Sanchez in July 1993 near Colon; *P. obliquum* Ruiz & Pav. near Wilson botanical garden; *P. guanacastense* C.DC. near Mirador, Guanacaste; and *P. carrilloanum* C.DC. and an unidentified *Piper* sp. on the Peninsula de Osa in October 1993 by J.T. Arnason, K. Downum, L. Poveda, N. Moreno, and P. Sanchez. The fruits of *P. guineense* Schum & Thom came from Nigeria and were provided by F. Ewete, while the leaves of *P. betle* L. were collected in 1991 in Java, Indonesia, by J.T. Arnason and Y. Razali. Identification of species was carried out by L. Poveda and P. Sanchez, and voucher specimens were retained at the Universidad Nacional de Heredia and the University of Ottawa.

Fresh leaves or fruits were immediately immersed on site in 95% ethanol (EtOH), and were stored in this state two weeks or more, until processed. This first EtOH extract was set aside, and leaves were blended in a similar volume of EtOH and macerated for another two days. Both EtOH extracts were combined, filtered, and concentrated to dryness on a rotary evaporator under reduced pressure at 30°C. Water was further removed by freeze-drying the material for 12 hr, which resulted in a powder or a gum, i.e., crude extract, depending on the plant species. Dry weight of plant material was calculated from the plant solids remaining on the filter paper and the dry crude extract.

Insect Bioassays

Larvae of *O. nubilalis* Hübner (Lepidoptera: Pyralidae) from a laboratory colony were maintained according to procedures described previously (Arnason et al., 1985). The freeze-dried crude EtOH extract of each *Piper* spp. (leaves, or fruits) was incorporated into a meridic diet at 30°C at final concentrations of 4% and 0.4% (w/w). Twenty naïve second instars (6-day-old larvae weighing 8–12 mg) per *Piper* diet were individually distributed in 12-ml glass vials, which were closed with a cotton plug, and returned to the rearing conditions of the colony culture. The weights (wt) of each larva, the diet deposited, the diet remaining, and the frass in each vial were determined at five-day intervals. The relative growth rate (RGR = wt gain/mean wt during the period of study), and nutritional indices were calculated by the following equations as described in Reese (1979).

Consumption index (CI) = wt of food ingested/wt of larvae

Approximate digestability (AD)
= [(wt of food ingested − fecal wt)/wt of food ingested] × 100

Efficiency of conversion of ingested food (ECI)
= (wt gained/wt of food ingested) × 100

Efficiency of conversion of digested food (ECD)
= [wt gained/(wt of food ingested − wt of feces)] × 100

All statistical analysis were performed using Tukey's general linear models procedure (Tukey, 1949) with SAS for PC.

A second insect species, the rock hole breeding mosquito, *Aedes atropalpus* L. (Diptera: Culicidae), was used to monitor the isolation of chemicals from the plants. The small amount of material needed for the bioassays with larvae of these species when compared to the ECB, as well as the rapidity of the toxic response, made it a system of choice to follow the activity of the successive fractions of the plant extracts. Rearing conditions were previously reported (Watt et al., 1981). Ten second instars were pipetted into each of the glass vials containing the plant crude extract at concentrations of 10, 50, and 100 ppm.

TABLE 1. INSECTICIDAL ACTIVITY OF *Piper* SPP. CRUDE EXTRACTS TO MOSQUITO LARVAE

	Concentration of extract		
Pipp spp.	10 μg/ml	50 μg/ml	100 μg/ml
P. aduncum	7(5)[a]	2(2)	0
P. aequale	9(1)	8(1)	3(2)
P. auritum	8(1)	1(1)	0
P. betle	10(0)	10(0)	9(1)
P. carrilloanum	10(0)	9(1)	9(1)
P. decurrens	7(1)	5(2)	0
P. guanacastensis	8(1)	7(1)	7(1)
P. guineense	0	0	0
P. hispidum	8(0)	6(1)	2(1)
P. marginatum	9(1)	8(1)	2(1)
P. obliquum	9(1)	7(2)	2(1)
P. pseudo-lindenii	9(1)	7(1)	6(1)
P. reticulatum	1(1)	0	0
P. tuberculatum	8(1)	7(2)	4(3)
P. not identified	9(1)	8(2)	8(2)

[a] Number of surviving larvae after 24 hr: mean (± SD) from three trials of 10 larvae/trial.

After 24 hr at room temperature, the surviving larvae were counted. The experiment was repeated three times. The pattern of toxicity of the 16 *Piper* extracts to mosquito larvae generally paralleled that to the ECB, confirming the suitability of these insect species as assay guides for the isolation of compounds with activity to both species (Table 1). For assays on fractions of the crude extracts, a concentration of 100 ppm was arbitrarily selected. The activity of the fraction was expressed as the percentage of the toxicity of the corresponding crude extract at the same concentration (Table 2).

TABLE 2. DISTRIBUTION OF INSECTICIDAL ACTIVITY TO MOSQUITO LARVAE OF FRACTIONS FROM THREE *Piper* SPP

	Mortality (%) at 100 ppm[a]			
Piper spp.	HEX	CH_2Cl_2	EtOAc	MeOH
aduncum	26(3)	72(7)	2(1)	0
decurrens	88(3)	8(2)	3(1)	1(1)
tuberculatum	54(6)	44(7)	2(1)	0

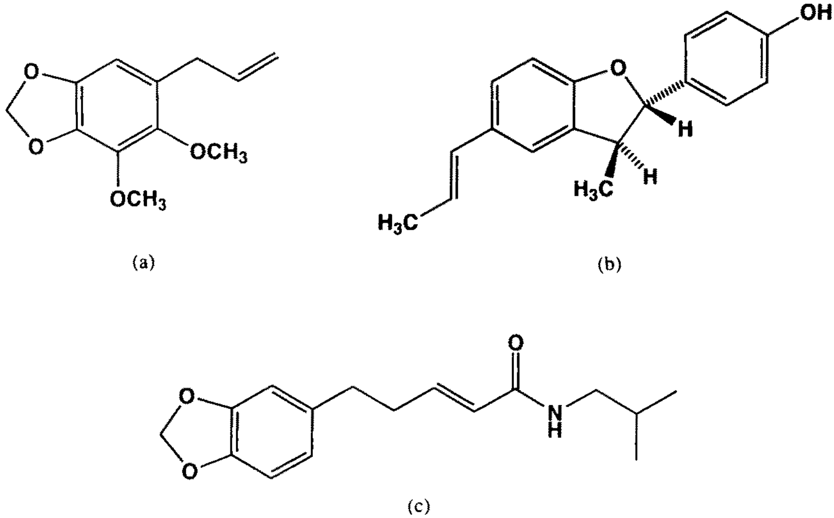
[a] Values in parentheses are SE.

Phytochemical Isolations and Identifications

P. aduncum. Fifteen grams of dry crude leaf extract were suspended in minimal volume of acetone, adsorbed on 30 g of silica gel 70–230 mesh, and the solvent evaporated under reduced pressure. The resulting powder (silica-crude extract) was deposited in a Buchner funnel. Five hundred milliliters of the following solvents were added successively: hexanes (HEX), methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), and methanol (MeOH). Each fraction was then evaporated to dryness, weighed (0.2 g, 1.02 g, 0.01 g, and 0.2 g, respectively), and redissolved in the water of the mosquito bioassay to achieve the proper concentration. Most of the activity was found in the first two fractions (Table 2). These two were combined, and a pale yellow oil, dillapiol (Figure 1a), the major component, was isolated following the method described by Orjala et al. (1989). Its identification was made by comparison with the spectral data of a sample of pure compound provided by one of us (H.G.K.).

P. decurrens. The chemistry of this species had not been previously reported. Sixty grams of the crude dried extract were adsorbed on silica gel and washed successively with four solvents (HEX, CH_2Cl_2, EtOH, and MeOH), as described above. This resulted in the repartition of the toxicity shown in Table 1. The HEX and CH_2Cl_2 fractions were combined and passed through a column of Silica gel (70–230 mesh; 200 g) with HEX and EtOAc as eluents (100:0 to 0:100). From the resulting fractions, a fine white solid (92 mg) was isolated by crystallization from cold HEX. Its physical and spectroscopic data allowed it to be identified as the neolignan, conocarpan (Figure 1b). IR (KBr pellet, cm^{-1}) 3410(br), 3242(br), 2963, 1607, 1607, 1520, 1476, 1239, 925, 830; ^1H Nmr ($CDCl_3$, ppm) δ 1.40 (3H, d, J = 6.8 Hz, $CH{-}C\underline{H}_3$), 1.87 (3H, dd, J = 6.5 Hz, 1.5 Hz, $CH{=}CH{-}C\underline{H}_3$), 3.40 (1H, apparent quint, J = 7.3 Hz, $C\underline{H}{-}CH_3$), 5.09 (1H, d, J = 8.8 Hz, $O{-}C\underline{H}$), 6.45 (1H, dq, J = 15.7 Hz, 6.5 Hz, $CH{=}C\underline{H}{-}CH_3$) 6.37 (1H, dd, J = 15.7 Hz, 1.5 Hz, $C\underline{H}{=}CH{-}CH_3$), 6.77 (1H, D, J = 8.2 Hz, Ar—H ortho to OR), 6.83 (2H, d, J = 8.5 Hz, Ar—H ortho to OH), 7.13 (1H, d, J = 8.2, Ar—H ortho to CH=CH), 7.30 (2H, d, J = 8.5 Hz, Ar—H meta to OH); MS (EI, *m/z*) 267(28), 266(100), 251(17), 233(10), 159(12), 133(12), 131(11), 121(12), 119(18), 107(17); Anal. Calcd. for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81.

P. tuberculatum. Seventy grams of crude EtOH extract from fresh leaves were treated as for the two former species. The bioactivity of the four basic fractions is shown in Table 1. The HEX and CH_2Cl_2 fractions were combined and chromatographed on a large silica gel column (70–230 mesh, 500 g) that was eluted successively with pure HEX, mixtures of HEX-EtOAc of increasing polarities, pure EtOAc, and finally with EtOAC-MeOH (1 : 1, v/v). The largest fraction contained one major compound, white silky needles, recrystallized from cold benzene-hexane mixtures. The physical (mp = 116–118°C) and spectro-



(a) (b)

(c)

FIG. 1. Compounds isolated in this study: (a) dillapiol, (b) conocarpan, (c) dihydropiperlonguminine.

scopic data were as follows: IR (KBr pellet, cm^{-1}) 3300, 2941, 1667, 1624, 1550, 1490, 1444, 1249, 1041, 928, 812; ^1H NMR (acetone-d6, ppm) δ 0.89 [6H, d, J = 6.9 Hz, $CH(C\underline{H}_3)_2$], 1.77 [1H, non, J = 6.9 Hz, $C\underline{H}(CH_3)2$], 2.44 (2H, dq, J = 7.5 Hz, 1.5 Hz, $C\underline{H}_2{-}CH{=}CH$), 2.69 (2H, t, J = 7.5 Hz, $Ar{-}CH_2$), 3.16 (2H, t, J = 6.9 Hz, $NH{-}C\underline{H}_2$), 5.95 (2H, s, $O{-}C\underline{H}_2{-}O$), 5.96 (1H, dt, J = 15.2 Hz, 1.5 Hz, $CH_2CH{=}CH$), 6.69 (1H, dd, J = 7.9 Hz, 1.7 Hz, Ar—H meta and para to $O{-}CH_2O$), 6.75 (1H, dt, J = 15.2 Hz, 7.5 Hz, $CH_2{-}C\underline{H}{=}CH$), 6.75 (1H, D, J = 7.9 Hz, Ar—H ortho to $O{-}C\underline{H}_2O$ and meta to R), 6.76 (1H, d, J = 1.7 Hz, Ar—H ortho to $O{-}CH_2{-}O$ and R); HR-MS (m/z M+275, $C_{16}H_{21}O_3N$). These data for this compound matched those given for dihydropiperlonguminine (Figure 1c).

RESULTS AND DISCUSSION

Activity of Crude Extracts to European Corn Borer Larvae. The present study establishes the growth-reducing activity to the European corn borer (ECB) of the majority of the leaf extracts of the *Piper* species collected (Figure 2). *Piper aduncum* (ad), *P. tuberculatum* (tu), and *P. guineense* (gi) had the highest activity in the diet (Figure 2). The mortality data (not shown) generally parallelled the growth-reducing data, and all larvae in the *P. aduncum*, *P. tubercu-*

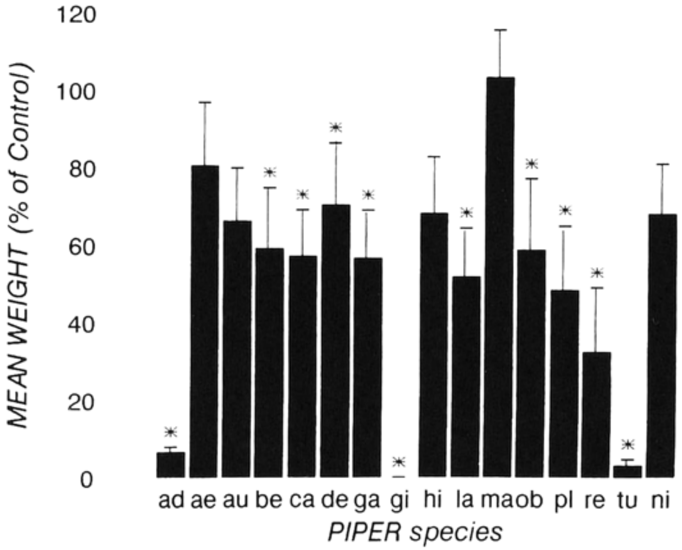


FIG. 2. Growth reducing activity of *Piper* extracts (0.4% in diet) to European corn borer larvae. ad: *P. aduncum*; ae: *P. aequale*; au: *P. auritum*; be: *P. betle*; ca: *P. carrilloanum*; de: *P. decurrens*; ga: *P. guanacastensis*; gi: *P. guineense*; hi: *P. hispidum*; la: *P. lanceiaifolium*; ma: *P. marginatum*; ob: *P. obliquum*; pl: *P. pseudo-lindenii*; re: *P. reticulatum*; tu: *P. tuberculatum*; ni: *P.* not identified. (*Piper* species with an asterisk are significantly different from their control, $P = 0.05$ in Tukey's general linear models procedure). Twenty second-instar larvae (day 6) were fed treated diets and weights were determined on day 20.

latum, and *P. guineense* groups died by day 12 of treatment. In addition, seven other species, *P. betle* (be), *P. decurrens* (de), *P. guanacastensis* (ga), *P. lanceiaifolium* (la), *P. obliquum* (ob), *P. pseudo-lindenii* (pl), *P. reticulatum* (re), and *P. carrilloanum* (ca) significantly reduced the growth of the larvae (Figure 2), with larval weights after a period of 10 days on these diets ranging from 27% to 68% of controls. For the larvae that survived the treatment, the percentage of pupation was decreased in all cases except for *P. marginatum*.

Nutritional indices (Table 1) showed limited effect of the extracts on the food consumption (CI) of the larvae, indicating little feeding deterrency (Table 3). In fact, the three most insecticidal species induced three to eight times higher consumption of food. The digestion of the insect was also little affected by the allelochemicals, but the ECD was lowered, indicating toxicity of the digested food components.

Phytochemical Isolations of Pure Compounds. Dillapiol was the main active

TABLE 3. EFFECT OF PIPERACEAE EXTRACTS (0.4%) ON INSECT GROWTH AND FOOD MANAGEMENT

	Percent of control				
Piper spp.	RGR	CI	ECI	AD	ECD
P. aduncum (leaves)	3.63[a]	591.11[a]	5.23[a]	148.62[a]	3.43[a]
P. aequale (leaves)	78.93	127.33	85.12	100.00	100.00
P. aequale (twigs)	59.26[a]	162.91[a]	58.45[a]	102.61[a]	56.02[a]
P. auritum (leaves)	68.58	164.48	55.42	109.63	49.83[a]
P. auritum (twigs)	56.17	161.69	63.37	101.24	62.66
P. betle (fruits)	55.56[a]	171.29	61.70	119.62	59.82[a]
P. decurrens (leaves)	68.31	155.73	78.55	119.80	64.87[a]
P. guanacastensis (leaves)	75.42	86.62	90.50	98.87	90.69
P. guineense (fruits)	0.18[a]	336.32[a]	0.35[a]	104.11[a]	0.33[a]
P. hispidum (leaves)	73.36	123.63	73.00	106.38	71.74
P. marginatum (leaves)	101.86	100.45	119.38	91.37	214.30[a]
P. pseudo-lindenii (leaves)	40.83[a]	256.15[a]	36.81[a]	113.62	32.48[a]
P. reticulatum (leaves)	30.65[a]	274.84[a]	29.46[a]	116.32[a]	25.72[a]
P. tuberculatum (leaves)	0.26[a]	831.24[a]	0.28[a]	121.85[a]	0.23[a]

[a] Data are significantly different from their control in Tukey's multiple range test ($P = 0.05$). The mean RGR, CI, ECI, AD and ECD (+SD) values of control groups were: 1.7(0.4), 2.7(0.6), 5.3(1.2), 0.7(0.1) and 0.07(0.03).

component of *P. aduncum* and caused 92% mortality of mosquito larvae at 0.1 ppm (Table 4). High concentrations of dillapiol were also obtained from *P. aduncum* from Brazilian species (74–88% of the leaf essential oil) (Gottlieb et al., 1981), and this monolignan occurred in the leaves, fruits, or wood of at least five other Piperaceae distributed worldwide—*P. banksii*, *P. hispidum*, *P. marginatum*, *P. novae-hollandiae*, and *P. umbellatum* (Loder and Nearn, 1972; Burke and Nair, 1986; Ramos et al., 1986; Loder et al., 1969; Bernhard and Thiele, 1978).

Dihydropiperlonguminine was isolated from the active fraction of *P. tuberculatum*. This isobutylamide has been isolated from other Piperaceae (Tabuneng et al., 1983), along with longuminine and other derivatives. Dihydropiperlonguminine is toxic to mosquito larvae (Table 4).

Conocarpan was isolated from the active fraction of *P. decurrens* extract and could account for part of the insecticidal activity of this plant (Table 4). Conocarpan has been previously isolated from the wood of *Conocarpus erectus* (Combretaceae) (Hayashi and Thomson, 1975), but this is its first isolation from the Piperaceae. Large quantities of sitosterol (118 mg) were also purified from this fraction, as well as a number of less abundant lignans of a similar type.

TABLE 4. ACTIVITY OF NEWLY ISOLATED PURE COMPOUNDS TO MOSQUITO LARVAE

Species	Mortality (%)[a]
P. aduncum	
Dillapiol	92(4)
P. decurrens	
Conocarpan	47(5)
Sitosterol	6(3)
P. tuberculatum	
dihydropiperlonguminine	47(2)

[a] Percent mortality (±SE) from three assays of 10 larvae assay. Concentrations in bioassays were 0.1 ppm for *P. aduncum* and *P. decurrens*, and 0.01 ppm for *P. tuberculatum*.

These are presently being analyzed and will be described elsewhere (Chauret, unpublished data).

Insecticidal Lignans and Amides of Piperaceae. More than 15 species of *Piper* have been reported in the literature to have insecticidal activity. According to Marquis (1991), the highly insecticidal activity of most of the *Piper* spp., which he observed in the field in relation to two phytophagous insects, could not be correlated with the leaf characteristics, the soil, or other environment factors, "leaving the individual chemistry as a possible factor to investigate."

The two species of *Piper* with the highest toxicity to the ECB, *P. tuberculatum* and *P. guineense*, contain large amounts of amides. The isobutylamide, dihydropiperlonguminine, is a major component of *P. tuberculatum* leaves, as described in the present study. In the only other chemical investigation reported on this species, the pyridone alkaloid piplartine and its dimer (Filho et al., 1981), were isolated from the root bark.

P. guineense is, by contrast, well studied. A series of isobutylamides, including pipercide, guineensinamide, guineensine, pellitorine, and kalecide, have been isolated from the roots of *P. guineense* and shown to be responsible for the insecticidal activity of this species (Gbewonyo et al., 1993). The first three amides were the most active against adults of *Musca domestica*. Each of these contain a methylenedioxyphenyl (MDP) structural moiety.

The isobutylamide newly isolated from *P. tuberculatum*, dihydropiperlonguminine (Figure 1c) also has an MDP ring and is the main component of the active fraction of the leaf extract. Greger (1988) pointed out that these types of amides, including olefinic and alkyl isobutylamides, are common to a restricted number of related plant families, namely the Piperaceae, the Asteraceae, and the Rutaceae. All three families are abundant in the tropics, particularly in the humid tropics in the case of the Piperaceae, where herbivory is a potent selective force. The isobutylamides found in the *Piper* family have low molecular weights, contain a single nitrogen, and presumably are not ''expensive'' to biosynthesize. The pungent, numbing sensation caused by some of the psychoactive species, such as *P. methysticum*, may be a reason for their avoidance by herbivores, as observed by Marquis (1991), in particular with *P. auritum*. Piperaceae are mostly vines or small shrubs in the tropics, and short life-cycle species such as these would be expected to protect themselves with simply synthesized, low-cost, molecules. The isobutylamides could be described as belonging to this category of defense compounds.

The most active insecticidal species in our bioassays was *P. aduncum*. Surprisingly, no amides have been reported from this species. It does, however, contain several lignans, including dillapiol, first found in the Indian dill, *Anethum sowa* (Fabaceae), where it constitutes up to 35% of the essential oil of the seeds of Indian cultivars (Tomar et al., 1979), and also in the leaves of *P. aduncum* from Panama, where it is the only major component (Gupta and Arias, 1983). Dillapiol can synergize several natural insecticides, including pyrethrum (Mukerjee et al., 1979), azadirachtin (Bertrand, 1992), and tenulin (Bernard et al., 1990), at the concentration of 100 ppm in the diet of the European corn borer, a concentration well below the level in the leaves of *P. aduncum* (approximately 60% of the oil). It previously was found to be toxic to the ECB (Bernard et al., 1989). It is the main insecticidal component of this *Piper* sp. Monolignans and lignans with the MDP moiety are characteristic chemicals from the Piperaceae. Lignan toxicity differs from that of classical neurotoxic insecticides, as it is chronic rather than acute. Several lignans inhibit a vital biochemical defense of herbivores, the polysubstrate monooxygenase (PSMO) detoxification enzymes (Ahmad, 1986; Bernard et al., 1989; Berenbaum and Neal, 1985). Thus, several potentially toxic components in the food of the insect that are usually gradually eliminated could accumulate to a toxic level. These specific lignans, myristicin, dillapiol, epiyangambin, ashantin, and eugenol, are distributed in most of the *Piper* spp. and often occur in large quantities in the plants.

The elucidation of the structure of conocarpan, a neolignan, as the main, but not highly, insecticidal component of *P. decurrens*, further suggests the importance of lignans in the Piperaceae. Only lignans have been isolated from extracts having a growth-reducing rather than an acute toxic effect on insect larvae, i.e., *P. marginatum*, *P. decurrens*, and *P. hispidum* of the present study. These produced the lowest larval mortality. This leads to the possibility that *Piper* lignans not associated with amides, excluding monolignols, may have a long-term effect and low toxicity, as further suggested by the low incidence of pupation in the groups fed on these three species. This possibility warrants further investigation.

In any case, isobutylamides and lignans combined in the same plant constitute an inexpensive and effective means of defense elaborated by a limited number of highly successful plants from the neotropics.

The present study confirms that tropical American *Piper* spp. have insecticidal activities comparable to previously studied African and Asian species. Bioassay-guided isolation suggests the importance of neolignans and amides as the active components.

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