Insecticidal Neolignans from *Piper decurrens*

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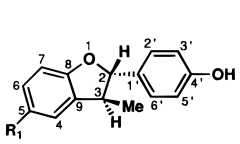
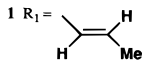
2,3-Dihydro-2-(4′-hydroxyphenyl)-3-methyl-5(*E*)-propenylbenzofuran (conocarpan) (**1**), 2-(4′-hydroxy-3′-methoxyphenyl)-3-methyl-5(*E*)-propenylbenzofuran (eupomatenoid-5) (**2**), and 2-(4′-hydroxyphenyl)-3-methyl-5(*E*)-propenylbenzofuran (eupomatenoid-6) (**3**), three known neolignans found for the first time in a species of the Piperaceae, were isolated from *Piper decurrens* via insecticidal bioassay-guided fractionation, along with a small quantity of a new related compound, 2,3-dihydro-5-formyl-2-(4′-hydroxyphenyl)-3-methylbenzofuran (decurrenal) (**4**), and 3,7,11,15-tetramethyl-2(*E*)-hexadecen-1-ol (*trans*-phytol).

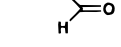
The Piperaceae, or pepper family, is a large family of plants indigenous to the tropics, where the native peoples have long used them in a variety of medicinal and pest-control capacities. Species from Africa, the Middle East, the Far East, and the Amazon have been examined to a considerable extent;[1] however, those from Central America are less well known. A recent study in these laboratories of the chemical defenses of the Piperaceae[2] revealed *Piper decurrens* C.DC. as one of the more active species, displaying significant delayed toxicity (Figures 1 and 2) during a life-cycle bioassay involving larvae of the European corn borer. In addition, the chemistry of this species had not previously been investigated. Knowing that the bioactivity of *Piper* species can generally be attributed to the presence of lignans and/or amides, such as alkyl or olefinic isobutylamides,[1,3,4] it was of interest to determine which of the two preceding types of compounds would be present in *P. decurrens*. Bioassay-guided fractionation of the crude ethanol extract of this species led to the isolation of four compounds new to the Piperaceae, 2,3-dihydro-2-(4′-hydroxyphenyl)-3-methyl-5(*E*)-propenylbenzofuran (**1**), 2-(4′-hydroxy-3′-methoxyphenyl)-3-methyl-5(*E*)-propenylbenzofuran (**2**), 2-(4′-hydroxyphenyl)-3-methyl-5(*E*)-propenylbenzofuran (**3**), and 2,3-dihydro-5-formyl-2-(4′-hydroxyphenyl)-3-methylbenzofuran (**4**), as well as 3,7,11,15-tetramethyl-2(*E*)-hexadecen-1-ol, or *trans*-phytol, which has been identified previously in the extracts of other *Piper* species.[5−7]

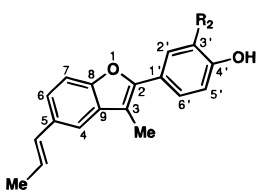


1 R_1 =



4 R_1 =





2 R_2 = OMe

3 R_2 = H

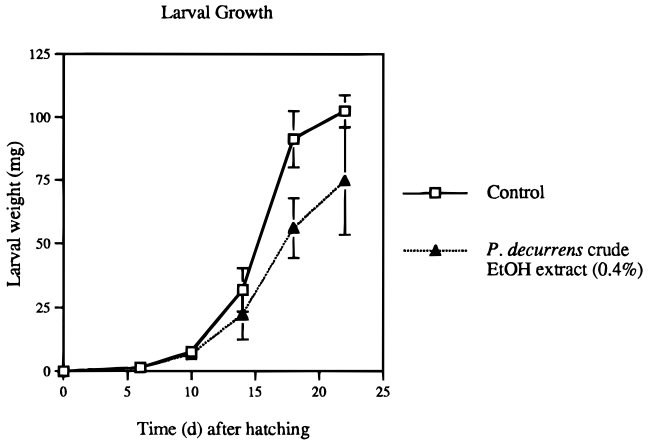


Figure 1. Growth of *O. nubilalis* larvae during a feeding bioassay, with *P. decurrens* crude EtOH extract incorporated into the diet at 0.4%. The *P. decurrens* results were found to be significantly different from the control in Tukey's multiple range test ($p = 0.05$).

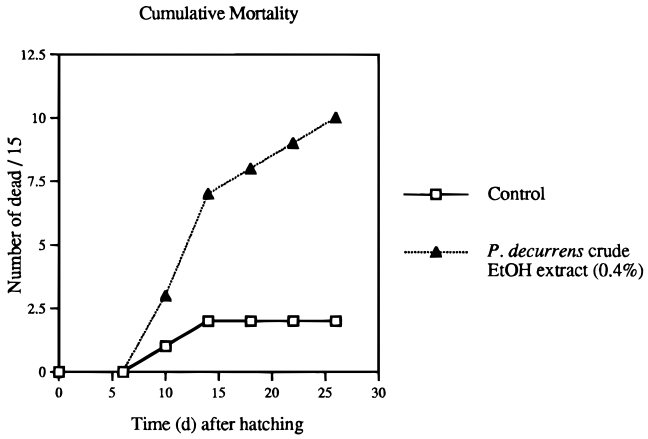


Figure 2. Cumulative mortality of *O. nubilalis* larvae during a feeding bioassay, with *P. decurrens* crude EtOH extract incorporated into the diet at 0.4%. Day 26 represents pupae.

Compounds **1**, **2**, and **3**, conocarpan,[8,9] eupomatenoid-5,[10] and eupomatenoid-6,[9−12] respectively, all possess a neolignan skeleton, and although they have each been reported in the literature in the past, they have never before been found in the Piperaceae. The fourth compound, named decurrenal (**4**), a neolignan missing two

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Table 1. Survival of Mosquito Larvae Exposed to Extracts and Purified Isolated Compounds Derived from *P. decurrens* Leaves

entry	extract/solvent fraction/purified isolate	concn (μg/mL) 10	100
1	crude ethanol extract	7 (1)[a]	0 (0)
2	hexane fraction	0 (0)	
3	CH_2Cl_2 fraction	7 (2)	
4	ethyl acetate fraction	9 (1)	
5	ethanol fraction	10 (0)	
6	conocarpan (**1**)	2 (2)	
7	eupomatenoid-5 (**2**)	3 (1)	
8	eupomatenoid-6 (**3**)	0 (1)	
9	decurrenal (**4**)	10 (1)	5 (3)
10	α-terthienyl	0 (0)	

[a] Mean number of surviving larvae (standard deviation) out of 10 after 24 h ($n = 3$).

carbon atoms, is an oxidized analogue of compound **1** and has not been previously described.

The freeze-dried ethanol extract of the leaves of *P. decurrens* (collected in 1993 in Costa Rica) was bioassayed using larvae of the European corn borer, *Ostrinia nubilalis* Hübner, an economically and ecologically relevant species. A simplified life-cycle study was carried out, consisting of monitoring larval growth (Figure 1) and cumulative mortality (Figure 2) every 4 days, beginning on day 6. A significant reduction in larval weight was observed, in particular after day 14, hence, and the term "delayed toxicity." A similar effect was observed in the cumulative larval mortality, with a substantial difference between *P. decurrens* and the control also becoming apparent by day 14.

The crude ethanol extract was thus impregnated onto an equivalent weight of Si gel and washed successively with hexane, CH_2Cl_2, ethyl acetate, and ethanol. The next series of bioassays were conducted using the larvae of the rock-hole breeding mosquito, *Aedes atropalpus* SAY. This type of bioassay was found to correlate well with the more ecologically relevant corn borer assay in our previous study[2] and provided a very fast (24 h) means by which to examine the relative toxicity of the solvent fractions using a minimum of material. The crude ethanol extract was tested via the mosquito larvae bioassay as well, as a basis of comparison between the results obtained using the two bioassays. The insecticidal activity of the crude extract (Table 1, entry 1) was shown to be contained primarily in the hexane fraction and to a much lesser degree in the CH_2Cl_2 fraction (Table 1, entries 2−5).

Si gel CC of both the hexane and the CH_2Cl_2 fractions led to the isolation of five compounds. Compound **1** was obtained in the greatest quantity. Its structure was determined primarily via high-field 1D and 2D NMR spectroscopy. This neolignan was certainly new in the Piperaceae[1] but had been isolated previously from the wood of *Conocarpus erectus* (Combretaceae) ("conocarpan")[8] as well as from the roots of *Krameria cystisoides* (Krameriaceae).[9] The ^{1}H-NMR spectra of compound **1** and those reported for conocarpan were in full agreement with one another, confirming the designation of the *trans* relative stereochemistry between C-2 and C-3 ($J_{H\text{-}2,H\text{-}3} = 8.8$ Hz).

The second and third neolignans, compounds **2** and **3**, were also new for the Piperaceae,[1] but both compounds had previously been isolated from the bark of *Eupomatia laurina* R.Br. (Eupomatiaceae)[10] as "eupomatenoid-5" and "eupomatenoid-6," respectively. In addition, compound **3** had also been isolated from *Ratanhiae radix* Ph. Eur. (Krameriaceae) ("ratanhiaphenol II")[11] and from *K. cystisoides*.[9,12] The melting point and ^{1}H-NMR spectrum of compound **2** were in agreement with those of eupomatenoid-5, and the ^{1}H-NMR spectrum of compound **3** was consistent with those of eupomatenoid-6 and ratanhiaphenol II, its ^{13}C-NMR spectrum agreeing with that of ratanhiaphenol II.

The last of the neolignan-type compounds, compound **4**, was isolated in only a very small quantity. Its ^{1}H-NMR spectrum was very nearly identical to that of conocarpan, except that the propenyl moiety had been replaced by an aldehyde (δ 9.65). This was confirmed by HMQC 2D-NMR spectroscopy, indicating a ^{13}C carbonyl resonance at 189.93 ppm. The remainder of the ^{1}H-NMR spectrum of compound **4** was so similar to that of conocarpan that the location for the aldehyde had to be at C-5. Final confirmation of this assignment was obtained with the aid of an NOE experiment. Irradiation of the aldehyde peak at δ 9.65 produced enhancement of both of the ortho aromatic proton signals which had, for conocarpan, been the signals ortho to the propenyl moiety. In addition, the coupling constant, $J_{H\text{-}2,H\text{-}3} = 8.7$ Hz, again confirmed the *trans* relative stereochemistry between C-2 and C-3. This unusual variation of a neolignan certainly did turn out to be an entirely new compound and was thus named "decurrenal."

The final compound to be isolated was *trans*-phytol, whose ^{1}H- and ^{13}C-NMR spectra agreed with those previously published.[13,14]

Having purified and identified these five compounds from *P. decurrens*, it was of interest to determine their insecticidal activity. A mosquito larval toxicity bioassay (Table 1, entries 6−10), including α-terthienyl as a control antimosquito agent, revealed eupomatenoid-6 (**3**) as the most toxic structure, giving complete toxicity at 10 ppm, with conocarpan (**1**) and eupomatenoid-5 (**2**) following quite closely behind. The aldehyde functionality of decurrenal (**4**) seemed to significantly reduce its activity, compared to that of compound **1**. A full life-cycle study using the European corn borer was not conducted due to the need for significantly larger quantities of material.

Experimental Section

General Experimental Procedures. Mps were taken on a Gallenkamp capillary melting point apparatus and are uncorrected. ^{1}H-, ^{13}C-, and DEPT NMR spectra were recorded using Varian Gemini-200 and XL-300 and Bruker AMX-500 spectrometers. COSY, HMQC, and TOCSY NMR spectra and NOE experiments were performed using the Bruker AMX-500 spectrometer. When $CDCl_3$ was used as a solvent, $CHCl_3$ (^{1}H, δ 7.25) or $CDCl_3$ (^{13}C, δ 77.00) was used as internal reference, and when C_6D_6 was the solvent, C_6D_5H (^{1}H, δ 7.15) or C_6D_6 (^{13}C, δ 128.00) was used as the internal reference. Mass spectra were obtained on a VG 7070E or Kratos concept 2H instrument using electron ionization. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. TLC was carried out on Si gel 60 F_{254} precoated 0.25-mm aluminum sheets (Merck 5554). Developed plates were visualized by UV light and by staining with a 5% solution of

phosphomolybic acid in EtOH. Prep. TLC was performed using Si gel 60 F_{254} precoated 1.00-mm or 2.00-mm glass plates (Merck), and CC was carried out with Si gel, 70−230 mesh.

Plant Material. Leaves of *P. decurrens* were collected in Ciudad Colon, Costa Rica, at El Rodeo field site in 1993. Voucher specimens were also collected and are retained in the herbarium at the University of Ottawa and the Universidad Nacional in Costa Rica. The fresh leaves were immediately immersed on site in 95% EtOH and were stored in this state for approximately 4 months.

Insect Bioassays. Larvae of *O. nubilalis* were maintained as a laboratory colony according to previously described procedures.[15] The crude EtOH extract of *P. decurrens* was incorporated into a meridic diet at 30 °C at a final concentration of 0.4%. Twenty naive second-instar larvae (6 days old, weighing 0.8−1.5 mg) were individually distributed, each with a cube of diet, into 12-mL glass vials stoppered with a cotton plug, and returned to the rearing conditions of the colony culture. The weight of each larva was measured after 10 days.

Larvae of *A. atropalpus* were maintained as previously reported.[16] Ten second-instar larvae were pipetted into glass vials containing 10 mL of water, with extracts or pure compounds at concentrations of 10 and 100 ppm. After 24 h at room temperature, the surviving larvae were counted.

Extraction and Isolation. The EtOH from approximately 2 kg of soaking leaves of *P. decurrens* was decanted off. The leaves were then ground and left to macerate in additional EtOH overnight. The EtOH from the combined extracts was removed under reduced pressure, maintaining a temperature of less than 35 °C. After freeze-drying, 67.4 g of a dark residue remained, 49.5 g of which was subsequently redissolved in a minimum (200 mL) of EtOH. Si gel (50 g, 70−230 mesh) was added, and after removal of the solvent, the resulting solid was deposited into a Büchner funnel and washed successively with hexane (2500 mL), CH_2Cl_2 (2500 mL), EtOAc (1500 mL), and EtOH (1500 mL), each until the solvent contained little or no color. The solvent of each fraction was removed under vacuum, leaving a 12.0-g hexane fraction, a 7.7-g CH_2Cl_2 fraction, a 3.5-g EtOAc fraction, and a 26.0-g EtOH fraction. Insecticidal bioassays indicated that the hexane and CH_2Cl_2 fractions were the most active. Therefore, the EtOAc and EtOH fractions were not processed further.

CH_2Cl_2:EtOAc (20:1) was found to effect optimal separation of the components in both the hexane and CH_2Cl_2 fractions. Thus, the entire hexane fraction was subjected to CC using 500 g of Si gel. Three major components, each either fluorescing under or absorbing ultraviolet light, eluted one after the other. Fractions containing each compound were separately pooled together and rechromatographed, using the same solvent system. The largest and most polar of these sets of fractions yielded, after relevant fractions from the second column were recombined and the residue was macerated in 0 °C hexane, 480 mg of an off-white amorphous solid, conocarpan (**1**). The other two sets of fractions yielded, in a similar fashion, eupomatenoid-5 (**2**), a white solid and the least polar of the three compounds (177 mg), and eupomatenoid-6 (**3**), an amorphous beige solid of medium polarity (50 mg). In addition, a small amount (13 mg) of another compound, decurrenal (**4**), slightly more polar than conocarpan (**1**), was also isolated form the original hexane fraction column. Purification of this compound was achieved via prep. TLC, using the previously mentioned solvent system.

The CH_2Cl_2 fraction was also subjected to CC because by TLC many of the same components could be observed. CH_2Cl_2:EtOAc (20:1) and 400 g Si of gel led to the isolation of a little more of each of the previously mentioned major isolates: conocarpan (**1**), 120 mg; eupomatenoid-5 (**2**), 10 mg; and eupomatenoid-6 (**3**), 22 mg. The latter two small amounts were purified by prep. TLC, again using the previously mentioned solvent system. Thus, in total, 600 mg of conocarpan (**1**), 187 mg of eupomatenoid-5 (**2**), 72 mg of eupomatenoid-6 (**3**), and 13 mg of decurrenal (**4**) were isolated from 49.5 g of crude freeze-dried extract.

The major contaminant in all fractions containing conocarpan (**1**), both from the hexane and CH_2Cl_2 fraction columns, was *trans*-phytol. In total, 160 mg of this pale yellow oil was isolated by prep. TLC using CH_2Cl_2:EtOAc (20:1). It is estimated that the remaining mixture of conocarpan (**1**) and *trans*-phytol comprises approximately 500 mg of conocarpan (**1**) and 1 g of *trans*-phytol. Therefore, the crude EtOH extract of *P. decurrens* in fact contains a very significant 2% conocarpan (**1**).

Conocarpan (**1**): Mp 124.5−126.0 °C (lit.[9] mp 133−135 °C); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.30 (2H, d, J = 8.5 Hz, H-2′, H-6′), 7.14 (1H, s, H-4), 7.13 (1H, d, J = 8.2 Hz, H-6), 6.83 (2H, d, J = 8.5 Hz, H-3′, H-5′), 6.77 (1H, d, J = 8.2 Hz, H-7), 6.45 (1H, dq, J = 15.7, 6.5 Hz, βH-5), 6.37 (1H, dd, J = 15.7, 1.5 Hz, αH-5), 5.09 (1H, d, J = 8.8 Hz, H-2), 3.40 (1H, apparent quint, J = 7.3 Hz, H-3), 1.87 (3H, dd, J = 6.5, 1.5 Hz, γH_3-5), 1.40 (3H, d, J = 6.8 Hz, Me-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.22 (C-8), 155.75 (C-4′), 132.73 (C-9), 132.37 (C-1′), 131.32 (C-5′), 130.77 (αC-5), 127.87 (C-2′, C-6′), 126.31 (C-6), 123.08 (βC-5), 120.76 (C-4), 115.50 (C-3′, C-5′), 109.30 (C-7), 92.68 (C-2), 45.17 (C-3), 18.39 (γC-5), 17.82 (Me-3); HRMS m/z found M^+ 266.130 57, $C_{18}H_{18}O_2$ requires M^+ 266.130 68. Anal. Calcd for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 80.98; H, 6.86.

Eupomatenoid-5 (**2**): Mp 114.7−115.0 °C (lit.[10] mp 114−115 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42 (1H, d, J = 1.8 Hz, H-4), 7.36 (1H, d, J = 8.6 Hz, H-7), 7.31 (1H, d, J = 1.9 Hz, H-2′), 7.27 (1H, dd, J = 8.2, 1.9 Hz, H-6′), 7.26 (1H, dd, J = 8.6, 1.8 Hz, H-6), 7.00 (1H, d, J = 8.2 Hz, H-5′), 6.50 (1H, dd, J = 15.7, 1.7 Hz, αH-5), 6.22 (1H, dq, J = 15.7, 6.5 Hz, βH-5), 3.97 (3H, s, OMe-3′), 2.41 (3H, s, Me-3), 1.89 (3H, dd, J = 6.5, 1.7 Hz, γH_3-5); ^{13}C NMR ($CDCl_3$, 75 Hz) δ 152.50 (C-2), 150.88 (C-8), 146.28 (C-4′), 145.31 (C-3′), 132.30 (C-5), 131.18 (C-9), 130.90 (αC-5), 123.83 (βC-5), 123.49 (C-1′), 121.82 (C-6), 120.03 (C-6′), 115.73 (C-4), 114.17 (C-5′), 110.22 (C-7), 109.45 (C-3), 108.83 (C-2′), 55.66 (OMe-3′), 18.11 (γC-5), 9.06 (Me-3); EIMS (70 eV) m/z 295 (30), [M^+] 294 (100), 293 (6), 279 (10), 251 (8), 147 (9), 28 (10); HRMS m/z found M^+ 294.125 71, $C_{19}H_{18}O_3$ requires M^+ 294.125 60. Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.61; H, 6.33.

Eupomatenoid-6 (**3**): Mp 142.7−144.5 °C (lit.[11] mp 149−150 °C); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.69 (2H, d, J = 8.9 Hz, H-2, H-6), 7.43 (1H, d, J = 1.6 Hz, H-4),

7.37 (1H, d, J = 8.4 Hz, H-7), 7.27 (1H, dd, J = 8.4, 1.6 Hz, H-6), 6.94 (2H, d, J = 8.9 Hz, H-3′, H-5′), 6.52 (1H, dd, J = 15.7, 1.6 Hz, αH-5), 6.23 (1H, dq, J = 15.7, 6.5 Hz, βH-5), 2.42 (3H, s, Me-3), 1.91 (3H, dd, J = 6.5, 1.6 Hz, γH_3-5); HRMS m/z found M^+ 264.116 33, $C_{18}H_{16}O_2$ requires M^+ 264.115 03. Anal. Calcd for $C_{18}H_{16}O_2$: C, 81.79; H, 6.10. Found: C, 81.05; H, 6.16.

Decurrenal (**4**): ^{1}H NMR (C_6D_6, 500 MHz) δ 9.65 (1H, s, αH-5), 7.54 (1H, d, J = 1.2 Hz, H-4), 7.34 (1H, dd, J = 8.2, 1.2 Hz, H-6), 6.98 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.67 (1H, d, J = 8.2 Hz, H-7), 6.60 (2H, d, J = 8.5 Hz, H-3′, H-5′), 4.85 (1H, d, J = 8.7 Hz, H-2), 3.01 (1H, apparent quint, J = 7.4 Hz, H-3), 0.91 (3H, d, J = 6.8 Hz, Me-3); ^{13}C NMR (C_6D_6, 125 MHz) δ 189.93 (αC-5), 164.70 (C-8), 156.69 (C-4′), 133.61 (C-9), 133.37 (C-6), 131.69 (C-1′), 131.08 (C-5), 128.04 (C-2′, C-6′), 124.42 (C-4), 115.50 (C-3′, C-5′), 109.49 (C-7), 93.65 (C-2), 44.32 (C-3), 17.26 (Me-3); EIMS (70 eV) m/z [M^+] 254 (12), 85 (10), 84 (100), 82 (13), 56 (25), 54 (22), 52 (13), 42 (15); HRMS m/z found M^+ 254.096 16, $C_{16}H_{14}O_3$ requires M^+ 254.094 29.

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