Piplaroxide, an Ant-Repellent Piperidine Epoxide from *Piper tuberculatum*

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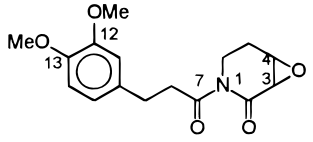
Investigation of leaves from the shrub *Piper tuberculatum* for compounds that might serve as repellents to leafcutting ants has led to identification of three cinnamic acid derivatives. One has been characterized as a new piperidine epoxide, piplaroxide (**1**), while the other two were recognized as the known compounds piplartine (**2**) and demethoxypiplartine (**3**). Both piplaroxide and demethoxypiplartine demonstrated significant activity in a laboratory bioassay measuring repellency to the leafcutter ant *Atta cephalotes*.

Leafcutter ants are classified as significant pests throughout the tropical and subtropical New World because they inflict great damage on a wide variety of agriculturally important plant species. Leafcutters also attack many native plant species, but when foraging in the natural forests these ants demonstrate clear and reproducible preferences.[1] For some years we have studied the chemistry of native American plants that are avoided by leafcutter ants, in an effort to identify natural products with ant-repellent activity.[2,3] During the course of this project, several plants of the genus *Piper* have been examined,[4−8] and a number of new natural products have been identified. In this report, we describe studies of the shrub *Piper tuberculatum* Jacq. (Piperaceae) that have led to the identification of three cinnamic acid derivatives.[9]

Air-dried leaves of *P. tuberculatum* were first steeped in hexane, and then in $CHCl_3$. After concentration of the $CHCl_3$ extract, the residue was purified by a sequence of column and radial dispersion chromatography on silica. The final purification gave pure compound **1**, and a second fraction containing two related compounds (**2** and **3**). Compound **1** crystallized from MeOH−$CHCl_3$ as small white needles (mp 91−92 °C).



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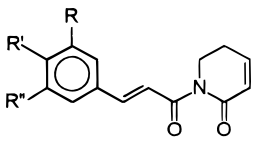

2 R = R' = R" = OMe
3 R = R' = OMe, R" = H

The mass spectrum of compound **1** contained an apparent molecular ion at 305.1266, corresponding to a molecular formula of $C_{16}H_{19}NO_5$, and the presence of nitrogen was supported by a positive Dragendorf's test. The ^{13}C NMR spectrum displayed 16 resonances, providing further support for the assigned molecular formula, and observation of resonances for six aromatic and two carbonyl carbons (δ 169.59 and 174.77) accounted for a total of 6 of the 8 degrees of unsaturation required by this formula. The remaining degrees of unsaturation were assumed to result from the presence of two additional ring systems.

When the ^1H-NMR spectrum of compound **1** in $CDCl_3$ was recorded, only a single resonance with a 3H intensity was observed in the aromatic region. However, when C_6D_6 was used as solvent, the aromatic region of the ^1H spectrum displayed a classic pattern for a 1,2,4-trisubstituted aromatic ring [δ 6.77 (1H, dd, J = 8.1, 2.0 Hz), 6.72 (1H, d, J = 2.0 Hz), 6.59 (1H, d, J = 8.1 Hz)]. In either solvent, two methoxy resonances were observed (3.84 and 3.81 ppm in $CDCl_3$), representing two of the aromatic substituents and explaining the two relatively deshielded aromatic carbon resonances (δ 148.84 and 147.40).

Two other major spin systems of compound **1** were identified by analysis of the ^1H COSY spectrum and homonuclear decoupling experiments. The first was identified as a $-CH_2CH_2-$ unit incorporating resonances at δ 2.90 and 3.20, while the second was a $-CH_2CH_2CHCH-$ system with non-equivalent methylene hydrogens. HMBC correlations indicated connection of the first ethylene unit to the aromatic ring and one carbonyl group, establishing the presence of a modified cinammyl unit. Hydrogens on both terminal carbons of the second spin system correlated to the remaining carbonyl group, leading to assignment of a piperidine system. The final ring was identified as an epoxide moiety based on the chemical shifts of the methine carbons and hydrogens in this fragment, leading to the structure assignment as shown for compound **1**. Although the natural product was optically active, and piplaroxide is the first member of this family to incorporate chirality,[10] the absolute stereochemistry was not readily established.

Compound **1**, which we have named piplaroxide, is a new natural product related to piplartine (**2**)[11] and piplartine dimer,[12] which have been reported from the roots of *P. tuberculatum*.[12] In addition, the second fraction described above was found to contain both **2** and another known compound, demethoxypiplartine (**3**).[13] In a laboratory bioassay[1,14] with a captive colony of the leafcutter ant *Atta cephalotes*, compound **1** displayed significant activity as a repellent at concentrations slightly greater than that recovered from the plant material (C/T = 30/17, $p < 0.005$, concn = 0.4 mg/g pressed rye flake). At higher concentrations, compound **3** also demonstrated significant ant-repellency in this bioassay (C/T = 30/11, $p < 0.001$, concn = 4.0 mg/g flakes; C/T = 32/21, $p < 0.01$, concn = 2.0 mg/g flakes), but compound **3** was also much more abundant in this plant sample. Thus, it is quite possible that both

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compounds **1** and **3** play significant roles in the natural defenses of this plant against leafcutter attack.

Experimental Section

General Experimental Procedures. The NMR spectra were recorded with Bruker AC-300, WM-360, or AMX-600 instruments on $CDCl_3$ solutions with TMS as an internal standard, except where otherwise noted. HREIMS and LREIMS were obtained at 70 eV.

Plant Material. Leaves of *P. tuberculatum* were collected near Gamboa, Panama. The leaves were air dried and then chopped in a Waring blender before storage in plastic bags. Voucher specimens have been deposited at the National Herbarium, University of Panama, Panama City, Panama in the Jerome J. Howard collection.

Extraction and Isolation. The *P. tuberculatum* leaves (300 g) were steeped in hexanes for 24 h. The rinsed leaves were then steeped in $CHCl_3$ for 24 h to give 11.04 g of a green residue after filtration and concentration *in vacuo*. A portion of this extract (10.43 g) was purified by dry column chromatography on silica, eluting first with an EtOAc/hexanes gradient and then with MeOH. A portion of the second most polar fraction (100% EtOAc, 720 mg of 1.44 g) was then subjected to flash column chromatography on silica eluting with $CHCl_3$. Fraction 4 (33.2 mg) was further purified by radial dispersion chromatography, yielding pure **1** (12.3 mg), after crystallization from a $CHCl_3$−MeOH mixture, and a mixture of compounds **2** and **3**.

The most polar fraction from the dry column (100% MeOH) was partitioned between $CHCl_3$ and H_2O leaving 1.99 g of the original 2.12 g in the $CHCl_3$ layer. This was subjected to flash column chromatography on silica eluting with MeOH in $CHCl_3$. The 1−5% MeOH fractions were identified as a mixture of compounds **2** and **3**. After removal of all volatiles, the residue (879 mg) crystallized from MeOH yielding pure **3** (374 mg). Repeated recystallization of the residue from the mother liquors did not afford pure **2**. The ^1H and ^{13}C NMR spectra of compound **3**, as well as those resonances for compound **2** observed in the mixture, were identical to literature data.[13]

Piplaroxide (1): white crystalline needles; mp 91−92 °C; $[\alpha]^{25}_D$ +67.7° ($CHCl_3$, *c* 0.8); ^1H NMR (300 MHz, $CDCl_3$) δ 6.76 (s, 3, H-11, H-14, H-15), 4.29 (dddd, J = 13.5, 5.6, 1.5, 1.5 Hz, 1, H-6a), 3.84 (s, 3, −OMe), 3.81 (s, 3, −OMe), 3.58 (dd, J = 4.0, 4.0 Hz, 1, H-4), 3.51 (d, J = 4.0 Hz, 1, H-3), 3.20 (m, 2, H-8), 3.13 (dd, J = 13.3, 4.0 Hz, 1, H-6b), 2.90 (t, J = 7.2 Hz, 2, H-9), 2.31 (dm, J = 15.0 Hz, 1, H-5a), 1.88 (ddd, J = 15.0, 13.2, 5.7 Hz, 1, H-5b); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.77 (s, C7), 169.59 (s, C2), 148.84 (s, C12), 147.40 (s, C13), 133.52 (s, C10), 120.33 (d, C15), 111.95 (d, C14), 111.30 (d, C11), 55.91 (q, OMe), 55.81 (q, OMe), 53.34 (d, C4), 52.31 (d, C3), 41.24 (t, C8), 35.57 (t, C6), 30.42 (t, C9), 23.79 (t, C5); HMBC (^1H irradiation → ^{13}C observed) δ 6.76 → (C9, C10, C11, C12, C13, C14, C15), 4.29 → (C2, C4, C5, C7), 3.51 → (C2), 3.84 → (C12), 3.81 → (C13), 3.20 → (C7, C9), 3.13 → (C5), 2.90 → (C7, C8, C10, C11/C14, C15), 1.88 → (C6); EIMS *m*/*z* (rel int %) [M^+] 305 (26), 192 (47), 191 (9), 165 (12), 164 (100), 161 (16), 151 (75), 149 (27), 91 (14), 77 (15); HREIMS *m*/*z* 305.1266 (calcd for $C_{16}H_{19}O_5N$, 305.1263).

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