

## Arieianal, a Prenylated Benzoic Acid from *Piper arieianum*

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A new prenylated benzoic acid, arieianal [3,4-dihydroxy-5-(*E,E,E*-11'-formyl-3',7',15'-trimethylhexadeca-2',6',10',14'-tetraenyl)benzoic acid, **1**], has been isolated from the leaves of *Piper arieianum* and assigned this structure on the basis of its spectral data.

Leafcutter ants of the genera *Acromyrmex* and *Atta* (Formica) pose a serious threat to agricultural endeavors in Central and South America, due to the staggering amount of plant material they harvest and the wide variety of plant species they decimate.<sup>1</sup> The ants, however, are not indiscriminate in their foraging, nor do they simply harvest the most abundant or most convenient plant species.<sup>2</sup> For many years our group has been investigating the chemistry involved in the leafcutters' plant selection, and through a bioassay-guided fractionation we have isolated many novel and biologically active natural products.<sup>3</sup> Field studies have suggested that the leaves of *Piper arieianum* C. DC. (Piperaceae), a small shrub found at lower elevations,<sup>4</sup> are not routinely harvested by leafcutting ants. Given the wide variety of novel terpenoids, prenylated benzoic acids, alkaloids, and flavanoids previously isolated from other *Piper* species examined by this group<sup>5</sup> and others,<sup>6</sup> an investigation of *P. arieianum* was initiated. This study is the first examination of the secondary chemistry of this plant.

Leaves of *Piper arieianum* were extracted consecutively with  $\text{CHCl}_3$ , MeOH, and  $\text{H}_2\text{O}$  in a Soxhlet apparatus, and the chloroform extract was washed with 50% aqueous methanol. The extracts were concentrated, and the greatest activity in our ant-repellent bioassay<sup>7</sup> was exhibited in the remaining chloroform extract. This extract was further fractionated with successive dry column chromatography and radial dispersion chromatography to yield 11 mg of compound **1** as a brown wax.

The  $^1\text{H}$  NMR spectrum of compound **1** revealed the presence of an aldehyde, a tetrasubstituted aromatic ring, four additional olefinic hydrogens, four vinylic methyl groups, and 14 additional  $\text{sp}^3$  hydrogens. The  $^{13}\text{C}$  NMR spectrum displayed 26 peaks, with shifts indicative of a conjugated carboxylic acid, a conjugated aldehyde, 14 aromatic/olefinic carbons, and 11  $\text{sp}^3$ -hybridized carbons. The spectral data were suggestive of a prenylated aromatic moiety with the diterpene side chain having one of the methyl units at a higher oxidation state. Observation of an  $[\text{M} - \text{H}]^-$  ion at  $m/e$  439.2492 in the ESIMS, and an  $[\text{M} + \text{H}]^+$  ion at  $m/z$  441.2632 in the FABMS, established  $\text{C}_{27}\text{H}_{36}\text{O}_5$  as the molecular formula with all 10 degrees of unsaturation already employed in the above features. Two hydrogens and two oxygens remained to be assigned, and the absence of any oxygenated  $\text{sp}^3$  carbons, along with the need for a tetrasubstituted aromatic ring, suggested a diphenol, which correlated with the observation of downfield aromatic carbon signals at 142.99 and 147.8 ppm.

An HMBC experiment was employed to address the regiochemical issues and to connect the various subunits. A broadened triplet methylene at  $\delta$  2.23 correlated to both the vinylic carbon doublet of the conjugated olefin (C-10') and the vinylic methine of the terminal isoprene unit (C-14'), thus establishing C-10', C-11', and C-18' as a conjugated system. Observation of correlations between the aldehydic hydrogen and the C-12' methylene carbon, and the complementary correlation of the C-10' olefinic hydrogen with the aldehydic carbon, established C-18' as the aldehyde group. The remaining regiochemical issue was the aromatic substitution pattern. Correlations between the aromatic hydrogen at  $\delta$  7.49 (H-6) and both the carboxylic acid carbon at 171.8 ppm and the benzylic carbon implied that the hydrogen was positioned between these two groups. The other aromatic hydrogen at  $\delta$  7.45 (H-2) also correlated to the acid, suggesting that hydrogen also was adjacent to the acid substituent and indicating a 1,3,4,5-substitution pattern with the two phenolic groups ortho to one another on the ring.

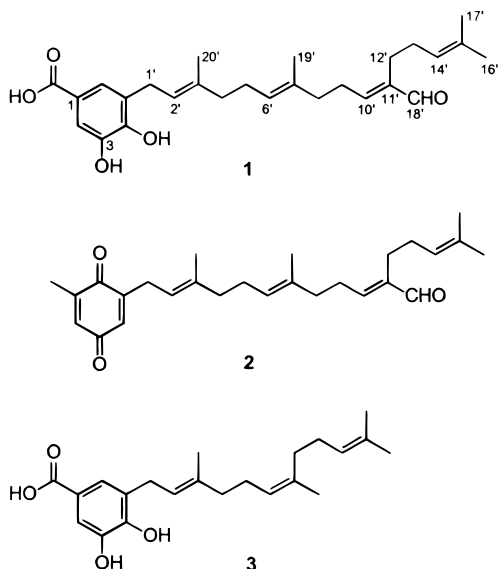
The carbon shifts of the vinylic methyl groups at C-3' and C-7' were indicative of *E* stereochemistry for both of these olefins. A NOESY correlation between the aldehydic hydrogen and the C-10' olefinic hydrogen suggested the *E* stereochemistry for the C-10',C-11' olefin. The geometry of the 10',11'-double bond was further supported by comparison to the aldehyde shifts reported for *cis*- and *trans*-2-methyl-2-pentenal ( $\delta$  9.38 for the *E* isomer and  $\delta$  10.11 for the *Z* isomer) by Rapoport et al.<sup>8</sup> In addition, the shift is in agreement with that of (*E,E,E*)-11-formyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol, where the stereochemistry was confirmed by reduction to the diol and comparison with naturally occurring (*E,E,E*)- and (*E,E,Z*)-11-hydroxymethyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol as reported by Sato et al.<sup>9</sup> The spectral data for arieianal (**1**) is comparable to that of sargaquinal (**2**), isolated by Kakisawa et al.<sup>10</sup> The hydrogen and carbon data for the aromatic portion of arieianal (**1**) also match those of piperolic acid (**3**), isolated from *Piper auritum*.<sup>11</sup>

The structure of compound **1**, therefore, was assigned as 3,4-dihydroxy-5-(*E,E,E*-11'-formyl-3',7',15'-trimethylhexadeca-2',6',10',14'-tetraenyl)benzoic acid, for which we propose the common name of arieianal.

### Experimental Section

**General Experimental Procedures.** NMR spectral data were recorded on Bruker AC-300 or AMX-600 instruments on  $\text{CDCl}_3$  solutions with TMS as an internal standard. LREIMS were obtained using a VG Trio 1 quadrupole mass spectrometer. HRFABMS were obtained using a VG ZAB-HF double-

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focusing mass spectrometer. HRESIMS were obtained using an Autospec mass spectrometer and were recorded on negative ions.

**Plant Material.** Leaves of *P. arieianum* were collected in Santa Rosa National Park, Costa Rica, in 1989. The leaves were air-dried and then chopped in a Waring blender before storage in plastic bags.

**Extraction and Isolation.** The *P. arieianum* leaves (ca 42 g) were successively extracted with 1.5 L of CHCl<sub>3</sub>, MeOH, and H<sub>2</sub>O for 24 h each using a Soxhlet apparatus. The CHCl<sub>3</sub> extract was further washed with 50% aqueous MeOH, and the remaining CHCl<sub>3</sub> solution was concentrated to yield 3.85 g of solid extract. This material was further purified by dry column chromatography with a hexane-EtOAc gradient to yield 496 mg of crude material. The material was split into three portions, and each portion was further purified by radial dispersion chromatography (toluene-EtOAc-HOAc gradient), yielding a combined 11 mg of an odorless, brown wax.

**Arieianal (1):** IR 3315 (br), 2919, 1684 cm<sup>-1</sup>; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 9.20 (1H, s, H-18'), 7.49 (1H, d, *J* = 1.9 Hz, H-6), 7.45 (1H, d, *J* = 1.9 Hz, H-2), 6.46 (1H, t, *J* = 7.3 Hz, H-10'), 5.31 (1H, br t, *J* = 7.1 Hz, H-2'), 5.15 (1H, br t, *J* = 7.1 Hz, H-6'), 5.05 (1H, br t, *J* = 7.3 Hz, H-14'), 3.37 (2H, d, *J* = 7.1 Hz, H-1'), 2.41 (2H, dt, *J* = 7.3, 7.3 Hz, H-9'), 2.23 (2H, t, *J* = 7.3 Hz, H-12'), 2.13 (4H, t, *J* = 7.3 Hz, H-5' and H-8'),

2.07 (2H, t, *J* = 7.4 Hz, H-4'), 2.00 (2H, dt, *J* = 7.6, 7.6 Hz, H-13'), 1.72 (3H, s, H-20'), 1.63 (3H, s, H-16'), 1.60 (3H, s, H-19'), 1.53 (3H, s, H-17'); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 196.1 (d, C-18'), 171.8 (s, CO<sub>2</sub>H), 156.4 (d, C-10'), 147.8 (s, C-4), 143.04 (s, C-11'), 142.99 (s, C-3), 137.5 (s, C-3'), 133.6 (s, C-7), 132.4 (s, C-15'), 127.5 (s, C-5), 125.3 (d, C-6'), 124.5 (d, C-6), 123.4 (d, C-14'), 121.4 (d, C-2'), 120.7 (s, C-1), 114.7 (d, C-2), 39.5 (t, C-4'), 38.2 (t, C-8'), 28.4 (t, C-1'), 27.5 (t, C-9'), 27.0 (t, C-13'), 26.4 (t, C-5), 25.7 (q, C-16'), 24.2 (t, C-12'), 17.6 (q, C-17'), 16.1 (q, C-20'), 15.9 (q, C-19'); EIMS (70 eV) 271 (3), 251 (18), 221 (11), 205 (29), 167 (28), 69 (100); HRESIMS found [M - H]<sup>-</sup> 439.2492, (calcd for C<sub>27</sub>H<sub>35</sub>O<sub>5</sub>: 439.2484); HRFABMS (thioglycerol matrix) found [M + H]<sup>+</sup> 441.2632, (calcd for C<sub>27</sub>H<sub>37</sub>O<sub>5</sub>: 441.2641).

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## References and Notes

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