Materials and Methods

**Insects and behavioral assays.** The *B. germanica* colony was maintained at 27 ± 0.5°C, ~50% relative humidity and 12:12 h light:dark photoperiod, and provided a continuous supply of rat chow 5012 (Purina Mills, St. Louis, MO) and water. Newly emerged adults were separated from the colony on the day of eclosion (day 0), and females and males were maintained in separate rooms.

For behavioral assays in the laboratory, a two-choice olfactometer device was used (S1, S2, S3). Briefly, a straight Plexiglas tube (55 cm long x 3 cm ID) was divided along 15 cm of its upwind end and air was drawn through it at 20 cm/min. A single cockroach was placed in a screen-gated cage at the downwind end 30 min before the start of an assay. A candidate attractant and control (solvent) were loaded in 5 µl dichloromethane onto separate Whatman #1 filter paper discs (5 mm diameter) and after the solvent evaporated (~30 sec) the discs were introduced simultaneously at the upwind end of the olfactometer. The percentage of insects running upwind, the latency of their responses (in sec), and the percentage choosing each arm of the olfactometer were recorded.

**Purification of blattellaquinone.** We followed a bioassay-guided chromatographic fractionation of the extract and purification of the active fraction. First, the total lipid extract of approximately 2000 pygidia was dried over anhydrous sodium sulfate and then separated by flash
chromatography on silica gel (800 mg) by eluting successively with 8 ml each of mixtures of pentane-ether in the sequence 100% pentane, 5%, 10%, 20% and 40% ether in pentane, and 100% ether. The active fraction (40% ether fraction) was concentrated under a N₂ stream in an ice bath and subjected to preparative high performance liquid chromatography (HPLC).

The HPLC system comprised a Rainin Rabbit-HP (Rainin Instrument Co., Inc., MA, USA) equipped with a Rheodyne injector with a 1.0 ml sample loop (Rheodyne, Inc., CA, USA). The column was an Econosil Silica 5 µm, 250 x 4.6 mm column (Alltech Associates, Inc., Deerfield, IL, USA). The solvent system comprised a hexane-ether gradient, programmed as 5% ether in hexane for 5 min, then increased linearly to 30% at 0.5%/min, to 100% at 5%/min, and kept at 100% ether for 20 min at a flow rate of 1 ml/min. A Dynamax UV-1 (Rainin Instrument Co., Inc., MA, USA) detector was used to monitor the eluate at 210 nm. The effluent was collected every 3 min between 0 and 24 min and from 46 to 73 min, and every 1 min from 24 to 46 min.

Gas chromatographic-electroantennographic detection (GC-EAD). GC-EAD instrumentation used in this study was the same as described previously (S4). An HP5890II GC equipped with either a non-polar Equity-1 capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness, Supelco, Bellefonte, PA, USA) or a slightly polar EC-20 capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness, Alltech Associates, Deerfield, IL, USA) was used for analysis in splitless mode. Nitrogen was used as the carrier gas at a head pressure of 79 kPa (flow rate, 1.0 ml/min). The oven temperature was 40°C for 2 min, increased at 15°C/min to 250°C and held for 10 min. Injector and flame ionization detector (FID) temperatures were set at 150 and 250°C, respectively. The column effluent was combined with nitrogen make-up gas (30 ml/min) and then split 1:1 to the FID and EAD. The EAD outlet was secured in a charcoal-filtered and
humidified air stream, refrigerated by a modified condenser flushed with 5°C water, flowing at 500 ml/min over the antennal preparation.

A live male was fixed on a custom-made acrylic holder and used for EAD recordings. The tips of the antennae were brought into contact with a capillary tube on the holder, which was filled with saline. A pure gold recording electrode was connected to the capillary tube, while the indifferent electrode was impaled directly into the mouth of the male. The output signal from the antennae was amplified by a custom-built high-input impedance DC amplifier and filtered by a high-pass filter with a cutoff frequency of ~0.5 Hz (S4). The signals were recorded on an HP3390A integrator synchronized with the GC integrator. GC-EAD analyses consistently revealed a single EAD-active compound (fig. S1).

**Preparative GC.** The behaviorally active HPLC fraction (34–35 min) was further separated on a preparative GC. In preliminary experiments, the pheromone appeared to be thermally unstable, because its FID peak declined by about 50% when the injector temperature was raised from 150°C to 250°C; it disappeared at 300°C. Therefore, we designed a micro-preparative GC system (S5) that was integrated with NMR sample preparation for minute amounts of thermally unstable volatile chemicals. The recovery efficiency of volatile chemicals with this technique was >80% with sample sizes of 0.05 to 0.5 µg, and the purity of the acquired NMR samples was sufficient for high sensitivity NMR analyses including two dimensional experiments. An HP5890 GC was equipped with a non-polar Equity-1 mega-bore capillary column (5 m x 0.53 mm ID, 1.5 µm film thickness, Supelco, Bellefonte, PA, USA). Nitrogen was used as the carrier gas at a head pressure of 6.9 kPa and a flow rate of 8.0 ml/min. The time for splitless injection was 1.0 min. The oven temperature was set initially at 40°C for 2 min, increased at 10°C/min to 250°C, and held for 10 min. The injector and collection port temperatures were set at 150°C and 220 °C,
respectively, and the septum purge flow rate was set at 1.5 ml/min with a total flow rate of 100 ml/min.

**GC-mass spectrometry (MS).** The behaviorally and GC-EAD active fractions from preparative HPLC were subjected to GC-MS analyses on a Shimadzu GC-17A equipped with a 30 m x 0.25 mm ID x 0.25 μm thickness DB-5ms column (J&W Scientific, Folsom, CA, USA) operated in splitless mode and coupled to a Shimadzu QP-5050 quadrupole MS running in the electron impact (EI) (ionization at 70 eV) scan mode. Helium was used as the carrier gas at a head pressure of 54 kPa (flow rate, 1.0 ml/min). Oven temperature was 40°C for 2 min, increased at 5°C/min to 220°C and held for 5 min, and injector and interface temperature were set at 150°C and 220°C, respectively.

**Nuclear magnetic resonance (NMR).** A total of ca. 5 μg of pure pheromone was isolated by preparative GC from the extract of ca. 5,000 female pygidia, dissolved in a minimum of “100%” benzene D₆, and used for the NMR analysis on a 600 MHz Burker Avance NMR spectrometer. The ¹H NMR spectrum showed, with a rather high degree of certainty, that it had 14 hydrogen atoms, suggesting either C₁₂H₁₄O₄ or C₁₆H₁₄O. The first molecular formula has 6 degrees of unsaturation while the second has 10 degrees of unsaturation. Since it is difficult to draw “reasonable” structures with the second formula, C₁₂H₁₄O₄ was selected as the most likely formula for the pheromone.

The presence of an isopropyl group is evident in the NMR spectrum. There is a six-proton doublet at δ 0.786 and a one-proton multiplet centered at δ 1.987 (Fig. 1). This methine-derived multiplet is obviously not a first order multiplet; it is clearly coupled to one or more protons in addition to the six-methyl protons. A likely candidate is the two-proton “doublet” at δ 1.920, which is distorted and “leaning” in the direction of the methine group. Assuming that the
methine and methylene groups are coupled, a rough calculation of $\Delta \nu/J$ gives an approximate value of five. This calculation indicates that the patterns should not be first order but that they should retain some resemblance to first order.

The chemical shifts and coupling patterns are reminiscent of esters of isovaleric acid and, in fact, matches NMR spectra of known isovalerate esters. This piece of the structure uses C$_5$H$_9$O$_2$ and accounts for one of the degrees of unsaturation. The other piece, the alcohol moiety, must have a formula of C$_7$H$_5$O$_2$, noting that the oxygen atom associated with the alcohol portion has already been accounted for in the acid portion.

Thus, a good place to start on the alcohol piece in the NMR spectrum is the two-proton doublet at $\delta$ 4.721 (Fig. 1). The coupling constant is small (~2 Hz), which is too small for typical vicinal coupling in a freely rotating system, meaning either long-range coupling or restricted rotation. Since the remaining piece is highly unsaturated, the likely case is that the small coupling is due to long-range coupling. The chemical shift of this methylene group indicates that it is deshielded by an oxygen atom and by some other group, likely some form of unsaturation.

The remaining three protons in the NMR spectrum are likely either olefinic or shielded aromatic protons. The proton at $\delta$ 6.356 is an apparent quartet, which is explained by long-range coupling to the methylene group at $\delta$ 4.721 and a similar (in magnitude) long range coupling to one of the other olefinic protons (Fig. 1). The other two protons form a multiplet at $\delta$ 5.928. This multiplet can be dissected into (1) vicinal coupling to give an AB multiplet, almost a quartet ($\Delta \nu/J \sim 1.7$) and (2) long range coupling of one of the AB protons to the proton at $\delta$ 6.356. This NMR pattern is consistent with a substituted para-benzoquinone. The primary alcohol of $p$-benzoquinone is gentisyl alcohol, and the corresponding quinone is gentisyl quinone (fig. S2). Thus, gentisyl quinone isovalerate was proposed as the structure for the pheromone compound.
**Synthesis and confirmation of chemical structure.** The synthesis is outlined in Fig. 2. Acylation of 2,5-dimethoxybenzyl alcohol in pyridine and CH₂Cl₂ with a catalytic amount of DMAP was carried out with isovaleryl chloride (Aldrich). The crude ester was oxidized with Ce(NH₄)₂(NO₃)₆ in acetonitrile and water to give crude blattellaquinone. This crude quinone ester was purified by flash chromatography on silica gel and was recrystallized from hexane-ether. ¹H NMR analysis in deuterobenzene (low concentration at 600 MHz) of the synthetic blattellaquinone (fig. S3), which was identical to the natural compound, confirmed the structure.

**References and Notes**


**Supplementary Figures**

**Figure S1.** GC-EAD traces of a behaviorally active HPLC-purified fraction. Puffs of the volatiles from a rotten apple are used to check for viability and response of the antenna before each GC-EAD run.

**Figure S2.** Structural formulas of blattellaquinone and related benzo- and hydroquinones.

**Figure S3.** NMR analysis of synthetic blattellaquinone.
Figure S1

Retention time (min)
Figure S2

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\begin{align*}
\text{p-benzoquinone} & \quad \text{gentsisyl alcohol} & \quad \text{gentsisyl quinone} & \quad \text{gentsisyl quinone isovalerate "blattellaquinone"}
\end{align*}
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Figure S3

![NMR Spectra]

- **Benzene d6**: peaks at 6.32, 5.92, 4.70, 1.98, 0.76 ppm.
- **Water**: peaks at 7.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.0 ppm.

*Note: The specific chemical shifts and peak assignments are for illustrative purposes.*