(2R,3S)-2,3-Octanediol, a Female-Produced Sex Pheromone of Megopis costipennis (Coleoptera: Cerambycidae: Prioninae)

Jacob D. Wickham, Jocelyn G. Millar, Lawrence M. Hanks, Yunfan Zou, Joseph C. H. Wong, Rhett D. Harrison, and Yi Chen

Abstract

During field screening trials of a number of known cerambycid pheromones in China, males of Megopis costipennis (White) (Coleoptera: Cerambycidae: Prioninae: Callipogonini) were found to be specifically attracted to racemic anti-2,3-octanediol, suggesting that one of the enantiomers of this compound might be a female-produced sex pheromone of this species. Analysis of volatiles produced by beetles of both sexes confirmed this hypothesis: females produced (2R,3S)-2,3-octanediol, whereas males did not, and in coupled gas chromatography–electroantennogram detection assays, antennae from male beetles responded strongly to this compound. In field trials, males were equally attracted to traps baited with either (2R,3S)-2,3-octanediol or racemic anti-2,3-octanediol, indicating that the enantiomeric (2S,3R)-2,3-octanediol does not antagonize attraction to the naturally produced enantiomer. Thus, the more economical racemic anti-2,3-octanediol can be used for trap baits for this species. Homologous 2,3-hexanediols previously had been identified as sex pheromones or sex attractants of prionine species in the genus Tragosoma Audinet-Serville of the tribe Meroscelisini.

Key words: Megopis, longhorned beetle, woodborer, monitoring

Materials and Methods

Collection of Volatiles from Beetles

One live female M. costipennis was captured while monitoring insects attracted to street lights on 17 May 2013 in the village of Mengsong (21° 30’29.39” N, 100° 29’50.60” E, Yunnan, China). On 22–25 May and 21 June 2013, live males for use in coupled gas chromatography–electroantennogram detection (GC-EAD) experiments were captured with flight-intercept panel traps (black corrugated plastic, 1.2 m in height by 0.30 m in width, AlphaScents, Portland, OR) fitted with dry collection cups. Panels and interior surfaces of collection cups were coated with Fluon® (Northern Products, Woonsocket, RI) to increase trapping efficiency (Graham et al. 2010). Pheromone lures consisted of resealable
polyethylene sachets (Cat. #018161A, 5.1 by 7.6 cm, Thermo-Fisher Scientific, Waltham, MA) loaded with 50 mg of racemic anti-2,3-octanediol diluted in 1 mL of ethanol. Males were only caught at night, confirming that they are nocturnally active.

Insect-produced volatiles were collected by aeration of beetles overnight (~12 h) in 0.5-liter Erlenmeyer flasks fitted with inlet and outlet ports. Beetles were provided with a cotton wick soaked with filtered water, which was changed daily. Charcoal-filtered air was pulled through the flasks at 500 mL/min, through two collectors in sequence, consisting of glass pipettes containing 200 mg of the adsorbent Porapak-Q (80–100 mesh; Supelco, Bellefonte, PA) packed between plugs of silanized glass wool. The Porapak-Q was cleaned before use by sequential rinses with 5 mL dichloromethane and 5 mL pentane. Trapped volatiles were eluted from the collectors with two 1-mL aliquots of pentane into separate glass vials, and vials were stored at ~20°C. Aerations were performed with the live female for five nights (18–22 May 2013) until she died, whereas volatiles were collected from five males on five nights from 25 May to 5 June 2013 (n = 25 separate extracts).

Analysis of Insect-Produced Compounds
To determine if one or both enantiomers of anti-3-octanediol were produced by female M. costipennis, compounds in a headspace extract from the female beetle were compared with authentic standards of the two enantiomers on a Cyclodex B chiral stationary-phase GC column (30m by 0.25 mm ID, 0.25-μm film; J&W Scientific, Folsom, CA) mounted in a gas chromatograph interfaced to a mass spectrometer (GCMS-QP2010 Plus, Shimadzu Corporation, Kyoto, Japan), with electron impact ionization (70 eV). The oven was programmed from 50°C (1 min), ramped 10°C/min, to 220°C (3 min hold); injector temperature was 150°C; and helium was the carrier gas (1 mL/min). Under these conditions, the anti-3-octanediol enantiomers eluted at 13.24 min (2R,3S) and 13.29 min (2S,3R). Extracts and synthetic standards also were analyzed by coupled GC-EAD on a Hewlett-Packard 5890 Series II gas chromatograph using the same column and temperature program as described above in the gas chromatography–mass spectrometry (GC-MS) analyses. Nitrogen makeup gas (10 mL/min) was added to the column effluent, which was then divided (~1:1) with a splitter (SGE Analytical Science, Melbourne, Australia) into two equal-length segments of deactivated capillary column (0.25 mm ID, Agilent Technologies, Palo Alto, CA), with one going to the flame ionization detector (FID) of the GC, and the second to the electroantennographic detector.

The second FID port of the GC was modified for use as an electroantennographic detector outlet. The transfer line was led into the FID heating block with a section of glass-lined stainless steel tube (1.6 mm OD by 0.3 mm ID, SGE Analytical Science), and the block was fitted with a stainless steel cap (68 by 18 mm) with a hole (16 mm diameter) that allowed a glass-lined tube to pass through, with its height adjustable with a set screw. This enabled the heated transfer line to project into a charcoal-filtered, humidified, and cooled (0°C) airstream, inside a modified condenser tube (40 by 1.5 cm ID), flowing at 500 mL/min over the antennal preparation.

The antennal preparations were prepared by removing an antenna from a live beetle, and mounting the antenna on an acrylic holding station, with the base contacting the reference electrode, and the tip contacting the recording electrode. The electrodes consisted of glass capillaries with a gold wire down the center, filled with saline solution (0.9% NaCl). Signals were recorded with a custom-built, single-step, high-impedance DC amplifier, with 100 × amplification. The output signal was filtered by a resistance/capacitance high-pass filter with a cutoff frequency of about 0.5 Hz. The outputs from the electroantennographic detector amplifier and the GC FID were routed to a two-channel analog-to-digital converter (PowerLab, AD Instruments Inc., Colorado Springs, CO), with signals analyzed using Chart 7.0 software (AD Instruments Inc.). An additional software-controlled, digital, low-pass filter was set at a cutoff frequency of 10 Hz. A minimum of three consecutive antennal responses were recorded before a compound was scored as bioactive.

Synthesis of Compounds
Racemic anti-2,3-octanediol 1 (Fig. 1) was prepared from (Z)-2-octene, as previously described (Lacey et al. 2004). (2R,3S)- and (2S,3R)-2,3-octanediols 2 and 6 were prepared by kinetic resolution of racemic anti-2,3-octanediol 1 with Amano Lipase PS, from Burkholderia cepacia (strain #534641, Aldrich Chemical Co., Milwaukee, WI), adapted from a previously reported procedure (Kim et al. 1995). Thus, racemic anti-diol 1 (5.85 g, 40 mmol), vinyl acetate (18.4 mL, 200 mmol), lipase PS (17.55 g), and methyl t-butyl ether (160 mL) were stirred at room temperature, monitoring the reaction progress with a Cyclodex B column as described above. The reaction was stopped when the enantiomeric excess (ee) of the slower-reacting (2S,3R)-enantiomer was >95% (~25 h). The enzyme was removed by filtration, and after concentration, the residue was purified by vacuum flash chromatography on silica gel (hexane/EtOAc = 2:1, then EtOAc) to give (2S,3R)-2,3-octanediol 2 (2.06 g, ~100% ee, 35% yield) and a mixture of acetates 3, 4, and 5, which was hydrolyzed with 5 M NaOH and MeOH overnight to give (2R,3S)-enriched (~85% ee) 2,3-octanediol.

This enantiomerically enriched diol was subjected to kinetic resolution again (12 mL vinyl acetate, 9.0 g lipase PS, 100 mL methyl t-butyl ether), stopping the reaction when analysis of a sample on the Cyclodex B column indicated a ~1:1 ratio of unreacted (2R,3S)- and (2S,3R)-2,3-octanediols (~20 h). After filtration and concentration, the residue was purified by vacuum flash chromatography (hexane/EtOAc = 2:1) to give a mixture of 3, 4, and 5, which was hydrolyzed with 5 M NaOH and MeOH overnight to give (2R,3S)-2,3-octanediol (1.72 g, ~100% ee, 34% yield).

To analyze the two enantiomers, racemic anti-2,3-octanediol was injected on the Cyclodex B column programmed isothermally at 95°C. The two enantiomers were resolved to baseline [(2R,3S)-2,3-octanediol 30.05 min, (2S,3R)-2,3-octanediol 31.03 min]. The enantiomers were identified based on their known relative reactivities with the Amano Lipase PS (Kim et al. 1995).

Field Bioassay
A field trial was conducted at the Bulong Nature Reserve (21° 30’ 29.39” N, 100° 29’ 50.60” E; elevation ~1,600 m), at the southern tip of Yunnan Province in China. The reserve is a seasonal, tropical, montane rain forest with mixed old growth and secondary forest interspersed with tea plantations and open landscapes. Average summer (May–June) temperatures are 15–21°C and average rainfall is ~1800–2400 mm/yr, ~80% of which falls in the wet season from May to October (Li et al. 2014, Zhao et al. 2015). Panel traps and pheromone lures were as described above. Lures were loaded with 1 mL of an ethanol solution of a test compound.

The experiment was conducted from 1 June to 11 June 2013, and consisted of four transects of traps baited with the following treatments (one treatment per transect): 1) (2R,3S)-2,3-octanediol...
Environmental Entomology, 2016, Vol. 45, No. 1

(25 mg); 2) (2S,3R)-2,3-octanediol (25 mg); 3) racemic anti-2,3-octanediol (a 1:1 mixture of the two enantiomers, 50 mg); and 4) an unbaited trap. Traps were spaced at least 20 m apart within transects, with at least 200 m between transects. Traps were serviced on 6 June 2013, after which treatments were rotated one position within each transect to control for positional effects. Beetles were collected again on 11 June 2013, when the experiment was terminated.

Differences among mean numbers of beetles caught in traps baited with the various treatments were determined using the non-parametric Friedman’s test (PROC FREQ, option CMH; SAS Institute 2011). Differences between pairs of means were tested with the REGWQ means-separation test, which controls for maximum experiment-wise error rates (PROC GLM, SAS Institute 2011).

Scanning Electron Microscopy
Scanning electron microscopy was used to examine the prothoraces of M. costipennis adults for the presence of pores that are associated with pheromone production in males of species in the subfamily Cerambycinae (Ray et al. 2006), and females of species in the genus Tragosaoma Audinet-Serville in the subfamily Prioninae (Ray et al. 2012). The male specimens were collected during the initial 2010 screening trials (Wickham et al. 2014), and the single female was hand-captured, as described above. Specimens were prepared following the methods of Ray et al. (2006), and were imaged with an environmental scanning electron microscope equipped with a field-emission electron gun (Philips XL30, FEI Company, Hillsboro, OR) at 5.0 kV.

Results
(2R,3S)-2,3-Octanediol was identified in the headspace volatiles collected from the female beetle on the first night that she was aerated, by both GC-MS (Fig. 2) and GC-EAD analyses (Fig. 3). (2R,3S)-2,3-Octanediol was not found in any of the 25 extracts of volatiles collected from five individual male beetles aerated on five separate nights, indicating that it is produced only by females. In GC-EAD analyses of the extract from the female, antennae of males responded only to (2R,3S)-2,3-octanediol (Fig. 3), indicating that the antennae are specifically tuned to this compound. The (2R,3S)-absolute configuration was determined by analysis of the extract on a chiral stationary-phase GC column (Fig. 3), in which the retention time of the insect-produced compound exactly matched that of the (2R,3S)-2,3-octanediol standard. This was further confirmed by coinjection of an aliquot of the extract with the racemic mixture, resulting in the enlargement of the first-eluting, (2R,3S)-2,3-octanediol peak.

In total, 121 male M. costipennis were trapped during the field bioassay, of which 65 males (~54%) were in traps baited with (2R,3S)-2,3-octanediol and another 41 males (~34%) were in traps baited with the racemic anti-2,3-octanediol. Means for those two treatments were significantly different from controls (Fig. 4; Friedman’s $Q_{5,25} = 18.3, p = 0.0004$), but not significantly different from one another. The mean for (2S,3R)-2,3-octanediol was not significantly different from controls. No female M. costipennis were captured during the bioassay.

Scanning electron microscopy of the prothoraces of male and female beetles revealed the presence of sex-specific pores in the cuticle of females (Fig. 5), which are the likely site of pheromone production.

Discussion
In our previous study in which a number of known cerambycid pheromones were tested in field trials in China, male M. costipennis were specifically attracted to racemic anti-2,3-octanediol (Wickham et al. 2014), suggesting that one of the enantiomers of this compound was a female-produced sex pheromone for this species. Analysis of headspace volatiles from beetles of both sexes by GC with a chiral stationary phase column confirmed this hypothesis, showing that females produced exclusively the (2R,3S)-enantior, and that production was sex-specific. However, the (2S,3R)-enantior did not appear to inhibit attraction, because catches in traps baited with either (2R,3S)-2,3-octanediol or the anti-2,3-octanediol (consisting of both enantiomers) were not significantly different. Furthermore, large numbers of males had been attracted in the initial field screening study in which traps were baited with the anti-2,3-octanediol (Wickham et al. 2014), providing further evidence that the (2S,3R)-enantior is not inhibitory. This finding is useful for practical purposes, because the anti-2,3-octanediol is considerably cheaper to synthesize than the pure (2R,3S)-enantior. What remains unknown is whether one or both of the syn-2,3-octanediol enantiomers (i.e., the [2R,3R]- or [2S,3S]-2,3-octanediols) might be inhibitory.

The bioassays reported here and in the previous field screening study in which this species was first caught (Wickham et al. 2014) were all carried out in Yunnan province, whereas this species has
been reported from a number of other Chinese provinces, as well as India, Laos, and Bangladesh (Hua et al. 2009). However, because the analytical and bioassay data suggest that the pheromone consists of a single compound, so that there is no possibility for blend variations, it seems likely that the sex pheromone in these geographically separated populations will be the same. Furthermore, the fact that we caught only a single Megopis species in the field trials reported here suggests either that any possible sympatric congeners

---

**Fig. 2.** Representative total ion chromatograms from extracts of headspace volatiles collected from an adult female (top trace) and male (lower trace, inverted) Megopis costipennis. The extract from the female contained (2R,3S)-2,3-octanediol, which was not detected in any of 25 aeration extracts from male beetles.

**Fig. 3.** Coupled gas chromatography–electroantennogram analyses of extracts of volatiles collected from male and female Megopis costipennis, and of synthetic standards. In each panel, top trace is response from the antenna of a male beetle, and bottom trace is the corresponding GC chromatogram. Panels show analyses of (a) headspace extract of a female, (b) headspace extract of a male, (c) (2R,3S)-2,3-octanediol, and (d) racemic anti-2,3-octanediol. The antennae of males responded specifically to (2R,3S)-2,3-octanediol. Extracts were analyzed on a Cyclodex B chiral stationary-phase GC column.
likely use a different pheromone, or have different seasonal activity periods than *M. costipennis*. Approximately 16 *Megopis* species have been reported from China (W. Lu, personal communication), but in field tests carried out in different regions of China with a panel of known cerambycid pheromones, the only other pheromone known from the subfamily Prioninae. The latter structure was identified from *Prionus californicus* Motschulsky (Rodstein et al. 2009), and has since been shown to attract a number of other North American and a European *Prionus* species (Barbour et al. 2011), as well as an Asian species, *Dorysthenes granulosus* (Thomson), which is in the same tribe (Wickham et al. 2015). Collectively, these data suggest that pheromone structures within the subfamily Prioninae are likely to be similar or identical among closely related species, as has been found with other cerambycid subfamilies such as the Lamiinae and Cerambycinae, but that there is clearly more than one basic structural motif within the subfamily overall. Furthermore, despite the substantial differences in the chemistry of the pheromones of the *Megopis/Tragosoma* and the *Prionus/Dorysthenes* groups of species, in all cases, these pheromones are female-produced sex pheromones, in contrast to the exclusively male-produced aggregation pheromones found in the subfamilies Cerambycinae and Lamiinae. Thus, the available evidence suggests a divergence along subfamily lines in the reproductive biology of the Cerambycidae. Possible reasons for the evolution of different reproductive strategies within the different subfamilies remain to be determined.

**Acknowledgments**

Scanning electron microscopy was made possible through a collaboration between J.C.H.W. and Bugscope, a project of the Imaging Technology Group at the Beckman Institute for Advanced Science and Technology, Urbana, IL (http://bugscope.beckman.illinois.edu). This work was supported financially by United States Department of Agriculture-Animal and Plant Health Inspection Service grant numbers 12-8130-1422-CA and 13-8130-1422-CA to J.G.M. and L.M.H., Chinese Academy of Sciences Young International Scientist Fellowship Grant No. 2012Y1JA0001 and National Science Foundation of China Fellowship fund for Young International Scientists grant number #21250110573 to J.D.W., and National Science Foundation of China grant 21235007 to Y.C.

**References Cited**


