ORGANIC LETTERS

2012
Vol. 14, No. 10
2576–2578

The Discovery of 2-Hydroxymethyl-3-(3-methylbutyl)-5-methylpyrazine: A Semiochemical in Orchid Pollination

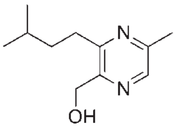
**Björn Bohman,[†,‡,§] Lynne Jeffares,[†,§] Gavin Flematti,[§] Ryan D. Phillips,[‡,‖,⊥]
Kingsley W. Dixon,[‖,⊥] Rod Peakall,[‡,§] and Russell A. Barrow*,[†]**

Research School of Chemistry, The Australian National University, Canberra ACT, 0200, Australia, Research School of Biology, The Australian National University, Canberra ACT, 0200, Australia, School of Chemistry and Biochemistry, The University of Western Australia, Crawley WA, 6009, Australia, Kings Park and Botanic Garden, The Botanic Garden and Parks Authority, West Perth WA, 6005, Australia, and School of Plant Biology, The University of Western Australia, Crawley, WA 6009, Australia

rab@anu.edu.au

Received April 5, 2012

ABSTRACT



Drakaea livida **(Orchidaceae) is pollinated by sexual deception of the wasp** ***Zaspilothynnus nigripes*** **(Thynnidae). It is shown that the orchid emits the same compound, 2-hydroxymethyl-3-(3-methylbutyl)-5-methylpyrazine, that females emit when calling for mates. This novel pyrazine was isolated and identified by GC-EAD and GC-MS and confirmed by synthesis. This compound may represent the first known case of pyrazines as sex pheromones in Hymenoptera.**

Sexually deceptive orchids employ mimicry of the sex pheromones of female insects to lure sexually excited males as pollinators.[1,2] This intriguing interaction provides unique opportunities to discover novel compounds through investigation of diverse but poorly studied insect families. For example, 2-ethyl-5-propylcyclohexan-1,3-dione, representing a novel class of natural products (2,5-dialkylcyclohexan-1,3-diones), is both the sex pheromone of the thynnine wasp *Neozeleboria cryptoides* and the semiochemical used by the orchid *Chiloglottis trapeziformis* to attract the male wasps as pollinators.[1,3]

One of the most sophisticated of all sexually deceptive systems is that of *Drakaea* (Orchidaceae), which mimics the flightless females of thynnine wasps.[4] In addition to chemical mimicry, the flower presents a female-like structure and exploits the precopulatory flight behavior of the male to precisely position the wasp for pollination.[4] *Drakaea* exploit a diversity of wasp genera,[5] potentially providing the opportunity to explore a range of pheromone systems.

Here we aim to identify the chemical compounds involved in the attraction of sexually deceived *Zaspilothynnus nigripes* (Thynnidae), the primary pollinator of *Drakaea livida*.[5] Flowers of *D. livida* were dissected and compounds extracted from the labellum using dichloromethane to provide samples for analysis using GC-EAD (gas chromatography coupled with electroantennographic detection) and GC-MS. Analysis of the solvent extracts using GC-EAD with antennae from male *Z. nigripes*

[†] Research School of Chemistry, The Australian National University.

[‡] Research School of Biology, The Australian National University.

[§] School of Chemistry and Biochemistry, The University of Western Australia.

[‖] The Botanic Garden and Parks Authority.

[⊥] School of Plant Biology, The University of Western Australia.

(1) Schiestl, F. P.; Peakall, R.; Mant, J. G.; Ibarra, F.; Schulz, C.; Franke, S.; Francke, W. *Science* **2003**, *302*, 437.

(2) Schiestl, F. P.; Ayasse, M.; Paulus, H. F.; Lofstedt, C.; Hansson, B. S.; Ibarra, F.; Francke, W. *Nature* **1999**, *399*, 421.

(3) Franke, S.; Ibarra, F.; Schulz, C. M.; Twele, R.; Poldy, J.; Barrow, R.; Peakall, R.; Schiestl, F. P.; Francke, W. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 8877.

(4) Peakall, R. *Funct. Ecol.* **1990**, *4*, 159.

(5) Hopper, S. D.; Brown, A. P. *Aust. Syst. Bot.* **2007**, *20*, 252.

10.1021/ol300864u

revealed an electrophysiological response to a single compound.

Concurrent analyses showed that the same EAD active constituent identified from flowers of *D. livida* was a major component eluting from the GC in extracts prepared from the dissected heads of female *Z. nigripes*. Furthermore, using SPME fibers, this same compound was detected in the headspace surrounding females exhibiting courtship behavior. Electrophysiological activity was confirmed for both samples of female head extracts and SPME headspace.

Owing to the small quantity of sample available in the natural extracts (~1−10 ng/flower and wasp), further identification of the physiologically active compound relied solely on GC-MS analysis. Using extracted ion chromatograms, a single peak and mass spectrum representing the unknown compound was identified. The tailing in the abundance of larger *m*/*z* fragments in the EI-MS made identification of the molecular ion difficult (Figure 1). However, utilizing the softer ionization technique available through chemical ionization (CI-MS) allowed confident assignment of the molecular ion.

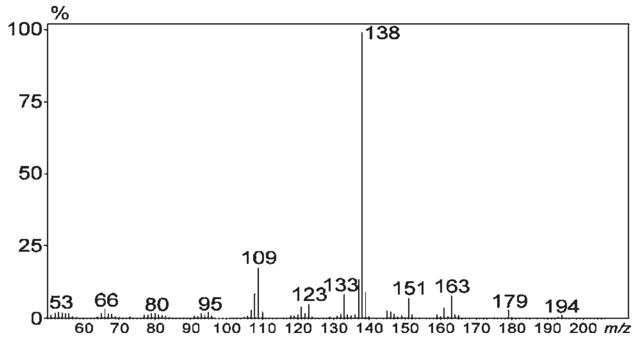


Figure 1. Mass spectrum (70 eV EI) of the electrophysiologically active compound in *Drakaea livida*.

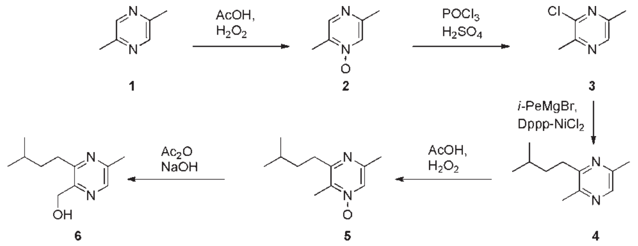
CI-HRMS confirmed the quasi-molecular ion $(M + H)^+$ of *m*/*z* 195.1504 supporting a molecular formula of $C_{11}H_{18}N_2O$.

Careful analysis of lower *m*/*z* fragments in conjunction with database searching supported a pyrazine skeleton. Consistent with this hypothesis, the even *m*/*z* ratio (*m*/*z* 138) observed for the daughter ion representing the base peak of the EI-MS spectrum (Figure 1) was indicative of a McLafferty type rearrangement commonly observed in the mass spectra of pyrazines possessing three alkyl side chains where one is able to rearrange.[6] An additional clue to the structure was provided by HRMS data for this fragment, which indicated a molecular formula of $C_7H_{10}N_2O$. A number of structures were proposed including pyrazinols, alcohols, and ethers. In an effort to broadly discriminate between these structural isomers microscale derivatization experiments were performed. Notably, when a simple esterification (DCC, DMAP, and acetic acid) was conducted on an aliquot of the female wasp head extract, analysis of the reaction mixture showed the disappearance of the EAD active compound from the sample and the appearance of a hitherto unseen compound, with a peak producing an EI-MS spectrum consistent with the formation of an acetate. These results provided evidence for the presence of a hydroxyl group within the EAD active compound.

After synthetic preparation of several compounds matching these criteria (i.e., trisubstituted pyrazines with primary or secondary hydroxyl groups including all six isomers of hydroxymethyl-(3-methylbutyl)-methylpyrazine)) and comparison of the retention times and fragmentation patterns, the natural product was tentatively identified as the new molecule 2-hydroxymethyl-3-(3-methylbutyl)-5-methylpyrazine.

The putative compound was prepared from 2,5-dimethylpyrazine (**1**) as a starting material in five steps (Scheme 1). 2,5-Dimethylpyrazine (**1**) was oxidized with hydrogen peroxide in acetic acid to return the *N*-oxide **2**[7] which was then chlorinated[8] utilizing phosphorus oxychloride in the presence of a catalytic amount of sulfuric acid to yield **3**. Appendage of the 3-methylbutyl side chain was achieved efficiently by employing the Kumada−Corriu cross-coupling[9] which provided access to 2,5-dimethyl-3-(3-methylbutyl)pyrazine (**4**). The pyrazine **4** was then subjected to a second *N*-oxidation to form the *N*-oxide **5** which was hydroxylated in a Boekelheide procedure[7] to yield the desired alcohol **6**.

Scheme 1. Synthetic Route to 2-Hydroxymethyl-3-(3-methylbutyl)-5-methylpyrazine



In addition to the observed match of the mass spectra, coinjection of the synthetic product with the natural sample showed peak enhancement on two separate GC columns. 1D NOESY NMR experiments were used to confirm the substitution pattern of the product by irradiating the aryl proton and observing enhancement of the C5 methyl proton resonance. This was supported by the reciprocal experiment. GC-EAD confirmed the electrophysiological

---

(6) Brophy, J. J.; Cavill, G. W. K. *Heterocycles* **1980**, *14*, 477.

(7) Cheng, X.-C.; Liu, X.-Y.; Xu, W.-F. *J. Chem. Res.* **2006**, *2006*, 577.

(8) Karmas, G.; Spoerri, P. E. *J. Am. Chem. Soc.* **1952**, *74*, 1580.

(9) Ohta, A.; Masano, S.; Iwakura, S.; Tamura, A.; Watahiki, H.; Tsutsui, M.; Akita, Y.; Watanabe, T. *J. Heterocycl. Chem.* **1982**, *19*, 465.

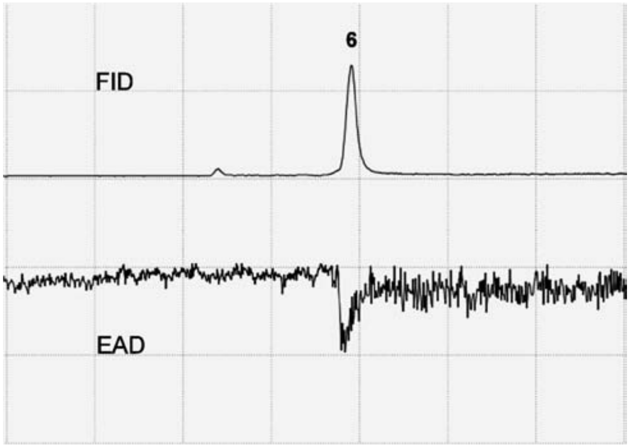


Figure 2. Antennal response in male *Zaspilothynnus nigripes* (bottom) generated by the synthetic product **6**. Upper trace: FID. Lower trace: EAD.

activity of the synthetic compound **6** (Figure 2) on the antenna of *Z. nigripes*.

Here we have discovered a novel pyrazine that is likely to be both an important component of the sex pheromone of the female wasp, and a semiochemical used by the orchid to sexually lure male pollinators. Given that there are more than 1600 species of thynnine wasps in Australia alone,[10] and several hundred sexually deceptive orchids exploiting them,[4,11] we predict that many more novel oxygenated pyrazines remain to be discovered in this system. While pyrazines are commonly utilized as trail and alarm pheromones,[6,12,13] to our knowledge the only confirmed instance of their use as a sex pheromone is by male fruit flies (Diptera).[14] If confirmed in field tests, this would represent the first case of pyrazines acting as a sex pheromone in Hymenoptera.

Acknowledgment. The study was undertaken with the support of an ARC linkage project to K.W.D., R.P., and R.A.B. (LP098338). L.J. was supported by a joint Honours scholarship from the ANU and KPBG. B.B. and R.P. conducted part of this work while visiting fellows at UWA. We thank Lindsay Byrne from the Centre for Microscopy, Characterisation and Analysis (UWA) for assistance with NMR and Myles Menz and Keith Smith for assistance with fieldwork.

Supporting Information Available. Experimental procedures and NMR of the final product. This material is available free of charge via the Internet at http://pubs.acs.org.

---

(10) Griffiths, K. E.; Trueman, J. W. H.; Brown, G. R.; Peakall, R. *Mol. Phylogenet. Evol.* **2011**, *59*, 195.

(11) Gaskett, A. C. *Biol. Rev.* **2011**, *86*, 33.

(12) Vander Meer, R. K.; Preston, C. A.; Choi, M. Y. *J. Chem. Ecol.* **2010**, *36*, 163.

(13) Tentschert, J.; Bestmann, H. J.; Hölldobler, B.; Heinze, J. *Naturwissenschaften* **2000**, *87*, 377.

(14) Chuman, T.; Landolt, P. J.; Heath, R. R.; Tumlinson, J. H. *J. Chem. Ecol.* **1987**, *13*, 1980.

The authors declare no competing financial interest.