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Discovery of Tetrasubstituted Pyrazines As Semiochemicals in a Sexually Deceptive Orchid

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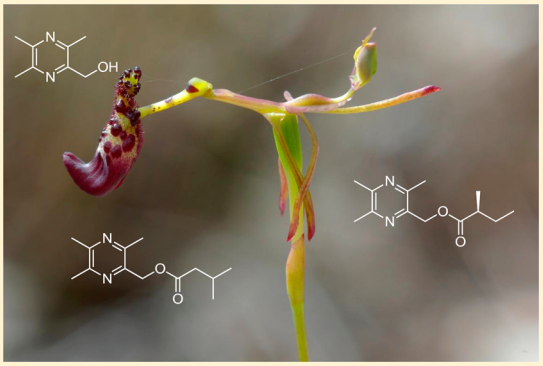
Supporting Information

ABSTRACT: Sexually deceptive orchids employ mimicry of insect sex pheromones to exploit a diverse group of pollinators. The chemical structures of five semiochemicals (**1**−**3**, **7**, **8**) produced by populations of the warty hammer orchid, *Drakaea livida*, pollinated by a thynnine wasp in the genus *Catocheilus* were elucidated. With the exception of (2,5-dimethylpyrazin-3-yl)methyl 3-methylbutanoate (**7**), all active compounds were tetrasubstituted pyrazines, including hydroxymethyl (**1**) and ester (**2** and **3**) trimethylpyrazine derivatives. Male *Catocheilus* wasps were responsive to all of these compounds in GC-EAD experiments.



Sexually deceptive orchids employ mimicry of the sex pheromones of female insects to lure males as pollinators.[1,2] The chemistry of sexual deception is best known in two genera, *Ophrys* in Europe and *Chiloglottis* in Australia.[3−8] One of the most sophisticated of all sexually deceptive systems is that of *Drakaea* (Orchidaceae), which mimics flightless thynnine wasps.[9] *Drakaea* exploit a diversity of wasp genera as pollinators,[10] providing exciting opportunities to discover a range of semiochemicals.

Here we build on our previous report of the novel 2-hydroxymethyl-3-(3-methylbutyl)-5-methylpyrazine indicated to be both a semiochemical in the floral odor of the warty hammer orchid *Drakaea livida* and a component of the sex pheromone of the pollinator *Zaspilothynnus nigripes* (Thynnidae).[11] In this study, we further investigate the role of chemicals in pollinator attraction by focusing on populations of *D. livida* that exploit a species of *Catocheilus* wasp as pollinator. Our specific objective was to identify electroantennographically active compounds produced by *D. livida* that are likely involved in pollinator attraction. We conclude the study by exploring the biological implications of our chemical findings.

RESULTS AND DISCUSSION

Solvent extracts were prepared from the labelum of *D. livida* flowers sourced from populations known by us to attract a species of *Catocheilus* as a pollinator. These extracts were used in gas chromatography−electroantennographic detection (GC-EAD) experiments against antennae from males of the *Catocheilus* pollinator. Replicated GC-EAD traces are shown in Figure 1, revealing multiple EAD-active compounds.

Guided by our previous discovery of a pyrazine-based semiochemical,[11] analyses of the mass spectra extracted from the GC-EAD active peaks indicated the presence of five compounds with pyrazine skeletons (**1**−**3**, **7**, **8**) (Figure 2). Four compounds were identified as tetrasubstituted pyrazines. The mass spectrum of the first of these compounds showed an ion of high abundance at m/z 152 and corresponded to a match in the commercial mass spectrometric library to 2-hydroxymethyl-3,5,6-trimethylpyrazine (**1**) (Figure 2a). This oxygenated pyrazine **1** was prepared in three steps from tetramethylpyrazine via the corresponding *N*-oxide that was acetylated in a Boekelheide reaction. The obtained acetate was hydrolyzed with base (Scheme 1).[12]

HRMS analysis of **2** and **3** indicated that both possessed a molecular formula of $C_{13}H_{20}N_2O_2$ (Figure 2b, c). The abundant ions occurring at m/z 152 were proposed to be rearrangement products generating the ion $[C_8H_{12}N_2O]^{\cdot+}$, consistent with 2-hydroxymethyl-3,5,6-trimethylpyrazine (**1**). A proposed McLafferty type rearrangement from the molecular ion (m/z 236) to produce an ion at m/z 152 indicated that an alkyl chain in excess of three carbons was appended to the hydroxymethylpyrazine core. Since $C_8H_{11}N_2O$ was accounted for by the presence of **1** as its core moiety, C_5H_9O remained to

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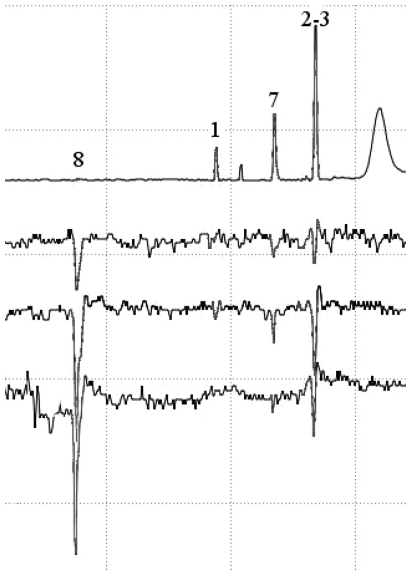
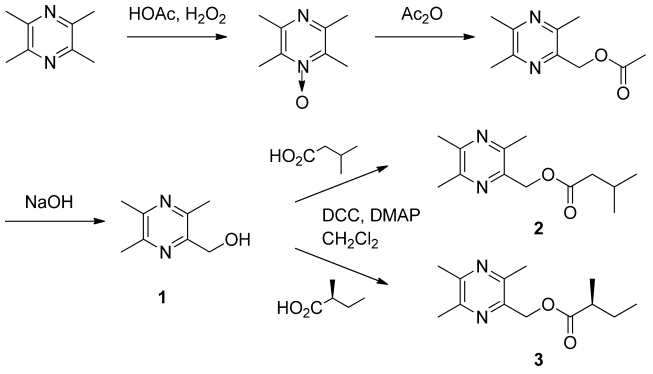


Figure 1. GC-FID chromatogram (top trace) of the solvent extract of *Drakaea livida*. GC-EAD chromatograms (lower traces) of the antennal responses of the pollinator, a species of *Catecheilus*, to the solvent extract of *D. livida*.

be assigned. Candidate compounds included esters with a five-carbon carboxylic acid moiety, and in this direction synthetically prepared **1** was esterified with a number of commercially available C_5 carboxylic acids. It was found that 3-methylbutanoic acid and 2-methylbutanoic acid resulted in esters possessing identical GC retention indices and with EI mass spectra that were identical to those in Figure 2b and c, respectively, indicating that the natural products were (3,5,6-trimethylpyrazin-2-yl)methyl 3-methylbutanoate (**2**) and (3,5,6-trimethylpyrazin-2-yl)methyl 2-methylbutanoate (**3**). Chirality was determined by enantioselective GC, showing the presence of a single enantiomer in the natural extract corresponding to one of the two isomers produced in the synthesis of *rac*-**3**. Repetition of the synthesis using (*S*)-2-methylbutanoic acid allowed unequivocal identification of the natural product as (+)-(3,5,6-trimethylpyrazin-2-yl)methyl (2*S*)-methylbutanoate (**3**) (Scheme 1).

Scheme 1. Synthesis of Compounds 1−3



Analysis of the molecular ion of **7** by HRMS indicated a molecular formula of $C_{12}H_{18}N_2O_2$ (Figure 2d). The fragmentation pattern for **7** was similar to the EIMS spectrum obtained for the 3-methylbutanoyl ester **2**, but with corresponding ions observed at 14 mass units lower compared to **2**. As a result, dimethyl-substituted pyrazine analogues **6** and **7** of ester **2** were proposed. Trimethylpyrazine was subjected to *N*-oxidation conditions to generate a mixture of the two possible mono *N*-oxides.[12] Boekelheide *β*-hydroxylation of both *N*-oxides yielded

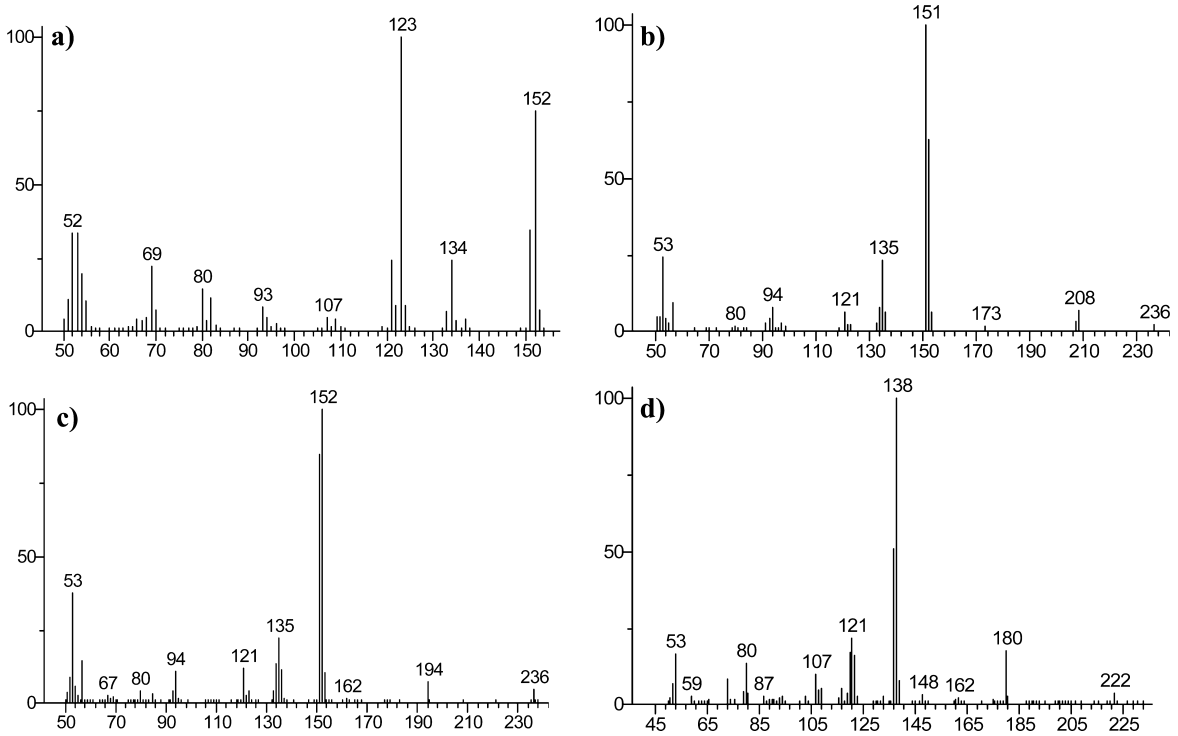


Figure 2. EI mass spectra of four pyrazine semiochemicals from the orchid *Drakaea livida* identified by GC-EAD analysis: (a) 2-hydroxymethyl-3,5,6-trimethylpyrazine (**1**), (b) (3,5,6-trimethylpyrazin-2-yl)methyl 3-methylbutanoate (**2**), (c) (3,5,6-trimethylpyrazin-2-yl)methyl (2*S*)-methylbutanoate (**3**), (d) (3,6-dimethylpyrazin-2-yl)methyl 3-methylbutanoate (**7**).

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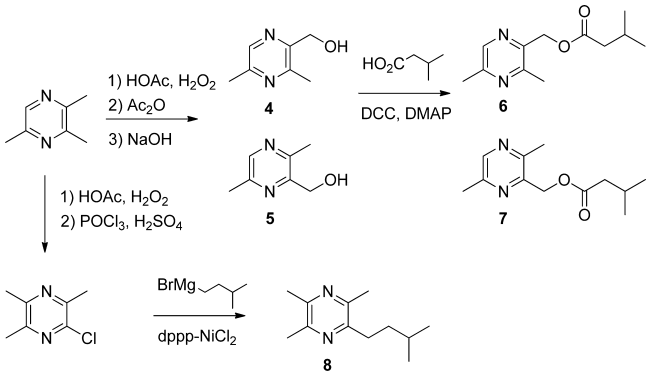
2-hydroxymethyl-3,5-dimethylpyrazine (**4**) and 3-hydroxymethyl-2,5-dimethylpyrazine (**5**).[12] Subsequent esterification with 3-methylbutanoic acid generated the dimethylpyrazine esters **6** and **7** (Scheme 2).[13]

Scheme 2. Synthesis of Compounds 4−8



The EI mass spectra for both **6** and **7** showed a strong correlation with the spectrum of the natural product. The ring substitution pattern could not be determined from the mass spectrometric data alone, but the two regioisomers could be readily separated by chromatography. The retention index of the first eluting synthetic product matched the retention index of the natural product. NMR analysis (NOESY, HSQC, and HMBC) of the intermediates **4** and **5** allowed the tentative assignment of the natural product as (3,6-dimethylpyrazin-2-yl)methyl 3-methylbutanoate (**7**).[14] Initially, 1D NOESY experiments were used to assign the resonances due to adjacent ring substituents in the ^{1}H NMR spectrum of each of the hydroxymethyldimethylpyrazine isomers **4** and **5**. Subsequently, $^2J_{CH}$ and $^3J_{CH}$ correlations in the HMBC spectrum of each isomer allowed the assignment of the individual structures. In particular, the significantly greater intensity of the two bond correlation signals from side-chain hydrogens to ring carbons compared to three-bond correlation signals allowed the tentative assignment of each structure. The intensity difference in these signals is due to the magnitude of $^2J_{CH}$ being twice that of the $^3J_{CH}$ from side-chain hydrogens to ring carbons in substituted pyrazines.[8] This assignment was confirmed by single-crystal X-ray crystallography of **4** (Figure 3 and Experimental Section).

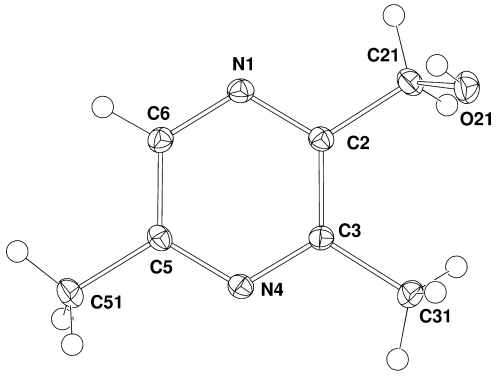


Figure 3. Structure of 2-hydroxymethyl-3,5-dimethylpyrazine (**4**) as determined by X-ray crystallography. Displacement ellipsoids have been drawn at the 50% probability level.

The mass spectrum of the last EAD-active compound matched a spectrum in the database, and this compound was confirmed to be 2-(3-methylbutyl)-3,5,6-trimethylpyrazine (**8**). The compound was prepared by ring-chlorination of 2,3,5-trimethylpyrazine-1-oxide, which afforded 2-chloro-3,5,6-trimethylpyrazine as the major product.[12,15] Kumada−Corriu cross-coupling of the chloride with freshly prepared 3-methylbutylmagnesium bromide gave the desired semiochemical **8**[16] (Scheme 2).

Thus we characterized one tri- and four tetrasubstituted pyrazines from the sexually deceptive orchid *D. livida* that are likely to be associated with mimicry of a sex pheromone produced by females of a *Catocheilus* sp. There are only two other cases where tetrasubstituted pyrazines have been reported as semiochemicals in insects: ponerine ants and melon fruit flies.[11−13]

We previously reported the discovery of 2-hydroxymethyl-3-(3-methylbutyl)-5-methylpyrazine in populations of *D. livida* that attract another pollinator, *Z. nigripes*. This same compound is produced by sexually calling females of *Z. nigripes* and is therefore likely to be a key component of the sex pheromone of that species. The finding that 2-hydroxymethyl-3,5,6-trimethylpyrazine (**1**) is likely to be involved in the sexual attraction of a species of *Catocheilus* suggests that hydroxymethylpyrazines are frequently used components of sex pheromones among thynnine wasps. The female of the *Catocheilus* wasp has not been discovered, leaving us unable to confirm if **1** is produced by the female. However, our finding of multiple EAD-active compounds indicates that the sex pheromone may involve a blend of pyrazines. Field bioassays with the synthetic compounds will ascertain their role in pollinator attraction.

The exploitation of more than one pollinator species by Australian sexually deceptive orchids is uncommon.[17−16] The present case of *D. livida* exploiting pollinators from *Zaspilothynnus* and *Catocheilus* represents the first known case of exploitation across wasp genera. Chemical analysis indicates that flowers using different species of pollinators also produce different EAD-active pyrazine derivatives, suggesting the existence of different chemotypes within *D. livida*. The presence of chemotypic differences among orchid populations may represent the early stages of speciation, where chemical-mediated reproductive isolation is evolving, but morphological divergence is yet to occur. From a conservation perspective, chemotypes may need to be treated as separate management units. This will be an important consideration for other *Drakaea* species, where five of the nine species are listed as endangered.[18]

■ EXPERIMENTAL SECTION

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded at 298 K, at 400 and 100 MHz, respectively, on a Varian 400 or at 600 and 150 MHz on a Bruker AV-600. Chemical shifts are reported in ppm. NMR experiments were run in $CDCl_3$ and are referenced to the resonance from residual $CHCl_3$ at 7.26 ppm for ^{1}H and to the central peak in the signal from $CDCl_3$ at 77.0 ppm for ^{13}C. The appearance and multiplicities of ^{1}H resonances are expressed by the following abbreviations: app (apparent), s (singlet), d (doublet), t (triplet), q (quartet), hept (heptet), m (multiplet), and combinations thereof. ^{1}H and ^{13}C NMR signals where appropriate are described by chemical shift (multiplicity, |*J*| (Hz), integration).

EIMS (70 eV) were recorded on an Agilent 5973 mass detector connected to an Agilent 6890 GC equipped with either a BP21 column [(TPA-treated polyethylene glycol), 30 m × 0.25 mm × 0.25 μm film thickness, SGE Australia] or a BPX5 column [(5% phenyl

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dimethylpolysiloxane), 30 m × 0.25 mm × 0.25 μm film thickness, SGE Australia], using He as a carrier gas. Mass/charge ratios (m/z) and relative abundances of the ions as percentages of the base peak intensity are reported. HREIMS (70 eV) were recorded on a Waters GCT Premier TOF-MS connected to an Agilent 5975 GC equipped with either a BP21 or a BPX5 column, using He as a carrier gas. All enantioselective GC was performed using a CYCLOSILB column (30 m × 0.32 mm × 0.25 μm film thickness, J & W Scientific, USA).

GC-EAD data were recorded using a 5890 Series II GC equipped with a BP21 column and a flame ionization detector (FID) using helium as carrier gas. A GC effluent splitter (split ratio 1:1) was used to split the flow to the FID and EAD. The split for EAD was passed through a Syntech effluent conditioner (Syntech, Kirchzarten, Germany) containing a heated transfer line, with the outlet placed in a purified and humidified airstream, where the electrodes holding the antenna were presented.

When necessary, solvents were dried and distilled following standard procedures. Where applicable compounds were purified by flash column chromatography on silica gel (230−400 mesh) using the solvent system specified.

Tentative identification of natural products was based on the comparison of retention index[19] and mass spectra with data from the literature and two spectrometric libraries (Wiley 275 L, 1998 and NIST-05). All tentative identifications were confirmed by peak enhancement using co-injections with synthetic samples on two columns.[20]

Plant and Insect Collection. Orchid flowers were collected in the Manjimup region of southwestern Australia from populations known to attract a species of *Catocheilus* as the pollinator and held at <10 °C until extraction. Wasp pollinators were collected by baiting with *Drakaea livida* flowers that were sourced from the same populations.

Wasp specimens of the pollinator were identified by Graham Brown of the Northern Territory Museum and Art Gallery as an undescribed species of *Catocheilus*. Voucher specimens of orchids are held at the Western Australian Herbarium (voucher number PERTH 05453232), and voucher specimens of the pollinators are held at the Western Australian Museum (voucher numbers W585, W598, W647, W659, W562, W604, W691).

Extraction and Isolation. Floral odor extraction and GC-EAD procedures followed Schiestl and Peakall,[21] with minor modifications. In brief, the labelum of the flower was excised and extracted in 100 μL of distilled DCM for 5 min in a 1 mL extraction vial. The solvent extract was then transferred to a sample vial and stored at −20 °C for future use. Live wasp specimens for GC-EAD were stored at <10 °C from the time of collection until required. For each EAD run, an excised antenna with the tip cut off was mounted on a holder consisting of two electrodes using electrode gel. The electrode was connected to a PC via a Syntech Intelligent Data Acquisition Controller (IDAC2) for simultaneous recording of the FID and EAD signals in the Syntech software package GC-EAD/2011 (freely available from http://gcead.sourceforge.net/download.html).

Synthesis. *2,3,5,6-Tetramethylpyrazine 1-Oxide.* Glacial HOAc (20 mL) was slowly added to 2,3,5,6-tetramethylpyrazine (6.86 g, 50 mmol) at 0 °C. The reaction mixture was allowed to reach room temperature, and H_2O_2 (30%, 20 mL) added. The mixture was heated to 50−60 °C prior to the further addition of H_2O_2 (30%, 20 mL), stirred for 16 h, and allowed to cool to room temperature before addition of H_2O (50 mL). The mixture was concentrated under reduced pressure to approximately 10% of its original volume, a further 50 mL of H_2O was added, and the procedure was repeated three times. A saturated aqueous solution of K_2CO_3 was added until the mixture reached pH 9, before being extracted four times with DCM. The organic phases were combined and washed with saturated brine, dried with anhydrous $MgSO_4$, and concentrated to give 6.20 g (40.0 mmol, 82%) of the title compound as a white crystalline solid with a pervasive sweet aroma. NMR data were in agreement with published data.[22]

(3,5,6-Trimethylpyrazin-2-yl)methyl Acetate. Excess Ac_2O (20 mL) was added to 2,3,5,6-tetramethylpyrazine 1-oxide (3.01 g, 19.7 mmol), and the mixture was stirred at 100 °C for 16 h. The solution was allowed to cool before being poured on ice and adjusted to pH 9 with solid K_2CO_3. The mixture was extracted three times with Et_2O. The organic phases were combined, washed with saturated brine, and dried using anhydrous $MgSO_4$ before being concentrated under reduced pressure to give the crude product (3.31 g) as a brown oil. This crude product was subjected to silica gel chromatography eluted with 60% EtOAc/petroleum ether, to give the purified product (2.36 g, 12 mmol, 61%) as a colorless oil: ^{1}H NMR (400 MHz) δ 5.14 (s, 2H), 2.48 (s, 3H), 2.46 (s, 6H), 2.07 (s, 3H); ^{13}C NMR (100 MHz) δ 170.5, 151.2, 149.0, 148.1, 144.6, 65.0, 21.6, 21.3, 20.7, 20.4; EIMS m/z (%) 194(15), 152(50), 151(100), 135(10), 134(15), 133(10), 123(10), 121(20), 93(10), 80(10), 53(25), 52(15); HREIMS found 194.1058 (calcd for $C_{10}H_{14}N_2O_2$ 194.1055).

*2-Hydroxymethyl-3,5,6-trimethylpyrazine (**1**).* (3,5,6-Trimethylpyrazin-2-yl)methyl acetate (2.4 g, 12 mmol) was hydrolyzed using 5 M NaOH (10 mL, 50 mmol). The product was extracted three times with DCM. The organic phases were combined, dried with $MgSO_4$, and concentrated to give the title compound as a cream, crystalline solid (1.5 g, 10 mmol, 81%): ^{1}H NMR (400 MHz) δ 4.68 (d, J = 4.4 Hz, 2H), 4.26 (t, J = 4.4 Hz, 1H, OH), 2.522 (s, 3H), 2.516 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (100 MHz) δ 149.5, 147.5, 147.4, 146.6, 60.8, 21.3, 21.2, 19.2; EIMS m/z (%) 152(80), 151(30), 134(30), 123(100), 121(30), 80(15), 69(20), 54(20), 53(30), 52(30); HREIMS found 152.0954 (calcd for $C_8H_{12}N_2O$ 152.0950).

(S)-2-Methylbutanoic Acid. This compound was prepared according to the procedure outlined by Tashiro and Mori.[23] (*S*)-2-Methylbutan-1-ol (2.45 mL, 23 mmol) was added to acetone (10 mL) at 0 °C. Freshly prepared Jones' reagent (4.47 g of $Na_2Cr_2O_7$, in 10 mL of 25% H_2SO_4(aq)) was added to the stirred solution and allowed to warm to room temperature. After 16 h, *i*-PrOH was added (10 mL) and the reaction mixture was concentrated under reduced pressure. H_2O and Et_2O were added until clear phases were formed, with the aqueous phase extracted a further three times with Et_2O. The organic phases were combined, washed with saturated brine, and dried ($MgSO_4$) before being concentrated under reduced pressure to give the crude product (840 mg). The crude product was purified by silica gel chromatography (eluent: 6% EtOAc/petroleum ether, TLC stain: bromocresol green) to give the title compound as a colorless oil (440 mg, 4 mmol, 19%). Spectroscopic data were in agreement with published data.[23]

*(S)-(3,5,6-Trimethylpyrazin-2-yl)methyl 2-Methylbutanoate (**3**).* 2-Hydroxymethyl-3,5,6-trimethylpyrazine (**1**) (400 mg, 2.6 mmol) was added to a stirred solution of (*S*)-2-methylbutanoic acid (0.22 mL, 2 mmol) and *N*,*N*-dimethylaminopyridine (DMAP) (5 mg, cat.) in DCM (5 mL). The mixture was cooled to 0 °C prior to the addition of *N*,*N*′-dicyclohexylcarbodiimide (DCC) (170 mg, 0.8 mmol). This temperature was maintained for 5 min before allowing the reaction mixture to warm to room temperature, after which the mixture was stirred for a further 3 h. The resulting mixture was filtered through a cotton plug before being dried with $MgSO_4$ and concentrated under reduced pressure to give the crude product as a yellow oil (302 mg). Purification by column chromatography gave the title compound as a pale yellow oil (126 mg, 0.5 mmol, 27%): $[\alpha]^{20}_D$ +8.7 (c 0.83, DCM); ^{1}H NMR (400 MHz) δ 5.17 (s, 2H), 2.50 (s, 3H), 2.48 (s, 3H), 2.47 (s, 3H), 2.42 (tq, J = 7.2, 7.2 Hz, 1H), 1.69 (ddq, J = 13.6, 7.2, 7.4 Hz, 1H), 1.46 (ddq, J = 13.6, 7.2, 7.4 Hz, 1H), 1.14 (d, J = 7.2 Hz, 3H), 0.89 (app t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz) δ 176.3, 151.1, 148.9, 148.8, 144.9, 64.9, 40.9, 26.7, 21.6, 21.3, 20.4, 16.6, 11.6; EIMS m/z (%) 236(7), 194(10), 152(100), 151(80), 135(25), 134(15), 121(10), 94(10), 53(15); HREIMS found 236.1532 ($C_{13}H_{20}N_2O_2$ calcd. 236.1525).

*(3,5,6-Trimethylpyrazin-2-yl)methyl 3-Methylbutanoate (**2**).* This compound was prepared according to the procedure described for (3,5-dimethylpyrazin-2-yl)methyl 3-methylbutanoate (**3**). DCC (250 mg, 1.2 mmol) and (3,5,6-trimethylpyrazin-2-yl)methanol (**1**) (397 mg, 2.6 mmol) were added to a solution of 3-methylbutanoic acid (0.22 mL, 2 mmol) and DMAP (5 mg, cat.) in DCM. After 3 h the reaction mixture was extracted with DCM, washed with saturated brine, and dried ($MgSO_4$) to give the crude product as a yellow oil (330 mg). The crude product was purified by silica gel chromatography (eluent: 10% EtOAc/petroleum ether) to give the

title compound as a pale yellow oil (191 mg, 0.8 mmol, 67%): ^{1}H NMR (400 MHz) δ 5.15 (s, 2H), 2.48 (s, 3H), 2.47 (s, 3H), 2.46 (s, 3H), 2.21 (d, J = 7.6 Hz, 2H), 2.09 (m, 1H), 0.92 (d, J = 8.0 Hz, 6H); ^{13}C NMR (100 MHz) δ 172.6, 151.1, 148.85, 148.82, 144.8, 64.7, 43.1, 25.6, 22.3 (2 × C), 21.6, 21.3, 20.4; EIMS m/z (%) 236(4), 208(10), 152(50), 151(100), 135(20), 121(10), 94(10), 53(20); HREIMS found 236.1528 ($C_{13}H_{20}N_2O_2$ calcd 236.1525).

2,3,6-Trimethylpyrazine 1-Oxide and 2,3,5-Trimethylpyrazine 1-Oxide. These compounds were prepared according to the method described for 2,3,5,6-tetramethylpyrazine 1-oxide using 2,3,5-trimethylpyrazine (6.17 g, 50.0 mmol), glacial HOAc (20 mL), and H_2O_2 (30%, 20 mL + 20 mL). After 16 h, water was added and the mixture was concentrated under vacuum. This process was repeated three times. A saturated aqueous solution of K_2CO_3 was added until the mixture reached pH 9, at which point a yellow precipitate formed and the solution was extracted four times with DCM. The organic phases were combined and washed with saturated brine, dried with $MgSO_4$, and concentrated to give the title compounds as a mixture in a ratio of 3:2 as a white, crystalline solid (4.61 g, 33.4 mmol, 67%). The derived spectroscopic data agreed with those reported.[24]

*2-Hydroxymethyl-3,5-dimethylpyrazine (**4**) and 3-Hydroxymethyl-2,5-dimethylpyrazine (**5**).* These compounds were prepared from 1.38 g (10 mmol) of the mixture of 2,3,6-trimethylpyrazine 1-oxide, 2,3,5-trimethylpyrazine 1-oxide, and acetic anhydride (1.1 mL, 12 mmol) followed by the hydrolysis procedure described for **1**. Upon workup, the crude products were recovered as a brown oil (1.58 g), which was purified by silica gel chromatography (eluent: 60% EtOAc/petroleum ether) to give a mixture of the title compounds (267 mg, 2.0 mmol, 20%). Partial separation of the two isomers on silica gel allowed a small amount of each alcohol to be isolated for analysis. Compound **4** crystallized as the hydrate upon standing, allowing the crystal structure to be determined. Compound **4**: ^{1}H NMR (400 MHz) δ 8.21 (s, 1H), 4.70 (s, 2H), 4.14 (s, 1H), 2.52 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (100 MHz) δ 151.2, 149.8, 148.6, 139.5, 61.2, 21.0, 19.9; EIMS m/z (%) 138(95), 137(35), 120(40), 109(100), 107(30) and 138(95), 137(35), 120(40), 109(100), 107(30); HREIMS found 138.0795 ($C_7H_{10}N_2O$ calcd 138.0793). Compound **5**: δ 8.25 (s, 1H), 4.70 (s, 2H), 4.32 (s, 1H), 2.53 (s, 3H), 2.42 (s, 3H); ^{13}C NMR δ 150.4, 149.1, 147.5, 141.6, 61.0, 20.8, 19.3; EIMS m/z (%) 138(95), 137(35), 120(40), 109(100), 107(30) and 138(95), 137(35), 120(40), 109(100), 107(30); HREIMS found 138.0797 ($C_7H_{10}N_2O$ calcd 138.0793).

*(3,5-Dimethylpyrazin-2-yl)methyl 3-Methylbutanoate (**6**) and (2,5-Dimethylpyrazin-3-yl) 3-Methylbutanoate (**7**).* These compounds were prepared from 3-methylbutanoic acid (72 mg, 0.74 mmol) and a mixture of **4** and **5** (100 mg, 0.7 mmol) obtained in the previous step, according to the procedure outlined for **2** and **3**. Upon workup, the crude products obtained (183 mg) were purified by silica gel chromatography (eluent: 20% EtOAc/petroleum ether) to return **7** (60 mg, 0.3 mmol, 40%) and **6** (40 mg, 0.2 mmol, 26%) as pale yellow oils. The two products were differentiated by confirmation of GC retention times of the corresponding products from microscale reactions of the purified fractions of **4** and **5**.

*Compound **6**:* ^{1}H NMR (600 MHz) δ 8.26 (s, 1H), 5.22 (s, 2H), 2.57 (s, 3H), 2.52 (s, 3H), 2.26 (d, J = 7.2 Hz, 2H), 2.12 (m, 1H), 0.95 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz) δ 172.7, 152.6, 151.8, 145.8, 141.2, 64.6, 43.1, 25.9, 22.4 (2 × C), 21.3, 21.1; EIMS m/z (%) 180(20), 138(100), 137(50), 121(20); HREIMS found 222.1365 ($C_{12}H_{18}N_2O$ calcd 222.1368).

*Compound **7**:* ^{1}H NMR (600 MHz) δ 8.26 (s, 1H), 5.22 (s, 2H), 2.55 (s, 3H), 2.52 (s, 3H), 2.26 (d, J = 7.2 Hz, 2H), 2.14 (m, 1H), 0.95 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz) δ 172.7, 150.5, 149.5, 147.7, 143.1, 64.8, 43.1, 25.7, 22.4 (2 × C), 21.0, 20.6; EIMS m/z (%) 222(4), 180(20), 138(100), 137(50), 121(20), 80(15), 53(15); HREIMS found 222.1362 ($C_{12}H_{18}N_2O$ calcd 222.1368).

2-Chloro-3,5,6-trimethylpyrazine. A mixture of 2,3,6-trimethylpyrazine 1-oxide and 2,3,5-trimethylpyrazine 1-oxide (2.76 g, 20 mmol) was added slowly to freshly distilled $POCl_3$ (24.8 mL) containing 1 drop of concentrated H_2SO_4 at 70 °C. The mixture was stirred for 5 h before being poured on ice and basified with 5 M NaOH to a pH of 9. The mixture was extracted three times with DCM, and the organic phases were combined and then washed with saturated brine, before being dried with $MgSO_4$ and concentrated to give 2.51 g of the crude product as a yellow oil. The crude material was purified by silica gel chromatography (eluent: 50% EtOAc/petroleum ether) to give the title compound as a pale yellow solid (1.66 g, 10 mmol, 50%). Spectroscopic data were in agreement with those previously published.[24]

*2-(3-Methylbutyl)-3,5,6-trimethylpyrazine (**8**).* 1,3-Bis(diphenylphosphino)propane]nickel(II) chloride (dppp-$NiCl_2$, 20 mg) and 2-chloro-3,5,6-trimethylpyrazine (200 mg, 1.3 mmol) were added to dry Et_2O (5 mL) to form a suspension, to which freshly prepared 3-methylbutylmagnesium bromide (4 M) in Et_2O (0.5 mL, 2.0 mmol) was added. Upon addition of the Grignard reagent, the solution turned orange, then red, before darkening to deep brownish-red. The solution was refluxed for 2 h before being poured on ice and acidified with HCl (1 M) in the presence of Et_2O. The organic layer was removed and replaced before the solution was basified to pH 9 with saturated aqueous K_2CO_3 solution. The basic aqueous phase was extracted three times with Et_2O. The combined organic phase was dried and evaporated under reduced pressure to give the crude product as a dark yellow oil (247 mg). The product was purified on a silica column eluted with 15% EtOAc/DCM to give the purified product (41 mg, 0.2 mmol, 16%): ^{1}H NMR (400 MHz) δ 2.72 (m, 2H), 2.48 (s, 3H), 2.46 (s, 3H), 2.45 (s, 3H), 1.65 (thept, J = 6.8, 6.8 Hz, 1H), 1.48−1.54 (m, 2H), 0.96 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz) δ 152.2, 148.3, 148.0, 147.5, 37.9, 32.8, 29.7, 28.3, 22.5 (2 × C), 21.4, 20.9; EIMS data were consistent with published data;[25] HREIMS found 192.1605 ($C_{12}H_{20}N_2$ calcd 192.1628).

X-ray Crystal Structure of Compound 4. Diffraction data were collected at 100(2) K on an Oxford Diffraction Xcalibur diffractometer fitted with Mo Kα radiation. Following multiscan absorption corrections and solution by direct methods, the structure was refined against F^2 with full-matrix least-squares using the program SHELXL-97.[25] The hydrogen atoms of the water molecule were located and refined with geometries restrained to ideal values. All remaining hydrogen atoms were added at calculated positions and refined by use of a riding model with isotropic displacement parameters based on those of the parent atoms. All non-hydrogen atoms were refined with anisotropic displacement parameters. Atomic parameters for **4** have been deposited with the Cambridge Crystallographic Data Centre with deposition number 880008. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

*Compound **4**:* $C_7H_{10}N_2O$, H_2O, MW = 156.19. Orthorhombic, space group *Pbca*, a = 14.8674(5) Å, b = 7.0370(2) Å, c = 15.2699(6) Å, V = 1597.57(9) Å^3, Z = 8, ρ = 1.299 Mg·m^{-3}, μ = 0.096 mm^{-1}, crystal size = 0.46 × 0.23 × 0.06 mm^3. Total no. of reflections = 17 484, no. unique reflections = 2705 (R_{int} = 0.0366), R_1 = 0.056, wR_2 = 0.125 [$I > 2\sigma(I)$], R_1 = 0.066, wR_2 = 0.130 (all data); $|\Delta\rho_{max}|$ = 0.49 e·Å^{-3}.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra (^{1}H and ^{13}C) of the semiochemicals **1**−**3**, **7**, and **8** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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