Structures of the Pyrazines from the Mandibular Gland Secretion of the Ponerine Ant *Dinoponera australis*

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Two aldehydes and eleven pyrazines have been identified in the mandibular glands of the ant *D. australis*. Three of the tetraalkylpyrazines are novel, and a further two have their structures confirmed for the first time. Microgram scale reactions and syntheses were used, together with mass spectrometry, to establish these structures.

Members of the genus *Dinoponera* are among the largest ants in the world. They are restricted to continental South America where they live in small queenless colonies.[1] Reproduction is carried out by fertile workers, known as gamergates. *Dinoponera australis* has only one gamergate per colony, with an average colony size of just 13 workers.† This has allowed us to study the chemistry, behaviour and morphology of entire nests of this species.[2] The mandibular gland secretion is dominated by tri- and tetra-substituted pyrazines. Here we report the determination of their structures.

Results and Discussion

Two colonies of *D. australis* were collected at Itirapina, São Paulo, Brazil. They were transported to Belgium where, after behavioural observations, they were dissected. One mandibular gland from each worker was sealed in a glass capillary and the complete set posted to Keele for chemical analysis by gas chromatography-mass spectrometry.

Fig. 1 shows a gas chromatogram of the contents of one mandibular gland of a *D. australis* worker. The numbering of Fig. 1, Table 1 and the Schemes are consistent.

The relative amounts of **2**, **3**, **4**, **5** and **6** *vs.* **7**, **8**, **9**, **10**, **11** and **12** varied between individuals, however in all but one case out of 25 analyses, **6** [2,5-dimethyl-3-(3-methylbutyl)pyrazine] was found to be the major component. Compounds **1a** and **1b** were present as trace components in some of the glands analysed, and absent (within the limit of detection) from others. The mass spectra of **1a** and **1b** were very similar, indicating a pair of isomers. Assuming a formula of $C_{10}H_{18}O$ for the molecular ion at *m/z* 154, two double-bond equivalents were indicated for the molecule. The loss of water to give an ion at *m/z* 136 was suggestive of an alcohol or aldehyde, but the intensity of the molecular ion made an alcohol unlikely. Loss of 43 ($C_3H_7^{\cdot}$) and 57 ($C_4H_9^{\cdot}$) occurred readily to give intense ions at *m/z* 111 and 97, respectively. The structures were confirmed as (*E*)- and (*Z*)-2-isopropyl-5-methylhex-2-enal (see Scheme 1) by comparison of GC retention times and mass spectra with those of authentic samples (Quest International).

Compounds **2**–**6** all gave spectra with a base peak at *m/z* 122, a characteristic McLafferty ion of monoalkyldimethylpyrazines where the alkyl group is at least 3 carbon atoms in length and there is no branching on the methylene group next to the ring. Comparison of retention times and mass spectra of **2**, **3** and **6** with standard samples confirmed their identification as 2,5-dimethyl-3-propyl-, 2,5-dimethyl-3-(2-methylpropyl)-, and 2,5-dimethyl-3-(3-methylbutyl)-pyrazine respectively (see Scheme 1). Compound **4** eluted just after, had the same molecular mass as **3** but possessed an ion at *m/z* 135 corresponding to M − 29 (due to loss of an ethyl radical). This was suggestive of an n-butyl group, and comparison of its mass spectrum with that of 3-butyl-2,5-dimethylpyrazine, reported in the literature,[3] produced a good match. Compound **5** eluted just before **6** and had the same molecular mass of 178, but, as with **4**, had a relatively intense M − 29 ion at *m/z* 149. Comparison of GC retention time with a sample of 2,5-dimethyl-3-pentylpyrazine excluded this compound as a possible structure. However, the

† Unpublished data from 36 colonies living in tropical dry forest in Itirapina, SP, Brazil.

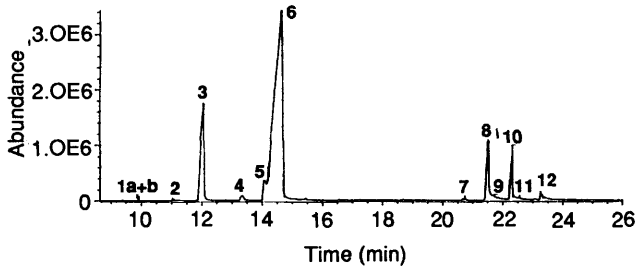
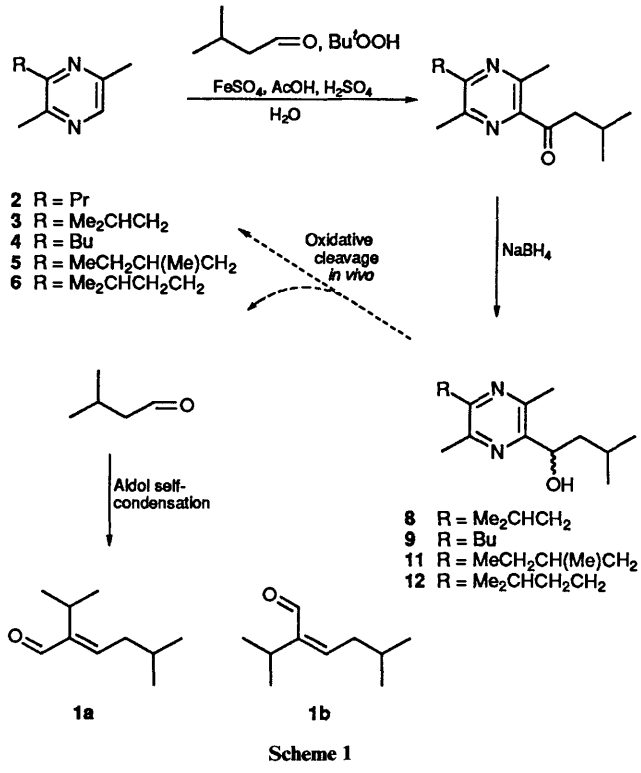


Fig. 1 Total ion chromatogram of one mandibular gland from a worker of *Dinoponera australis*, using the solvent-less technique. Numbers correspond to those in Table 1, Schemes and text.



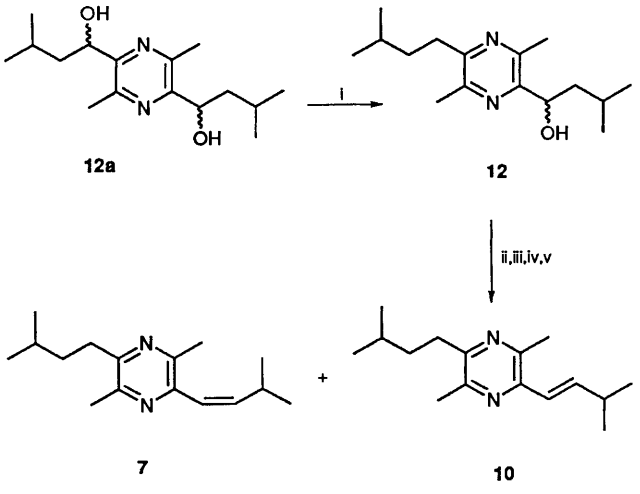
Scheme 1

Table 1

Compd.	Mass spectrum, *m/z* (intensity)
1a	154 (M^+, 13), 139 (61), 136 (15), 111 (80), 97 (88), 69 (70), 55 (41), 43 (85), 41 (100)
1b	154 (M^+, 10), 139 (44), 136 (21), 111 (87), 97 (69), 69 (67), 55 (41), 43 (81), 41 (100)
2	150 (M^+, 19), 135 (31), 122 (100), 107 (6), 81 (6), 66 (11), 56 (7), 38 (12)
3	164 (M^+, 4), 149 (8), 122 (100), 107 (4), 80 (5), 66 (2), 39 (15)
4	164 (M^+, 0.9), 149 (7), 135 (10), 122 (100), 107 (3), 80 (4), 53 (5), 39 (9)
5	178 (M^+, 0.3), 163 (6), 149 (4), 135 (2), 122 (100), 107 (2), 80 (3), 53 (5), 39 (8)
6	178 (M^+, 0.1), 163 (8), 149 (0.8), 135 (13), 122 (100), 107 (3), 80 (3), 53 (6), 39 (12)
7	246 (M^+, 85), 231 (45), 190 (100), 189 (69), 175 (91), 135 (19), 110 (7), 91 (12), 77 (11), 43 (15), 41 (15)
8	250 (M^+, 0.5), 235 (2), 233 (2), 232 (0.9), 208 (7), 194 (65), 151 (100), 121 (7), 80 (5), 53 (9), 41 (18)
8a	232 (M^+, 19), 217 (16), 190 (100), 189 (42), 175 (53), 160 (13), 135 (6), 91 (11), 77 (11), 53 (22), 41 (25)
9	250 (M^+, 0.2), 235 (4), 233 (2), 221 (0.7), 208 (17), 207 (45), 194 (19), 151 (100), 121 (6), 80 (1), 53 (3), 41 (6)
10	246 (M^+, 0.8), 231 (8), 190 (100), 189 (10), 175 (55), 135 (5), 110 (0.5), 91 (8), 77 (6), 43 (5), 41 (15)
11	264 (M^+, 0.1), 249 (3), 235 (2), 221 (2), 208 (60), 175 (5), 151 (100), 121 (5), 80 (3), 53 (5), 41 (10)
12	264 (M^+, 0.3), 249 (3), 235 (0.3), 221 (4), 208 (63), 175 (2), 151 (100), 121 (6), 80 (3), 53 (6), 41 (16)

mass spectrum of **5** was found to be identical with that of 2,5-dimethyl-3-(2-methylbutyl)pyrazine from the literature.[4]

Peaks **8**, **9**, **11** and **12** (see Fig. 1) all gave similar spectra, with a base peak at *m/z* 151 (see Table 1). The molecular ion of **12** was at *m/z* 264 and its spectrum was a close match with 3-(1-hydroxy-3-methylbutyl)-2,5-dimethyl-6-(3-methylbutyl)pyrazine reported by Fales *et al.*[5] To confirm this identification hydrogenolysis was carried out on a sample of 3,6-bis(1-hydroxy-3-methylbutyl)-2,5-dimethylpyrazine **12a** (supplied by E. Southwick, Philip Morris Research Centre) using Pd-on-C and hydrogen gas (see Scheme 2). This produced **12** in a 50%



Scheme 2 *Reagents and conditions:* i, H_2–Pd/C; ii, NaOH; iii, CS_2; iv, MeI; v, 220 °C

yield, with the synthetic and natural compounds possessing identical GC retention times and mass spectra.

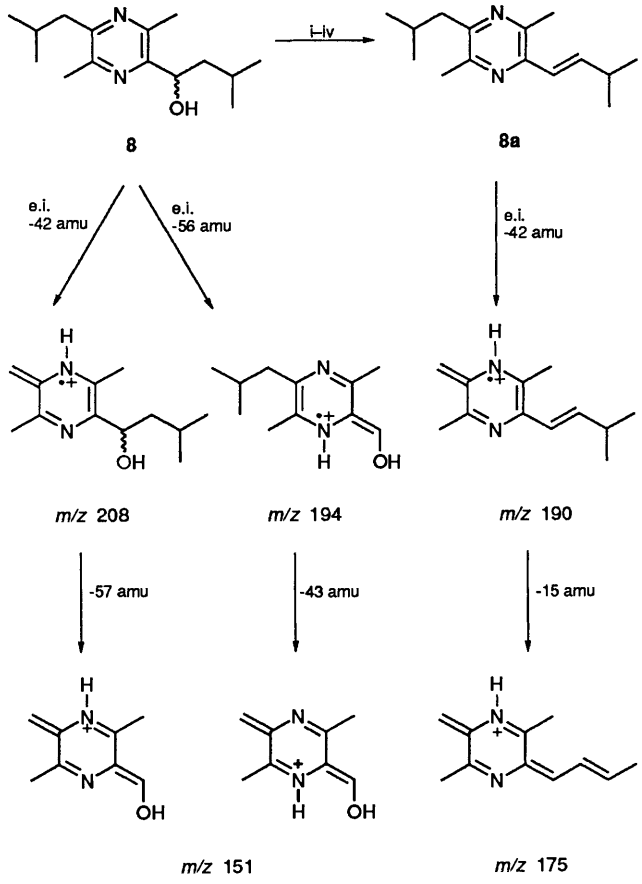
We suspected compounds **7** and **10**, to be (*Z*)- and (*E*)-2,5-dimethyl-3-(3-methylbut-1-enyl)-6-(3-methylbutyl)pyrazine, the dehydration products of **12**. Both had molecular ions at *m/z* 246, corresponding to M − H_2O of **12**, and both had very similar spectra to two unidentified compounds found by Fales *et al.* in *Mesoponera*.[5] To confirm their structures **12** was dehydrated by the Chugaev reaction. The *S*-methyl xanthate of **12** was readily prepared by reaction with sodium hydroxide, carbon disulfide and methyl iodide at room temperature. The xanthate was then pyrolysed in the GC injection port at 220 °C to yield **7** and **10** in the ratio 1:99 (see Scheme 2). The major component was known to be the (*E*)-isomer since the elimination involved extraction of the *syn*-β-hydrogen by the xanthate moiety, and where the incipient double bond has large substituents the transoid transition state is known to be favoured.[6] To confirm that **7** and **10** were actually components of the secretion, and not decomposition products of **12** brought about by heating in the injection port, a synthetic sample of **12** was injected into the GC-MS with the injection port at high temperature (300 °C). The absence of peaks corresponding to **7** and **10** in this experiment established them as real components of the secretion. In addition, we have identified the two compounds 3α and 3γ (numbering of their paper) found by Fales *et al.* in *Mesoponera*.[5] Compound 3α is our compound **7** with the *cis*-isopentenyl group and 3γ is our compound **10** with the *trans*-isopentenyl group.

Compound **8** had a molecular ion at *m/z* 250 (14 mass units less than **12**) and showed McLafferty ions at *m/z* 208 and 194, corresponding to the loss of C_3H_6 and C_4H_8 respectively (see Table 1). This suggested **8** was another tetrasubstituted hydroxyalkylpyrazine with one C-4 and one C-5 side-chain, however, it was unclear which alkyl group possessed the hydroxy function. In order to establish this, a glandular solution from colony 2 which was known to contain a relatively large amount of **8** was treated by the Chugaev reaction as described for **12**. GC–MS analysis of the products showed an additional peak eluting just before **8** with a molecular ion at *m/z* 232, corresponding to the elimination product **8a** (see Table 1). The presence of a double bond on the alkyl group which possessed the hydroxy function before elimination prevented this portion of the molecule from undergoing the McLafferty rearrangement, so only one McLafferty ion was seen at *m/z* 190 (see Scheme 3). This arose from the loss of 42 mass units (C_3H_6) and indicated the alkyl group was a C-4 chain and the hydroxyalkyl group was a C-5 chain (see Scheme 3).

No M − 29 peak (due to ethyl group loss) was observed in the mass spectrum of **8**, suggesting 3-(1-hydroxy-3-methylbutyl)-2,5-dimethyl-6-(2-methylpropyl)pyrazine as the probable structure (the prominent mass spectral fragmentation is shown in Scheme 3, demonstrating both side-chains undergo the McLafferty rearrangement followed by the loss of an alkyl radical to give the base peak at *m/z* 151).

Compounds **8** and **9**, eluting close together, had very similar mass spectra, with the same molecular mass 250, but **9** had an ion at M − 29 due to the loss of an ethyl radical. Assuming a 1-hydroxy-3-methylbutyl group, this indicated that **9** was the n-butyl isomer 3-butyl-6-(1-hydroxy-3-methylbutyl)-2,5-dimethylpyrazine (see Scheme 1).

The mass spectrum of **11** was very similar to **12**, but again had a more intense M − 29 ion, assuming a 1-hydroxy-3-methylbutyl side-chain (as before), the alkyl side-chain could have been n-pentyl or 2-methylbutyl. The latter seemed more likely since all the tetrasubstituted hydroxyalkylpyrazines would then have been analogues of the trisubstituted alkylpyrazines, differing by the 1-hydroxy-3-methylbutyl group only. To confirm this hypothesis the trisubstituted alkylpyrazines from a glandular extract (which contained only trace amounts of tetrasubstituted pyrazines) were acylated with isovaleraldehyde[7] and the keto pyrazines reduced ($NaBH_4$) to yield the corresponding 3-(1-hydroxy-3-methylbutyl) derivatives (see Scheme 1). The mass spectra and GC retention times of these compounds were in excellent agreement with **8**, **9**, **11** and **12**



Scheme 3 *Reagents and conditions:* i, NaOH; ii, CS_2; iii, MeI; iv, 220 °C; e.i. = electron impact ionization in the mass spectrometer

confirming their structures, and demonstrating the structural relationship between the tri- and tetra-substituted pyrazines present in the secretion.

There is an interesting structural relationship between the aldehydes, trisubstituted and tetrasubstituted pyrazines in the mandibular gland of *D. australis*. Oxidative cleavage of the 1-hydroxy-3-methylbutyl side-chain of the tetrasubstituted pyrazines **8**, **9**, **11** and **12** *in vivo* would yield the corresponding trisubstituted pyrazines **3**, **4**, **5** and **6** and isovaleraldehyde (see Scheme 1). Isovaleraldehyde itself was not detected in the secretion, but its aldol self-condensation products **1a** and **1b** were. This suggests these compounds are biosynthetically related, with the tetrasubstituted pyrazines as possible precursors to the trisubstituted pyrazines. We think this route is more likely than the reverse because we found a much higher proportion of the tetrasubstituted pyrazines (**8**, **9**, **11** and **12**) in the gland of a newly emerged worker than in older individuals. Over a period of time these compounds may be converted into their trisubstituted analogues.

The function of the mandibular gland secretion in *D. australis* is unknown. Where alkylpyrazines have been found in the mandibular glands of ants before, they have been identified as alarm pheromones.[3,8] However, no such response was observed on exposing *D. australis* to a glandular extract. Our observation that foragers tend to contain more secretion than those acting as nurses within the nest[2] suggests the secretion is associated with foraging, possibly as a home-range marking pheromone.

Experimental

Gas chromatography-mass spectrometry was carried out on a Hewlett Packard 5890 gas chromatograph coupled to a 5970B Mass Selective Detector. BP 1 and BPX 5 fused silica columns of dimensions 12 m × 0.2 mm × 0.25 μm and 12 m × 0.32 mm × 0.25 μm respectively were used for chromatography. The mandibular glands of colony 1 were analysed using the solventless technique of Morgan and Wadhams by heating the glass capillaries in the GC injection port at 200 °C for 2 min before crushing with the solid sampler.[9] The mandibular glands of colony 2 were individually extracted with 50 mm^3* of hexane, 1 mm^3 of which was injected into the GC-MS. The remainder of each solution was kept for chemical reactions. For the analysis of both sets of samples the oven of the gas chromatograph was held isothermally at 35 °C for 2 min before being raised to 250 °C at 7 °C min^{-1}.

(*E*)- and (*Z*)-2-Isopropyl-5-methylhex-2-enal, **1a** and **1b** respectively, were obtained from Quest International Ltd. Standard samples of 2,5-dimethyl-3-propyl-, 2,5-dimethyl-3-(2-methylpropyl)- and 2,5-dimethyl-3-(3-methylbutyl)-pyrazine, **2**, **3** and **6**, respectively, were synthesized in this laboratory.

3-(1-*Hydroxy*-3-*methylbutyl*)-2,5-*dimethyl*-6-(3-*methylbutyl*)*pyrazine* **12**.—Palladium-on-carbon (10%; 20 mg) was suspended in a solution of 3,6-bis(1-hydroxy-3-methylbutyl)-2,5-dimethylpyrazine **12a** (5 mg; supplied by E. Southwick, Philip Morris, USA) in ethyl acetate (0.2 cm^3). Hydrogen was then bubbled through the solution for 2.5 h. The suspension was filtered through a cotton wool plug in a Pasteur pipette, and the filtrate analysed by GC-MS. Approximately 50% of the dihydroxy compound was converted into the monohydroxypyrazine (Table 1, compound **12**).

(Z)- *and* (E)-2,5-*Dimethyl*-3-(3-*methylbut*-1-*enyl*)-6-(3-*methylbutyl*)*pyrazine* **7** *and* **10**.—To a solution of **12** (contaminated with **12a** from the previous reaction) in dichloromethane, powdered sodium hydroxide was added (5 mg) and the resulting suspension was shaken for 30 min in a microreactor vial. To the suspension, carbon disulfide (10 mg) was added, the vial was then shaken for a further 10 min before the addition of methyl iodide (10 mg). Pyrolysis of the resulting *S*-methyl xanthate ester occurred readily in the injection port of the GC-MS (220 °C) to yield the isomeric products **7** and **10** (see Table 1), formally the dehydration products of **12**, in the ratio 1:99. Also present in the mixture was the doubly unsaturated dehydration product of **12a**.

Dehydration of 3-(1-*Hydroxy*-3-*methylbutyl*)-2,5-*dimethyl*-6-(2-*methylpropyl*)*pyrazine* **8** *from a Mandibular Gland*.—A glandular extract containing a relatively large amount of **8** (total amount of secretion 98 μg) was selected from the analysed samples of colony 2 and treated with the xanthate-forming conditions as above. The resulting xanthate mixture was pyrolysed in the GC injection port as above to give a mixture with **8a** as the major compound with a trace of **10** (for mass spectra see Table 1). The dehydration products of **9** and **11** were not observed, as these hydroxypyrazines were only present as trace components in the gland.

Acylation of the Trisubstituted Alkylpyrazines from one Mandibular Gland.—A solution of the glandular contents (total amount of secretion 44 μg) containing only trace amounts of tetrasubstituted pyrazines was evaporated under a stream of nitrogen and the residue redissolved in a solution of glacial acetic acid (20 mm^3), water (20 mm^3), concentrated sulfuric acid (5 μg) and isovaleraldehyde (10 μg). To this was added 70% *tert*-butyl hydroperoxide (10 mg) and a solution of ferrous sulfate (20 mg) in water (40 mm^3). The reaction mixture was stirred at room temperature for 20 h after which saturated

* 1 mm^3 ≡ 1 μl.

aqueous sodium sulfite (10 mm^3) was added to it, followed by dichloromethane (100 mm^3). The mixture was neutralized by the dropwise addition of saturated sodium hydrogen carbonate and the organic layer removed. GC-MS analysis showed almost complete conversion from the trisubstituted pyrazines into their corresponding acylated derivatives.

Reduction of the Acylated Pyrazines to **8**, **9**, **11** *and* **12**.—The solution of acylated pyrazines from the previous stage was evaporated by exposure to a stream of nitrogen, the residue redissolved in ethanol (50 mm^3) and transferred to a 'Keele Microreactor'.[10] A solution of sodium borohydride (2 mol dm^{-3}; 50 mm^3) was slowly added to the mixture which was then left for 6 h. Aqueous sulfuric acid (2 mol dm^{-3}) was added to neutralize the solution, which was then extracted with dichloromethane (10 mm^3). GC-MS analysis showed quantitative conversion of the ketopyrazines into the four hydroxyalkyl tetrasubstituted pyrazines, which gave mass spectral and GC retention times identical with those of **8**, **9**, **11** and **12**.

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