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Alkylpyrazine Alarm Pheromones in Ponerine Ants

Abstract. *The mandibular gland secretions of the ponerine ants* Odontomachus hastatus, O. clarus, *and* O. brunneus *contain alkylpyrazines. These compounds, 2,5-dimethyl-3-isopentylpyrazine in* O. hastatus *and* O. clarus, *and 2,6-dimethyl-3-pentyl-, -butyl-, -propyl-, and -ethyl-pyrazines in* O. brunneus, *have previously not been found as animal natural products. These compounds function as powerful releasers of alarm behavior for* Odontomachus *workers and are probably also utilized as defensive compounds.*

The mandibular glands of ants, particularly species in the subfamilies Myrmicinae and Formicinae, have proved to be an outstanding source of natural products which function as releasers of behavior (*1*). Aliphatic carbonyl compounds are characteristically produced in these exocrine structures (*1*) and are often employed as powerful alarm pheromones. Similar behavior is displayed by many species in the primitive subfamily Ponerinae, but the mandibular gland products liberated by ponerine species are generally identified with distinctive odors which we have not encountered in other subfamilies of ants.

Disturbed worker ants of the large neotropical species *Odontomachus hastatus* (*2*), as well as other ponerine species, discharge a secretion having the characteristic odor of chocolate. Similarly, the mandibular gland secretions of *O. brunneus* and *O. clarus* (*2*), two remarkably protean species in this subfamily (*3*), also possess this aroma. The mandibular gland secretions (*4*) of these three species of *Odontomachus* contain various alkylpyrazines, none of which have been previously identified as exocrine products of animals. Significantly, these compounds possess the chocolate odors that are identified with the glandular exudates.

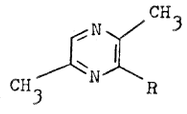
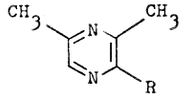
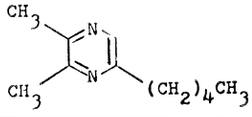
Methylene chloride or *n*-pentane extracts of *O. hastatus* and *O. clarus* heads were analyzed by combined gas chromatography and mass spectrometry (*5*). The major volatile component exhibited a base peak at *m/e* (mass/charge) 122 and additional peaks at *m/e* 177, 163, 149, 135, 80, and 42. Similar examination of extracts of *O. brunneus* heads indicated an apparent isomer of the compound from *O. hastatus*. Although both isomers showed a base peak at *m/e* 122, they could be distinguished by the relative intensities of the peaks at *m/e* 177, 163, 149, and 135. Of more significance, the isomer from *O. brunneus* exhibited a molecular ion at *m/e* 178 which was conspicuously absent in the other isomer. The second extract had, in addition, three lower homologs with molecular weights of 164, 150, and 136. The first two of these also exhibited a base peak at *m/e* 122.

A computer search of mass spectra (*6*) indicated that 2,5-dimethyl-3-isopentylpyrazine (**1**) has a fragmentation pattern very similar to that of the *O. hastatus* volatile. Although only 2,5-dimethyl-3-alkylpyrazines (**1**, **4**, and **5**) have been identified as major components of cocoa aroma (*7*), 2,6-dimethyl-3-alkylpyrazines give essentially identical mass spectra (*8*). Therefore, each of the alkylpyrazines (**1** to **12**) was synthesized from either 2,5- or 2,6-dimethylpyrazine (*9*) and the appropriate alkyl lithium (*10*). The mass spectra and retention times of **7**, **9**, **10**, and **11** were identical to those of the compounds present in *O. brunneus* extracts, and the mass spectrum and retention time of **1** were identical to that of the compound present in *O. hastatus* and *O. clarus*. *Odontomachus clarus* also contained a small amount of **5**.

Although the mass spectra of the 2,6-dimethyl-3-alkylpyrazines are essentially identical to those of the 2,5-isomers, the retention times of the 2,5-isomers are significantly shorter than the 2,6-isomers under isothermal conditions. Further confirmation of the occurrence of the 2,5-isomer in *O. hastatus* and *O. clarus* and the 2,6-isomers in *O. brunneus* was obtained by quaternization of the pyrazines with methyl iodide. The quaternized products were reduced with sodium borohydride to piperazines. The monomethylated piperazines from *O. hastatus* and *O. clarus* and from 2,5-dimethyl-3-isopentylpyrazine exhibited a base peak at *m/e* 72 and were identical. On the other hand, the monomethylated piperazines from *O. brunneus* and from 2,6-dimethyl-3-*n*-pentylpyrazine exhibited a base peak at *m/e* 128 and were identical. Similar treatment of other isomers showed that this difference is attributable to the ring substitution rather than differences in the side chain.

Pyrazine **1** constitutes 77 percent of the *O. hastatus* secretion, and pyrazines **7**, **9**, **10**, and **11** are present in *O. brunneus* extracts in 91, 7, 1.4, and 0.6 percent, respectively. An unidentified species of *Odontomachus* from Puerto Viejo, Costa Rica, contains 2,6-dimethyl-3-*n*-pentylpyrazine as the major component along with small amounts

Table 1. Mass spectra of 2,5- and 2,6-dimethyl-3-alkylpyrazines.

Compound number	R	Solution (°C) temperatures*	Mass spectra [*m/e* (relative intensity)]
		155	
1	$CH_2CH_2CH(CH_3)_2$	155	177(1) 163(9) 149(1) 135(14) 122(100) 80(3) 53(6) 42(12) 41(7)
2	$CH_2CH_2CH_2CH_2CH_3$	155	178(1) 177(1) 163(3) 149(8) 135(13) 122(100) 80(3) 53(6) 42(10) 41(7)
3	$CH_2CH_2CH_2CH_3$	140	164(1) 163(1) 149(5) 135(12) 122(100) 80(3) 53(7) 42(15) 41(7)
4	$CH_2CH_2CH_3$	115	150(6) 149(6) 135(20) 122(100) 108(3) 107(6) 80(3) 53(10) 42(22) 41(6)
5	CH_2CH_3	95	137(8) 136(85) 135(100) 121(6) 108(24) 107(13) 80(4) 56(33) 42(74) 41(15)
6	$CH_2CH(CH_3)CH_2CH_3$	155	177(1) 163(7) 149(4) 135(2) 122(100) 80(3) 53(8) 42(14) 41(9)
			
7	$CH_2CH_2CH_2CH_2CH_3$	160	178(1) 177(1) 163(2) 149(8) 135(13) 122(100) 80(4) 53(10) 42(14) 41(10)
8	$CH_2CH_2CH(CH_3)_2$	155	177(1) 163(12) 149(1) 135(19) 122(100) 80(5) 53(7) 42(15) 41(12)
9	$CH_2CH_2CH_2CH_3$	145	164(1) 163(1) 149(5) 135(10) 122(100) 80(4) 53(9) 42(15) 41(11)
10	$CH_2CH_2CH_3$	130	150(9) 149(6) 135(22) 122(100) 108(3) 107(3) 80(6) 53(10) 42(22) 41(16)
11	CH_2CH_3	110	137(8) 136(87) 135(100) 121(14) 108(56) 107(10) 80(4) 56(62) 42(47) 41(22)
12	$CH_2CH(CH_3)CH_2CH_3$	155	177(1) 163(7) 149(5) 135(2) 122(100) 80(3) 53(8) 42(15) 41(15)
13			178(2) 177(1) 163(1) 149(4) 136(1) 135(7) 122(6) 121(16) 109(9) 108(100) 80(1) 66(8) 53(4) 42(7) 41(12)

* Determined on a 10 percent SP-1000 column temperature programmed from 70°C at 8°C per minute.

of *n*-C_3, C_4, and C_6 side chain compounds.

Since the base peak of each pyrazine (except the 3-ethyl) corresponds to a McLafferty rearrangement (*11*), the loss of an alkene from the side chain is involved. This rearrangement also occurs in 2,5- and 2,6-dimethyl-3-(2-methylbutyl)pyrazine (**6** and **12**). The mass spectrum of synthetic **6** (and **12**) were sufficiently different from those of **2** and **1** (**7** and **8**) to distinguish them.

The pyrazines were presented to workers of a strong colony of *O. brunneus* by putting them on filter paper squares (1 cm^2) which were placed on the foraging platform of the ants. Excited workers approached the papers with their linear mandibles spread at an angle of nearly 90° to the long axis of the body and they frequently attacked the papers with their poised mandibles. Agitated workers exhibited characteristic alarm behavior (*3*) by snapping their mandibles together which resulted in their being projected backward if they closed on small pebbles. Sister workers were frequently attacked in the vicinity of the pyrazine source, and fatal injuries were sometimes inflicted.

The pyrazines effectively repelled workers in laboratory colonies of the fire ant *Solenopsis invicta*, and it appears that these compounds, in common with other alarm pheromones (*1*), may also function as defensive exudates.

Several pyrazines (**1**, **4**, and **5**) constitute major components in the aroma of cocoa (*7*) and some pyrazines have been identified as volatile products of coffee (*12*). On the other hand, natural products containing nitrogen are uncommon in arthropod secretions (*13*) and quinazolinones are the only compounds containing two nitrogens which had been previously identified (*14*). Furthermore, the mandibular gland products of ants which function as alarm pheromones are usually identified with C_6 to C_{11} aldehydes or ketones (*1*). However, *Paltothyreus tarsatus* workers produce alkyl sulfides in their mandibular glands (*15*), and this genus, like *Odontomachus*, is a taxon in the subfamily Ponerinae. It thus appears that some species of ponerine ants synthesize a variety of atypical insect exocrine products and the chemistry of other genera in this primitive subfamily forms the subject of continued investigations in our laboratories.

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Role of Relative Humidity in the Synergistic Effect of a Sulfur Dioxide–Aerosol Mixture on the Lung

Abstract. *Experimental evidence concerning the physicochemical and biological factors involved in the potentiation of the irritant property of sulfur dioxide in combination with an aerosol is reported. Relative humidity is an important variable for those aerosols capable of absorbing water at relative humidities below 95 percent.*

During many of the episodes of urban air pollution associated with increased rates of illness and death, elevated levels of sulfur dioxide and suspended particulates, low ambient temperature, and high relative humidity (RH) have been recorded (*1*). Still, the individual pollutants in these episodes did not approach levels required to impair the function or structure of the lungs in laboratory tests. To account for this paradox, it has been suggested that urban pollutants interact with each other giving rise to more potent products, usually in the form of aerosols. To date, only Amdur has shown convincingly an intensified or synergistic effect of gas-aerosol mixtures on pulmonary function (*2*). Her studies were done on guinea pigs; subsequent attempts to duplicate the results in man (*3*) and cats (*4*) have been unsuccessful. We now report an experiment testing the hypothesis that an elevated RH, by enhancing the interaction between SO_2 and certain aerosols (*5*), is important in determining whether gas-aerosol synergism occurs (*6*).

A modification of the method of Amdur and Mead (*7*) was used to measure pulmonary flow resistance (R_L) in guinea pigs (average weight, 334 ± 31 g). The animals were anesthetized with intramuscular injection of ketamine (100 mg/kg), to permit insertion of the pleural catheter. The measurements were made in a pressure plethysmograph, with the animals under light sedation (*8*) with sodium pentobarbital (10 mg/kg). Sulfur dioxide (1.1 ± 0.1 ppm), a polydispersed sodium chloride (NaCl) aerosol (900 to 1000 $\mu g/m^3$), and water vapor were mixed with filtered air to provide a flow of 10 liter/min, and a residence time in the reaction chamber of about 8 to 10 minutes. A polydisperse aerosol was produced by flowing filtered air through a fritted glass sparger submerged in a 5 percent NaCl solution. The generator and the size distribution of the aerosol have been studied by Pueschel *et al.* (*9*) and more recently by Covert *et al.* (*10*). The size distribution is essentially the same as that of urban air, as described by Junge (*11*) and Butcher and Charlson (*12*). The peak particle count occurs at 0.1 μm and because of settling in the reaction chamber, no particles greater than 2 μm reach the guinea pig; these observations were confirmed by electron photomicrographs.

There were six modes of exposure: (1 and 2) SO_2 at low (< 40 percent) and high (> 80 percent) RH; (3 and 4) NaCl at low and high RH; (5 and 6) SO_2 + NaCl at low and high RH. The temperature throughout the reaction chamber was 22°C. As the guinea pig breathes, the inspired gases and aerosols are rapidly brought to or near body temperature and humidity by the upper airways.

After a control period, each animal was exposed to one mode for 1 hour, allowed to recover on ambient, filtered air for 1 hour, and exposed again to another mode for a second hour. The sequence of modes was random. The latter half (30 minutes) of the recovery period served as the control for the second exposure. Thirty-six animals were studied to provide 72 exposures: 12 for each mode, half being first and half second exposures.

The average change in R_L during the period of exposure, relative to the average R_L during the control period preceding exposure, is shown (Fig. 1a) as a percentage. The control values of R_L were variable (average, 0.566 ± 0.32 cm-H_2O ml^{-1} sec^{-1}). The tendency for R_L to decrease during exposure in four of the modes is unexplained. This same tendency has been seen in control animals breathing filtered air for 5 hours. The R_L (average value for the entire exposure) increased significantly only in mode 6 when SO_2 plus NaCl were administered at high RH. We also divided the 1-hour exposure into 15-minute periods and obtained average values for these four periods (Fig. 1b). The increase in R_L, although pulsatile in character, was present throughout the exposure and tended to increase with time.

The concentration of gaseous SO_2 decreased significantly in mode 6. A decrease of approximately 10 percent was observed in mode 5 (SO_2 plus NaCl at low RH) which was probably due to adsorption of the gas on the dry aerosol.

This decrease in gas phase SO_2 was greater than expected from calculated uptake of gas on the aerosol, both in the dry and droplet form. Some wall loss of SO_2 is expected at high RH and also some loss of SO_2 on the filter in the SO_2 sampler line. This difference between the calculated and observed uptake of the gas by the droplet is not critical to the interpretation of the results. The *p*H of the droplets in mode 6, measured with a *p*H meter (Instrumentation Laboratory, Inc., model 245) was 3.2 ± 0.5. The reduction in *p*H could be due only to absorption of SO_2 by the aerosol (a droplet at high RH) (*10*). No decrease in SO_2 concentration was