Biochemical Systematics and Ecology 38 (2010) 875–879

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Identification of the alkaloidal venoms of some *Monomorium* ants of Saudi Arabia

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ARTICLE INFO

Article history:
Received 8 July 2010
Accepted 7 September 2010
Available online 24 November 2010

Keywords:
Dufour gland
Venom gland
Hydrocarbons
Alkylpyrrolidines
Alkylpyrrolines

ABSTRACT

The major volatile compounds in the poison glands of two *Monomorium* ant species from Saudi Arabia have been identified. *Monomorium niloticum* and *Monomorium najrane* both contain mixtures of alkyl- and alkenyl-pyrrolidines and -pyrrolines in their venom glands but no Dufour gland volatile compounds were detected. *Monomorium mayri* showed neither Dufour gland compounds nor venom components detectable by gas chromatography.

1. Introduction

The use of irrigation in agriculture and the introduction of ornamental plants into formerly dry areas of Saudi Arabia have created changes in the indigenous fauna, and unintentionally introduced new species, some of them undesirable (Collingwood et al., 1997).

A number of unfamiliar ant species have recently established themselves in parts of Saudi Arabia. Some of these can be undesirable and dangerous pests, such as the venomous "samsum" ant *Pachycondyla sennaarensis* (Dib et al., 1995; Al-Shahwan et al., 2006; Al-Anazi et al., 2009), and the black fire ant *Solenopsis richteri* (Khan et al., 1999), and potentially, the red fire ant *S. invicta* (Morrison et al., 2004). *Solenopsis* sp. are dangerous pests in the USA, where they were introduced accidentally. In an examination of introduced venomous ants now resident in Saudi Arabia, we have analysed the venom of three species of *Monomorium* (Hymenoptera: Formicidae: Myrmicinae) now found there. *Monomorium* is a very diverse genus, with over fifty species now recorded from the Arabian peninsula (Collingwood and Agosti, 1996). Two of these have been shown to contain poison reservoirs filled with mixtures of alkyl- and alkenyl-pyrrolidines and -pyrrolines.

As part of a wider study of ant trail pheromones, the Dufour glands of these species were also examined. No volatile substances of the types used by myrmicine ants as trail pheromones were identified in any of them.

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doi:10.1016/j.bse.2010.09.005

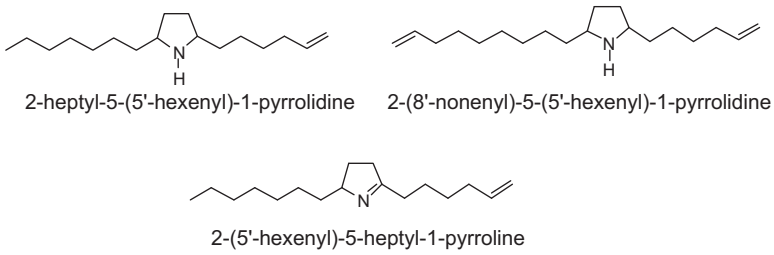

Fig. 1. Structures of examples of pyrrolidines and pyrrolines found in the venom glands of *M. niloticum* and *M. najrane*.

Table 1
Alkaloids identified in the venom glands of *M. niloticum* with their chromatographic retention times, means and standard deviation ($n = 9$).

Ret time	Compound	Mean SD
16.60	2-Heptyl-5-(5′-hexenyl)-1-pyrroline	4.11 ± 2.13
16.99	2-Heptyl-5-(5′-hexenyl)-1-pyrrolidine	37.86 ± 4.78
19.03	2-(8′-Nonenyl)-5-(5′-hexenyl)-1-pyrroline	5.97 ± 2.43
19.46	2-(8′-Nonenyl)-5-(5′-hexenyl)-1-pyrrolidine	45.69 ± 6.04
19.95	2-(8′,x-Nonadienyl)-5-(5′-hexenyl)-1-pyrrolidine	6.37 ± 1.76

2. Materials and methods

Monomorium niloticum (Emery), *Monomorium najrane* (Collingwood & Agosti) and *Monomorium mayri* (Ford) ants were collected from Ar Riyadh province, Kingdom of Saudi Arabia. Species identifications were by C. A. Collingwood, Skipton, England, and specimens deposited in his collection.

The poison apparatuses were dissected from the worker ants of each species in Riyadh, and separated into venom glands with reservoir, and Dufour glands. Each gland was separately placed in a soft glass capillary and sealed in a small flame, according to the method of Morgan (1990). The sealed capillaries were transported to Keele, where they were analysed by gas chromatography-mass spectrometry, using an Agilent 6890N Gas Chromatograph coupled to a 5973N Mass Selective detector (a quadrupole mass spectrometer using 70 eV electron impact ionization). The system was controlled by a Hewlett Packard computer with MSD ChemStation. The chromatography was carried out on a non-polar column (Hewlett Packard HP-5, 30 m × 0.25 mm with a 0.25 μm film thickness of methylsilicone) using helium as carrier gas at a constant flow rate of 1 ml min^{-1}. The samples in the sealed glass capillaries were introduced into the gas chromatograph using the solid sampling device described by Morgan (1990). The injector temperature was held at 250 °C. The samples were heated in the injector for 2 min before crushing the sealed glass capillary and beginning the chromatography. The column was initially at 50 °C, held at that temperature for 5 min, the temperature programme was started, increasing at 8 °C min^{-1} to 300 °C and holding at this temperature for 10 min.

Identification was made initially by comparison of retention times with a set of standard alkanes, which covered the range found, and mass spectra using the NIST 2003 computer library of spectra. The mass spectra of the pyrrolines and pyrrolidines from venom glands could be identified from their characteristic fragmentation patterns, and in most cases were confirmed by comparison with mass spectra recorded in the NIST Library 2003. The mass spectra of some of the compounds were also in our collection of mass spectra at Keele, having been identified in other species. Except for the terminal double bonds in the pyrrolidines, which could be easily identified, the position of double bonds were not determined.

Table 2
Alkaloids identified in the venom glands of *M. najrane* with their chromatographic retention times, means and standard deviation ($n = 8$).

Ret Time	Compound	Mean SD
14.32	2-Pentyl-5-(5′-hexenyl)-1-pyrrolidine	0.06 ± 0.13
14.41	2-Butyl-5-heptyl-1-pyrrolidine	1.16 ± 1.32
16.58	2-(5′-Hexenyl)-5-heptyl-1-pyrroline	11.11 ± 4.25
17.05	2-Heptyl-5-(5′-hexenyl)-1-pyrrolidine	82.88 ± 6.21
19.10	2-(5′-Hexenyl)-5-(8′-nonenyl)-1-pyrroline	1.28 ± 1.53
19.48	2-(5′-Hexenyl)-5-(8′-nonenyl)-1-pyrrolidine	3.51 ± 2.76

Table 3
Summary of analyses made on poison gland of three ant species.

Species	Dufour gland	Venom gland
M. niloticum	nf[1]	Alkaloids (Table 5)
M. najrane	nf[1]	Alkaloids (Table 6)
M. mayri	nf[1]	nf[1]

[1]nf, no compounds found.

3. Results

A mixture of pyrroline and pyrrolidine alkaloids was found in the venom glands of *M. niloticum* and *M. najrane*. The major alkaloids found are shown in Fig. 1. The secretion of *M. niloticum* was chiefly 2-(8'-nonenyl)-5-(5′-hexenyl)-1-pyrrolidine and 2-heptyl-5-(5′-hexenyl)-1-pyrrolidone (Table 1). The venom of *M. najrane* contained more than 80% 2-heptyl-5-(5′-hexenyl)-1-pyrrolidine (Table 2). No volatile material was identified in either the Dufour gland or venom gland of *M. mayri*.

The overall results of examining the poison apparatus of the three species of ant are summarized in Table 3. We found no detectable volatile compounds in the Dufour glands of the three *Monomorium* species.

4. Discussion

The alkaloids found in the two species are very similar, or the same as, those found in *Solenopsis* thief ants (subgenus *Diplorhoptrum*), and other species of *Monomorium* (Numata and Ibuka, 1987). For example, 2-heptyl-5-(5′-hexenyl)-1-pyrrolidine has been found in the venom of *Monomorium subopacum* (Jones et al., 1982a), and 2-(8′-nonenyl)-5-(5′-hexenyl)-1-pyrrolidine has been found in seven species of *Monomorium* (summarised in Numata and Ibuka, 1987), while 2-(5′-hexenyl)-5-(8′-nonenyl)-1-pyrroline has been idenitified in *Monomorium ebeninum* and *M.* sp. near *metoecus* (Jones et al., 1982b). The results for *M. mayri* need confirmation. We have occasionally found empty venom reservoirs in individual workers of the other species. The possibility of all ten workers sampled having empty glands seems remote, but we would wish to repeat the analysis, when another colony can be found.

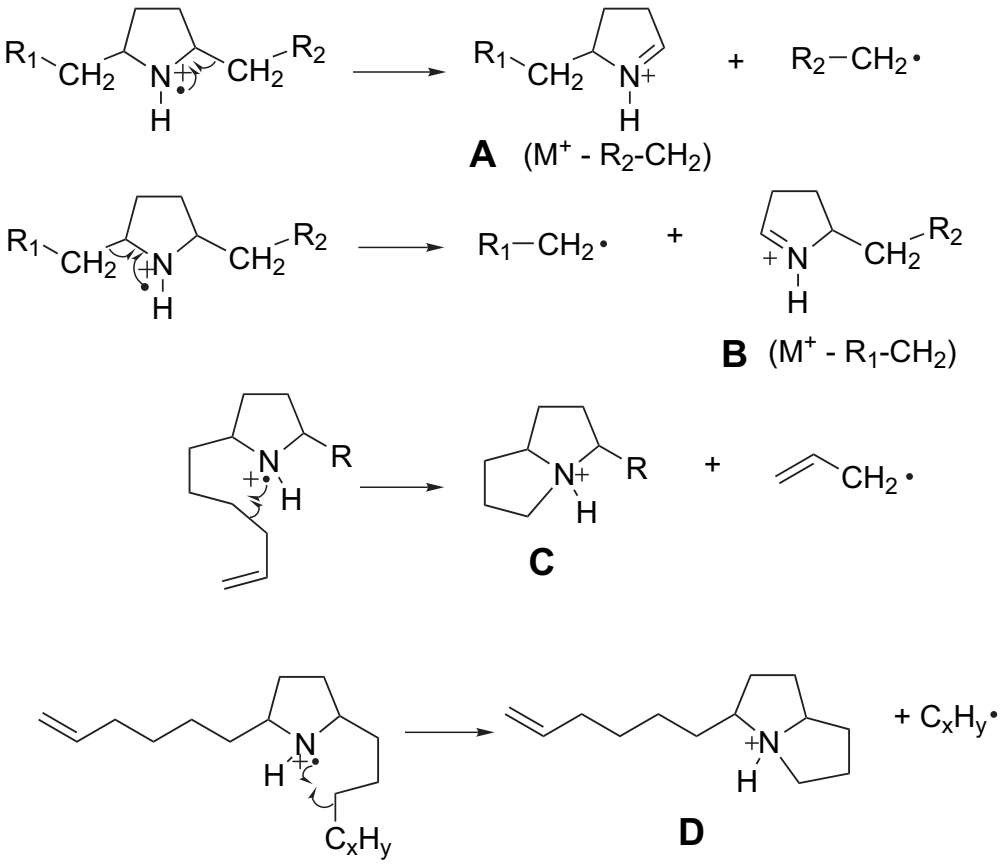

Fig. 2. Cleavage patterns of 2,5-dialkylpyrrolidines to give major ions, and information on the position of double bonds in alkenyl-pyrrolidines.

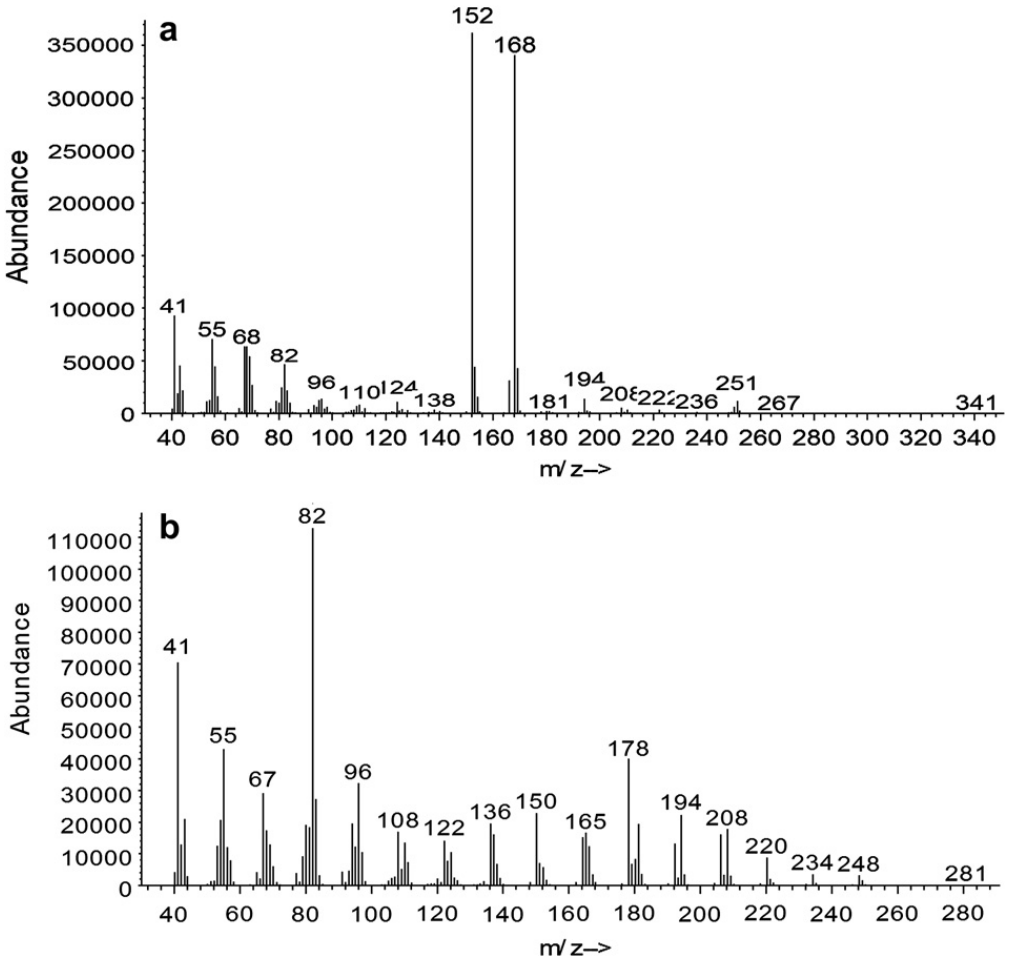

Fig. 3. Examples of pyrrolidine and pyrroline mass spectra. (a) The mass spectrum of 2-heptyl-5-(5′-hexenyl)-1-pyrrolidine from *M. niloticum*. (b) The mass spectrum of 2-(5′-hexenyl)-5-heptyl-1-pyrroline from *M. Najrane*.

M. niloticum and *M. najrane* can be readily distinguished by rapid gas chromatographic analysis, showing their different patterns of pyrrolines and pyrrolidines, most of which have already been identified in other species. The identification of these alkaloids was made entirely from their mass spectra. 2,5-Dialkylpyrrolidines and –alkenylpyrroldines characteristically cleave to lose one side chain and give two prominent ions (Fig. 2a and b). From the losses from the molecular ion and the ion masses, the masses of the two side chains are immediately known. This is illustrated for 2-heptyl-5-(5′-hexenyl)-1-pyrrolidine in Fig. 3a. If the side chains contain no other functional group, only low mass patterns are seen for the alkyl ions (*m/z* 43, 57, 71, etc.). Alkenyl side chains give ion patterns for *m/z* 41, 55, 69 etc. Reaction of the molecular ion with the side chain gives cyclic products (Fig. 2c and d), with loss of an allyl radical, which show that the double bond of the alkenyl group must be at the 5th or greater carbon atom along the chain. The mass spectra of dialkylpyrrolines give more complex patterns as seen in the spectrum of 2-(5′-hexenyl)-5-heptyl-1-pyrroline (Fig. 3b), but similar cleavages are seen and the same reasoning applies.

None of the three species of *Monomorium* contained detectable volatile substances in their Dufour glands, which could be candidate trail pheromones, though in field observation they display typical trail-following behaviour. Mashaly (2010) has shown that the trail pheromone is located in the poison glands of both *M. niloticum* and *M. najrane*, and no species specificity was found between them. The trail pheromone of *M. mayri* was located in the Dufour gland (Mashaly, 2010). It is highly possible the alkaloids of the two species also serve as the trail pheromone, and the intermediate volatility of the pheromone supports such an identification.

Acknowledgements

This work was supported by Research Centre, College of Sciences, University of King Saud, project no. Zoo/2009/44. The authors thank C. A. Collingwood for the species identifications, and Mr Gamal Tolba for his help in ant collection from the field.

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