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Journal of Chromatography A, 873 (2000) 73–77

JOURNAL OF
CHROMATOGRAPHY A

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Short communication

Use of solid-phase microextraction in the investigation of chemical communication in social wasps

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Abstract

Solid-phase microextraction has been used to investigate chemical communication in several social wasp species. Using the technique to analyse exocrine gland secretions, we demonstrate that the results are comparable with those obtained with the more classical methods that use solvents, eliminating, in many cases, the shortcomings of these methods in insect pheromone analysis. As a result of its simplicity this technique is very suitable for research on the chemical ecology of social wasps, and on insect communication in general. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Pheromones

1. Introduction

Chemical communication is widely used by insects, relying on substances produced in numerous exocrine glands, and often released by the correspondingly well defined structures of the cuticle. Chemical signals are of extreme importance in social insects (ants, bees, wasps, termites and other minor groups) where they are used for controlling social organization, in the co-ordination of mass activities, and for the defence of the colonies [1,2]. The efficient extraction of both these cuticular compounds and other glandular contents is thus clearly necessary if chemical communication in social insects is to be fully investigated.

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Classical methods of analysing glandular contents involve extraction by solvents. Extracts are then injected into a GC or GC–MS system for analysis. This method, though, can be time consuming and, more importantly, results may vary depending on factors such as time of extraction and type of solvent used [3]. This procedure may also result in the extraction of unwanted contaminants that originate from sources other than the gland in question. Alternate methods, such as direct mounting of the gland in the GC injector, have also been proposed [4], but this method is cumbersome to use and involves a modification of the GC inlet. These problems have recently been solved for the extraction of insect cuticular hydrocarbons by the use of solvent-less solid-phase microextraction (SPME), where extraction of the sample onto a fused-silica fibre coated with a stationary phase occurs [5]. In

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PII: S0021-9673(99)01176-0

this study we extend the use of SPME to the analysis of glandular contents in two social wasp species. Firstly, we show that headspace extraction of glandular contents is comparable with that of solvent analysis, and secondly we describe a novel use of SPME in extracting glandular contents.

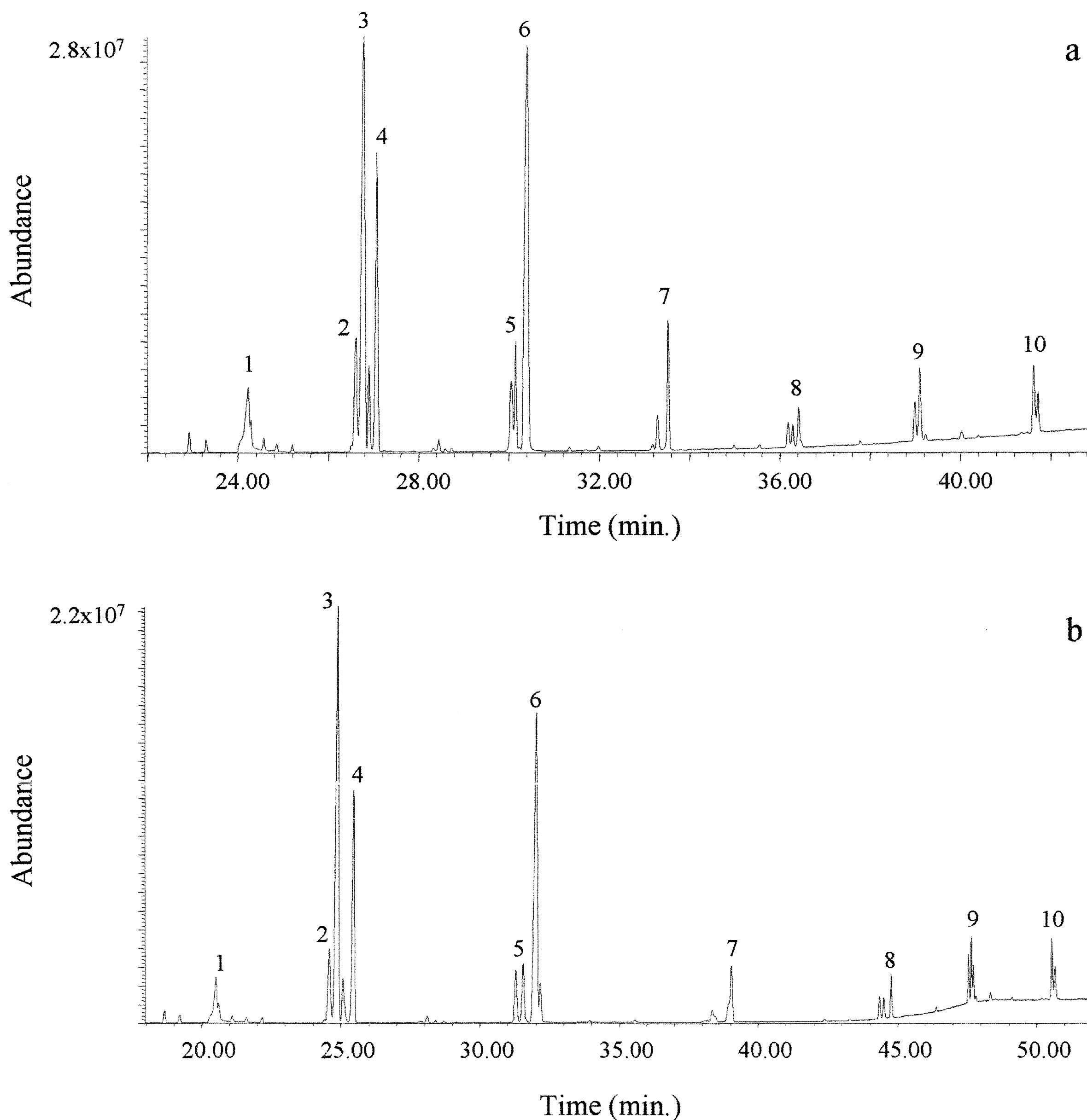

Fig. 1. Comparison of solvent (a) and SPME headspace (b) extraction of the Dufour's gland of the stenogastrine wasp *Parischnogaster* sp. (sim. *jacobsoni*). For solvent analyses, the gland was extracted in 20 μl heptane. SPME extraction was performed at 170°C for 10 min. Numbered peaks correspond to those in Table 1. Only the more abundant compounds are labelled here.

2. Experimental

Extraction by SPME was performed with a 7-μm polydimethylsiloxane (PDMS) fibre (Supelco, Bellefonte, PA, USA). Whole Dufour's glands were dissected from two or three wasps and placed individually in vials and then extraction performed in the headspace at 170°C for 10 min. Solvent extractions were made by placing glands in 20 μl heptane. A 2-μl volume was then injected into the GC–MS for analysis.

Sample analyses were performed using a Hewlett-Packard 5890A series II gas chromatograph coupled to an HP 5971A mass-selective detector (using 70 eV electron ionization) and a Varian (Walnut Creek, CA, USA) Saturn 2000 GC–MS ion-trap system in positive chemical ionization mode with acetonitrile. This allowed for the positive identification of alcohols and aldehydes, as well as the determination of the double bond position in unsaturated alcohols and hydrocarbons [6]. The ion trap temperature was set at 140°C with an ionization time of 2 ms and reaction time at 40 ms. In both cases a fused-silica capillary column coated with 5% diphenyl – 95% PDMS [Restek (Bellefonte, PA, USA) Rtx-5MS, 30 m×0.25 mm, thickness 0.5 μm] was used. The injector port and transfer line were set at 280°C. The temperature program was as follows: from 150 to 200°C at a rate of 5°C/min., from 200 to 260°C at 2°C/min., and from 260 to 310°C at 10°C/min. The carrier gas was helium at 1 ml/min. Analyses were performed in splitless mode. For SPME analyses a narrow-bore Supelco 0.75 mm I.D. GC inlet liner was used.

3. Results

Headspace extraction of the Dufour's gland of *Parischnogaster* sp. compared favourably with that of solvent extraction (Fig. 1). The chromatograms for each of the analyses have very similar patterns, showing that the two methods of extraction produce similar results. The Dufour's gland in this wasp was found to contain a series of unsaturated and saturated hydrocarbons, as well as several alcohols (saturated and unsaturated) (Fig. 1, Table 1).

Table 1
Composition of the Dufour's gland of the stenogastrine wasp *Parischnogaster* sp. (sim. *jacobsoni*); numbers correspond to those presented in Fig. 1, only the more abundant compounds are listed

Peak no.	Compound
1	Octadecanoic acid
2	9-Tricosene
3	7-Tricosene
4	*n*-Tricosane
5	7-Pentacosene
6	*n*-Pentacosane+1-docosanol
7	*n*-Heptacosane+1-tetracosanol
8	Hexacos-23-en-ol
9	*n*-Hentriacontane+1-octacasonol
10	Triacontenol

We have furthered the use of SPME in a novel way. The size of the fibre allows for the direct piercing of individual glands either dissected out of the wasp body, or directly from live wasps. This procedure produces comparable results to both solvent and headspace analysis, and may, in some cases, be more suitable for extracting hydrocarbons of long chain lengths (Fig. 2a and b). Using this method we have confirmed the presence of hydrocarbons in the Dufour's glands of the wasp *Polistes dominulus* [7] (Table 2).

4. Discussion

SPME has been shown to be a very suitable method for the extraction of cuticular hydrocarbons in insects [5,8]. We show here that it is also an appropriate method for extraction of glandular contents. SPME has allowed us to describe the chemical composition of the Dufour's gland in two species of social wasp in two separate ways, headspace extraction and direct extraction. The ability to extract directly from glands provides more accurate analyses, avoiding possible contamination due to degeneration of living tissues which is often found in solvent, solid or SPME headspace techniques. As has been shown for the extraction of cuticular hydrocarbons from live insects [9], this method may also prove to be extremely useful in extracting glandular

secretions in live insects. Direct sampling with an SPME fibre at glandular openings is non-destructive, and will allow for the tracking of changes in exocrine secretions in live (and the same) individuals over time.

In conclusion, solvent-less extraction by SPME solves, in many cases, the shortcomings of classical methods in insect pheromone analysis, and proves to be a very useful and practical technique in research on the chemical ecology of social wasps.

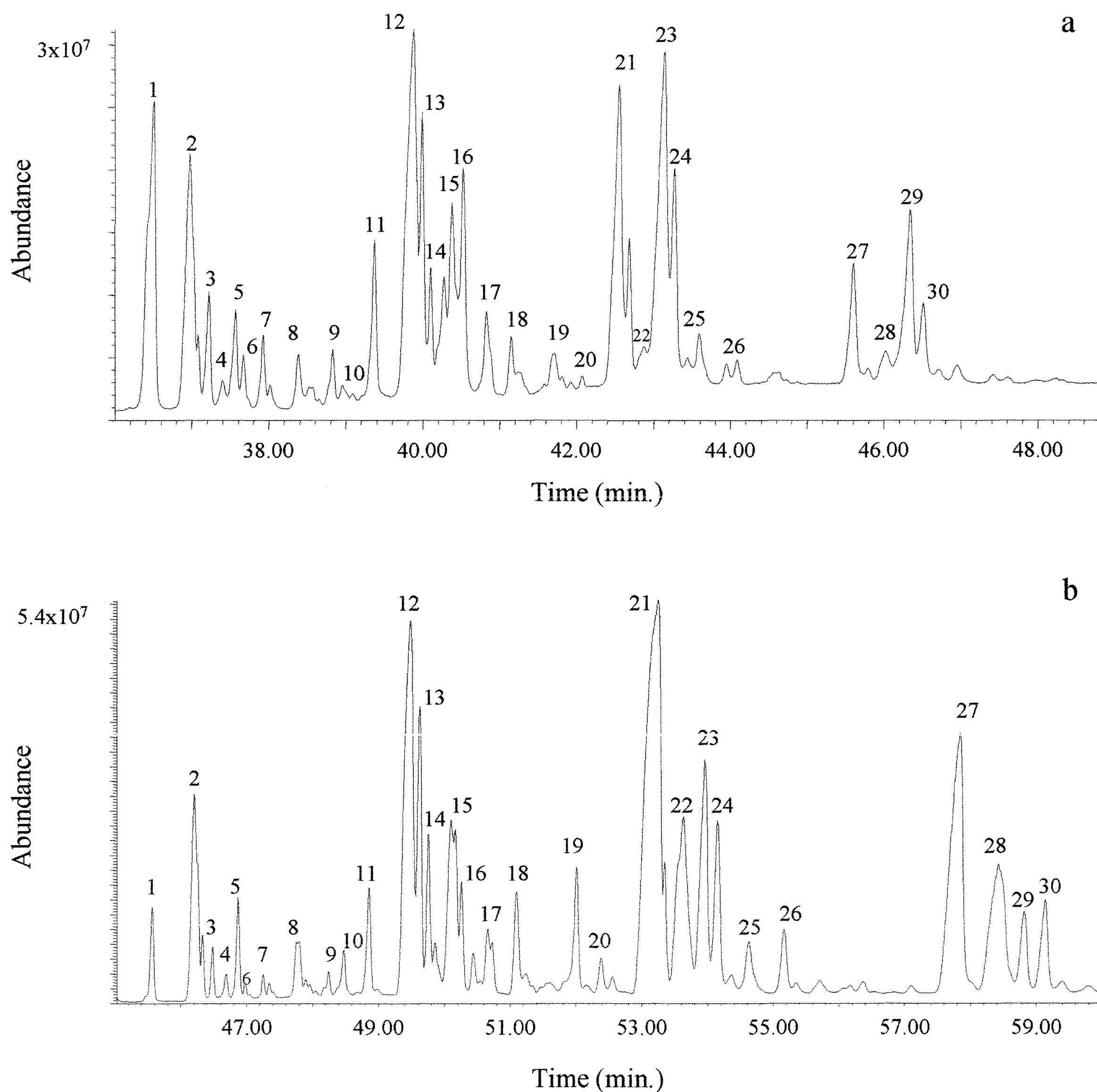

Fig. 2. Comparison of solvent extraction (a) and direct piercing with an SPME fibre (b) of the Dufour's gland of the wasp *Polistes dominulus*. Numbered peaks correspond to those in Table 2. Only the more abundant compounds are labelled here.

Table 2
Dufour's gland contents of the social wasp *Polistes dominulus*; numbers correspond to peaks in Fig. 2, only the more abundant compounds are listed here

Peak no.	Compound
1	*n*-Heptacosane
2	13-, 11-Methylheptacosane
3	5-Methylheptacosane
4	9,13-Dimethylheptacosane
5	3-Methylheptacosane
6	5,*y*-Dimethylheptacosane
7	*n*-Octacosane
8	14,- 13,- 12-Methyloctacosane
9	2-Methyloctacosane
10	*n*-Nonacosene
11	*n*-Nonacosane
12	15,- 13,- 11-Methylnonacosane
13	7-Methylnonacosane
14	5-Methylnonacosane
15	7,15-Dimethylnonacosane+3-methylnonacosane
16	5,*y*-Dimethylnonacosane
17	*n*-Triacontane+*x*,*y*-dimethylnonacosane
18	14,- 13,- 12-Methyltriacontane
19	*n*-Hentriacontene
20	*n*-Hentriacontane
21	15,- 13,- 11-Methylhentriacontane
22	13,17-+11,*y*-Dimethylhentriacontane
23	7,15-Dimethylhentriacontane
24	5,*y*-Dimethylhentriacontane
25	*n*-Docotriacontane
26	14,- 13,- 12-Methyldocotriacontane
27	17,- 15,- 13-Methyltritriacontane
28	13,17-Dimethyltritriacontane
29	7,*y*-Dimethyltritriacontane
30	5,*y*-Dimethyltritriacontane

Acknowledgements

We are grateful to Francesca R. Dani, Paolo Zanetti, Muriel Soleilhavoup and Giancarlo Bucelli for their kind assistance. Funding was obtained through the research network Social Evolution of the Universities of Aarhus, Firenze, Keele, Sheffield, Uppsala, Würzburg and the ETH Zürich, financed by the European Commission via the Training and Mobility of Researchers (TMR) programme.

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