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# GAS CHROMATOGRAPHIC DETECTION AND STRUCTURE ANALYSIS OF VOLATILE PHEROMONES IN INSECTS

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## SUMMARY

Methods are described for obtaining gas chromatographic separations of the substances in biological samples of glands or tissues without the use of solvents. The whole tissue may be introduced into the gas chromatograph or the liquid withdrawn with a fine capillary.

The technique, first applied to packed columns has been adapted to capillary columns, without using a splitter, to take advantage of the small sample size, sensitivity and increased resolution, without appreciable peak broadening compared to conventional split injection. Highly volatile substances and gases can be conveniently analysed as well as substances of higher molecular mass.

On-column reactions can be carried out to learn something of the nature of the functional groups encountered.

The methods have been applied to studies of the mandibular glands, poison glands and Dufour glands of a number of species of ants.

## INTRODUCTION

Currently there is great interest in the chemistry of substances produced by insects (pheromones, hormones or defensive secretions), because of the long-term possibility of using knowledge of these substances to achieve more effective and selective control of insect pests. Because of the very small amounts of these substances, ($10^{-6}$ to $10^{-12}$ g per individual) and their suitable volatility, they are largely studied by the sensitive techniques of gas chromatography (GC) with or without linked mass spectrometry (MS).

We describe here techniques we have found effective in handling small samples of insect material for GC without manipulation with solvent or distillation and which we have used to identify and quantify substances in the molecular-weight range from 32 to 280. These techniques are not limited to our applications, nor to the molecular-weight range given, but can be applied in any area, either where small samples of biological tissue, or small volumes of liquid are to be examined and contamination with solvent is unnecessary, or to be avoided.

The techniques were first applied to packed columns, however we can now demonstrate that they are applicable to capillary columns as well.

## EXPERIMENTAL

GC was performed with a Pye Series 104 gas chromatograph with flame ionisation detectors which had been modified to take capillary columns. Make-up carrier gas was added at the detector to produce a flow-rate of 50 ml $min^{-1}$. The detector jet was drilled out so the end of the capillary column was well above the point at which the make-up gas was introduced. Both helium and nitrogen have been used as carrier gases. For capillary work the gas was preheated in the column oven.

Tissue samples and fine capillaries (for packed columns) were sealed in 1.5 cm lengths of 1 mm O.D. soda glass melting point tubing. For removing liquid by a capillary tube, the required gland was dissected under water, blotted dry and then pierced with a 50–70 $\mu$m diameter hard glass capillary.

For capillary column work, the open glass capillary containing liquid was introduced on-column with the device in Fig. 3. An "Interflon" PTFE three-way stop-cock, 2 cm diameter (Springham, Harlow, England) modified with a glass liner was used.

## RESULTS AND DISCUSSION

In the simplest case, substances for GC can be extracted from the body, gland or tissues of an organism with a solvent, but solvents introduce impurities, obscure volatile components and increase sample volume to be applied to the chromatograph, a particularly important point with capillary columns. To avoid the use of solvents, various techniques have been used, such as trapping in absorbants (charcoal or Tenax GC), and then desorbing on to the column by flash heating (see for example, Bergström[1]). Another alternative is to introduce the biological tissue directly onto the gas chromatograph.

We have described earlier the device we use to introduce insect tissue samples directly onto the gas chromatograph[2]. The tissue is sealed in a glass ampoule made from a short length of melting point tubing and placed in the solid sampling device in the heated zone at the top of a GC column. The design of the Pye series of gas chromatographs is particularly suitable, since they have a small area flash heater about 7 cm from the top of the column. The solid sample can be placed in a vertical position with the sealed glass tube accurately placed in the heated zone above the column packing. The effect of heating the tissue sample above 100° and the sudden change of pressure on crushing the sealed tube effectively disrupts the cells, the water vapour and volatile organic components are swept on to the column in a compact plug.

In some cases, the glands to be studied can be easily dissected out, as we have found with the poison gland complex of some ants, and by piercing the sac of the gland with a fine glass capillary, under the microscope, the contents of the gland can be removed completely into the glass tube by capillarity. That fine tube is then sealed in a larger glass capillary and heated and crushed in the top of the column with the solid sampler. We have described this technique[3] and used it to analyse the Dufour gland contents of some ant species.

Some preliminary chemical information about the substances present can be

gained before resorting to mass spectrometry, or confirmatory to MS by carrying out on-column reactions (see, for example, Beroza[4], or Tumlinson and Heath[5]). For example, bromine in carbon tetrachloride, sealed up in the capillary was used to remove unsaturated hydrocarbons[6] and sodium borohydride can be added to the tube so that aldehydes and ketones are reduced to alcohols before chromatography. This is illustrated in Fig. 1 for the mixture of ketones and alcohols present in the mandibular gland of the ant *Myrmica rubra*. Capillary column techniques can be used with surprisingly little modification of these procedures. With a pre-column made of glass capillary of 1 mm I.D. with a plug of glass wool 8 cm below the septum, it is possible to unscrew the septum cap, insert a fine glass capillary containing glandular liquid, replace the cap and obtain capillary GC results comparable to those obtained by injection. An example of a capillary column separation of the substances from an ant Dufour gland, obtained in this way is given in Fig. 2. A small modification of replacing the septum cap wtih a three-way PTFE tap with a glass liner (Fig. 3) produces a more convenient device. The column pressure is maintained, the carrier gas is interrupted to place the fine capillary in the bore of the tap which is then turned so the carrier gas stream is opened and the glass capillary drops into the heated zone of the pre-column.

Bowman and Karmen[7], twenty years ago first described a device for crushing

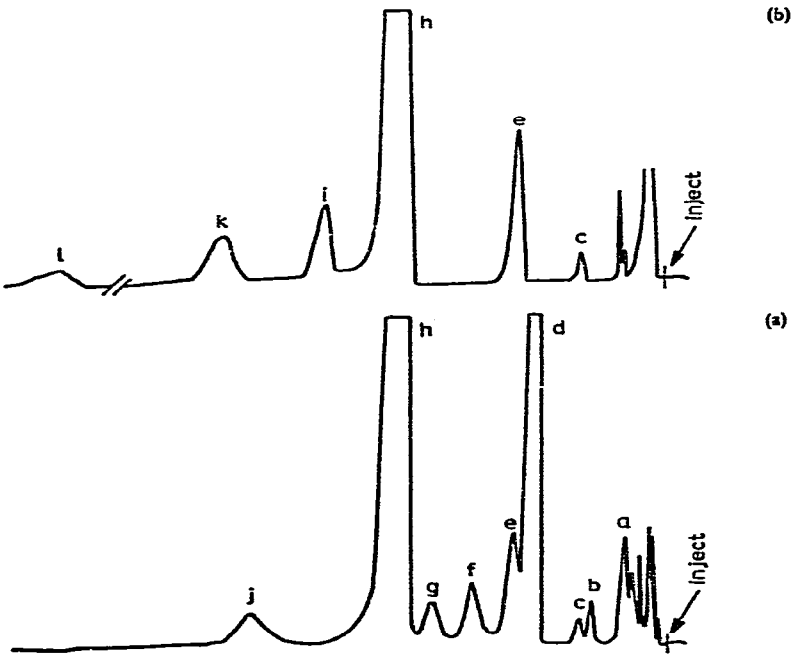


Fig. 1. (a) GC trace of analysis of mandibular gland components of the ant *Myrmica rubra*, on a 5 ft. × 4 mm I.D. glass column of 10% PEG 20M using 3 ant heads sealed in a glass tube and crushed with the sampling device described[1]. Column temperature, 90°, nitrogen carrier gas at 45 ml $min^{-1}$. (b) The same, except sample sealed with finely powdered sodium borohydride to reduce ketones. Peaks: a = 3-hexanone; b = 3-heptanone; c = 3-hexanol; d = 3-octanone; e = 3-heptanol; f = 6-methyl-3-octanone; g = 3-nonanone; h = 3-octanol; i = 6-methyl-3-octanol; j = 3-decanone; k = 3-nonanol; l = 3-decanol.

small samples in the injection area of a gas chromatograph, but the idea has not attracted wide attention. However, chromatographers' needs vary widely, and in our work we have found the solid sampling method very useful. Some insect glands are difficult to disect, or the volatile chemicals are not concentrated in a resevoir as in the case of the poison or Dufour gland. For example, the mandibular gland in the head of Myrmicine ants is difficult to reach. Moreover, an accurate measure of the amount of volatile substances in each individual insect is required. It is more convenient to place the whole head (volume approx. 0.5 $\mu$l) in a glass tube and crush it "on-column" with a solid sampler. We are presently working on modifying the sampler described earlier[2], so that it can be used with the low flow-rates and low dead volume requirements of capillary columns.

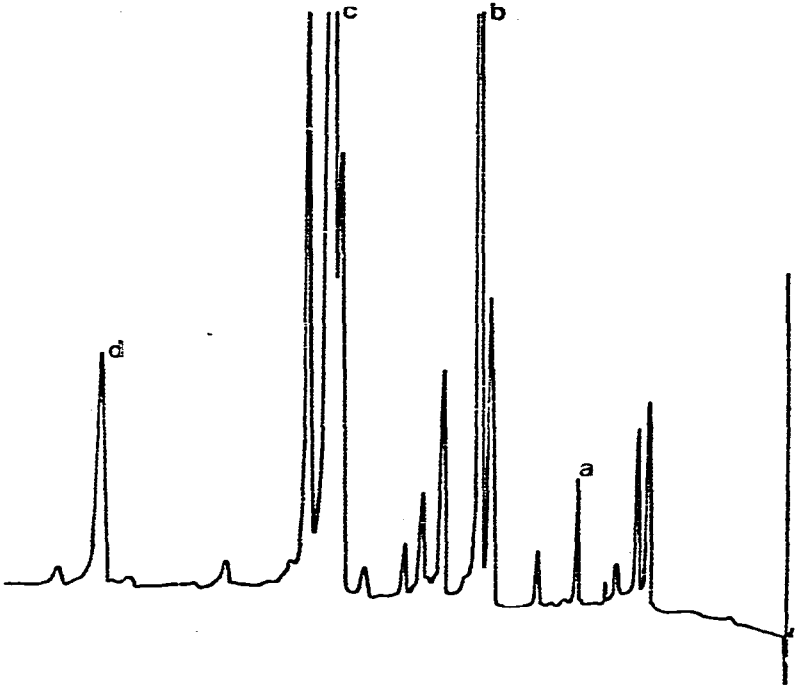


Fig. 2. Separation of the components of the Dufour gland of the ant *Myrmica rubra* on a 30 m wall-coated open tubular capillary column coated with OV-101 silicone. Carrier gas, nitrogen at linear flow-rate of 15 cm $sec^{-1}$, temperature programmed from 165° to 198° at 2° $min^{-1}$. The gland was pierced with a fine capillary, which was dropped into the pre-column while the carrier flow was interrupted. Peaks: a = tridecane; b = pentadecane; c = 8-heptadecene; d = 9-nonadecene. The number of theoretical plates for this separation was 92,500 or 3090 plates $m^{-1}$.

As well as pre-column reactions, we have used extraction loops to gain some knowledge of the functional groups present in insect substances, preliminary to combined GC–MS, and confirmatory to it. Specifically we have used boric acid to remove alcohols[8] and *o*-dianisidine to remove aldehydes[9], when investigating the volatile components of the poison gland and Dufour gland secretion of ants[3]. Other examples are given by Beroza[4]. The same technique can be applied to capillary

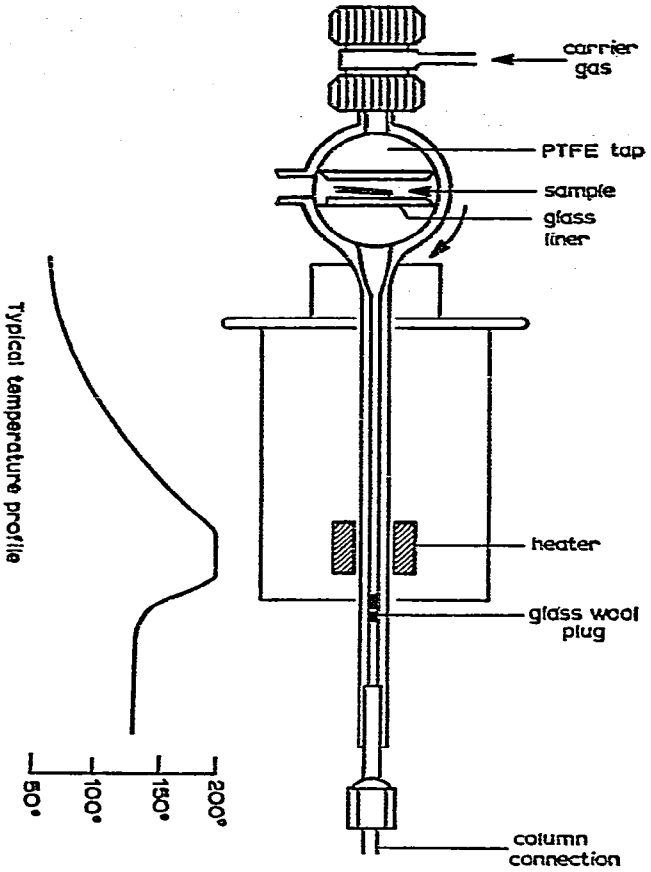


Fig. 3. Device for inserting fine glass capillary into heated zone of pre-column for capillary column GC. The connection to the column is through a piece of glass-lined metal tubing sealed to the glass pre-column. A typical temperature profile down the pre-column is shown.

columns by attaching a packed post-column loop as used for the packed columns provided make-up gas is introduced at the loop and dead space is avoided.

With the increasing use of direct linking of capillary columns to a mass spectrometer with fast pumping and omitting a separator, the usefulness of this sampling technique is enhanced further. A complete tissue sample can be introduced to the mass spectrometer, without the need for an injection splitter and without contamination by organic solvent, but diluted only by the water present in the tissue itself. Therefore the maximum amount of sample possible reaches the mass spectrometer.

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