

Note

Isolation and characterization of trace amounts of volatile compounds affecting insect chemosensory behaviour by combined pre-concentration on Tenax GC and gas chromatography

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In the course of an investigation of *Trichothecium roseum* (RV 12851 strain) for its attractancy against *Tyrophagus putrescentiae* (cheese mite), the volatile compounds emitted at a very low concentration by the fungi were analysed by a gas chromatographic (GC) method adapted to trace analysis. They were removed from the collected microorganisms with a flow of nitrogen at room temperature and pre-concentrated following the method of Bertsch *et al.*¹ in a vaporizer tube packed with a porous polymer. Thermal desorption of the volatile substances and their direct injection on to the head of the GC columns were achieved with an external flash vaporizer inlet with very low dead volumes.

EXPERIMENTAL

Apparatus and materials

A Packard-Becker gas chromatograph (Model 421) was equipped with a dual flame-ionization detector and a dual digital mass flow controller (Model 794). Glass columns packed with 5% Carbowax 20M on 60-80-mesh Gas-Chrom Z, 2 m × 3.17 mm I.D., and glass capillary columns coated with FFAP, 100 m × 0.5 mm I.D., were used. Connections of glass capillaries (graphite ferrules) were achieved according to Schomburg *et al.*² The flash vaporization inlet and the vaporizer tubes (length 95 mm; I.D. 2.5 mm) were obtained from Hamilton (Reno, Nev., U.S.A.) (Model 86801); a chromatographic-type needle (I.D. 0.15 mm) was soldered to the outlet fitting of this device.

Tenax GC (60-80 mesh) was purchased from Applied Sciences Labs. (State College, Pa., U.S.A.) and conditioned with a flow of nitrogen (flow-rate 10 ml/min) at 275° for 5 h.

Diethyl ether and dichloromethane were purified by adsorptive filtration through a column packed with basic alumina (activity I).

Anhydrous sodium sulphate was heated for 48 h at 150° and stored in glass containers.

Spherical joints or Teflon tubing were used for all connections.

Procedure

Fungi (10 g), stored at -18°, were crushed at 4° with 1 part of anhydrous sodium sulphate; the mixture was quickly transferred into a tube (150 mm overall length, diameter 20 mm) equipped with a trap head. After 12 h at 4°, a vaporizer tube packed with Tenax GC and plugged with silanized glass-wool at both ends was connected to the trap head with a short Teflon connection. High-purity nitrogen at a flow-rate of *ca.* 5 ml/min was then passed at room temperature through the treated fungi, carrying the volatiles on to the polymer.

After 72 h, the tube was disconnected from the flask and inserted in the flash vaporization inlet pre-heated at 230° and conditioned at this temperature under a flow of nitrogen. The glass inlet cap, the septum and the septum retainer of the inlet were immediately assembled and, after 90 sec, the desorbed volatiles were injected for 60 sec by means of the inlet terminal needle and of an external flow of nitrogen set at a pressure (3.0 bar) slightly higher than the column carrier gas pressure (2.8 bar). The flow-rate of the resulting effluent through the needle was adjusted to *ca.* 2 ml/min by preliminary tests. To avoid any back-flushing of the inlet, the flow of carrier gas was stopped during injection when capillary columns were used. This precaution was not taken with the packed columns, because interruption of the flow of carrier gas, even for a short period, leads to the appearance of artefacts on the chromatograms.

For bioassays, the volatiles desorbed from Tenax GC were trapped by dipping the inlet needle in a small volume of dichloromethane cooled to 0°.

For preparative studies, pre-concentration of the volatiles emitted from the fungi (150 g) was conducted under the same conditions but using a capillary tube (length 300 mm, I.D. 2.5 mm); after the pre-concentration step, the polymer was removed from this tube, homogenized and further used in small aliquots for several direct injections.

RESULTS AND DISCUSSION

Tenax GC was chosen as the porous polymer because of its high trapping performance. Pre-concentration on this polymer, under the experimental conditions, leads to chromatographic patterns that are very similar to those obtained from the headspace sampling performed with a gas syringe; the volatiles collected and desorbed under conditions used are thus qualitatively and quantitatively representative of the original composition of the odour emitted by the fungi.

The comparison of chromatographic tests was supported by bioassays following the method of Yoshizawa *et al.*³; volatile material recovered from Tenax GC shows a greater attractancy against *Tyrophagus putrescentiae* (95%) compared with that isolated by steam distillation (76%), direct solvent extraction (78%) or trapping in cooled solvents (80%).

Cryogenic trapping procedures are not suitable for the isolation of trace amounts of volatiles present in our biological material in amounts sufficient for micro-analysis; in the course of pre-concentration, water vapour and ice condensed in the

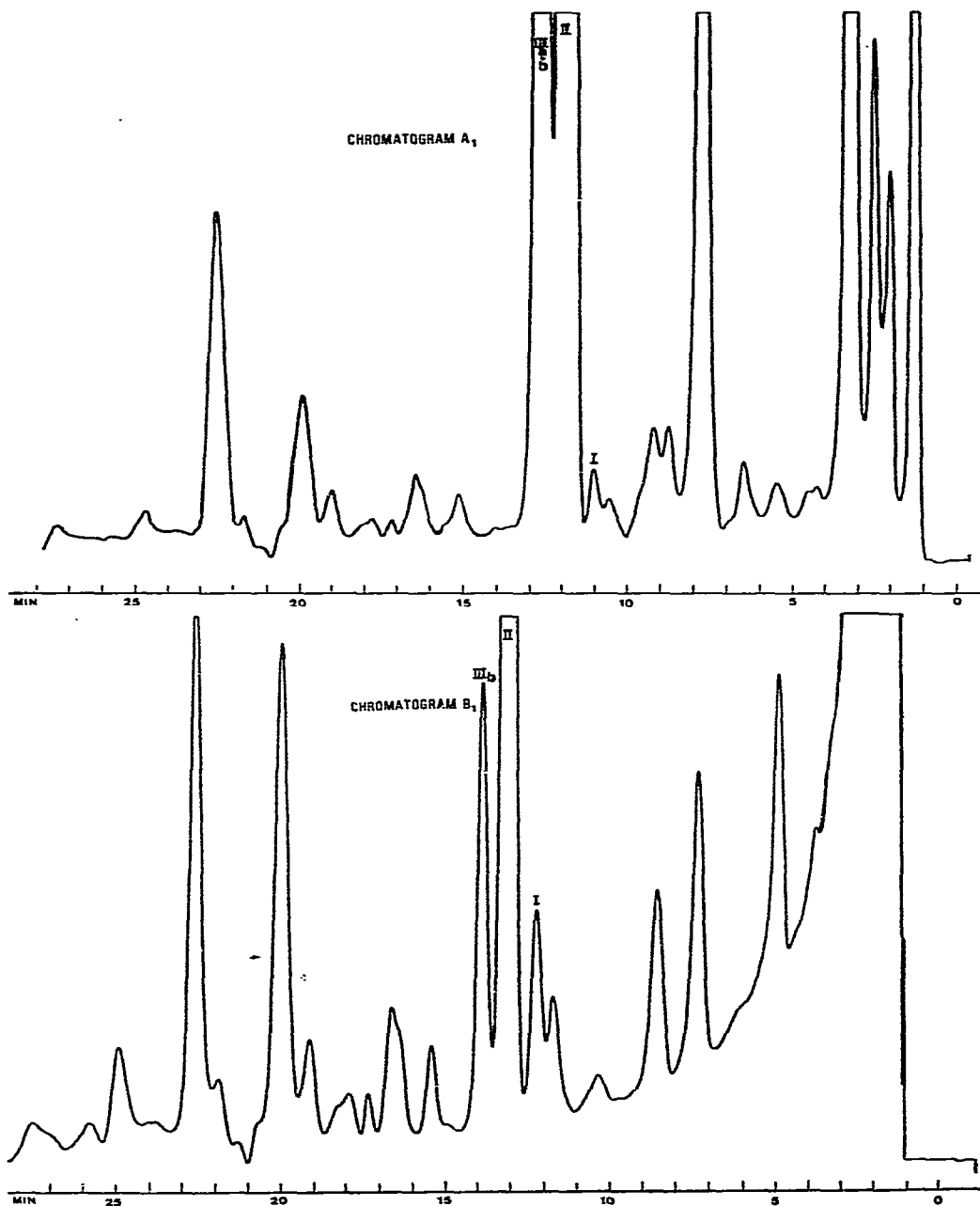


Fig. 1. Gas-liquid chromatograms of volatile extracts isolated from *Trichothecium roseum* by methods A and B on packed glass columns (5% Carbowax 20M on 60-80-mesh Gas-Chrom Z; 2 m \times 3.17 mm I.D.; nitrogen flow-rate, 30 ml/min; oven temperature, 60-160° at 5°/min then isothermal; injector and detector temperature, 220°). Peaks: I = octa-1,5-dien-3-one; II = octa-1-en-3-ol; III_a = *cis*-octa-1,5-dien-3-ol; III_b = *trans*-octa-1,5-dien-3-ol.

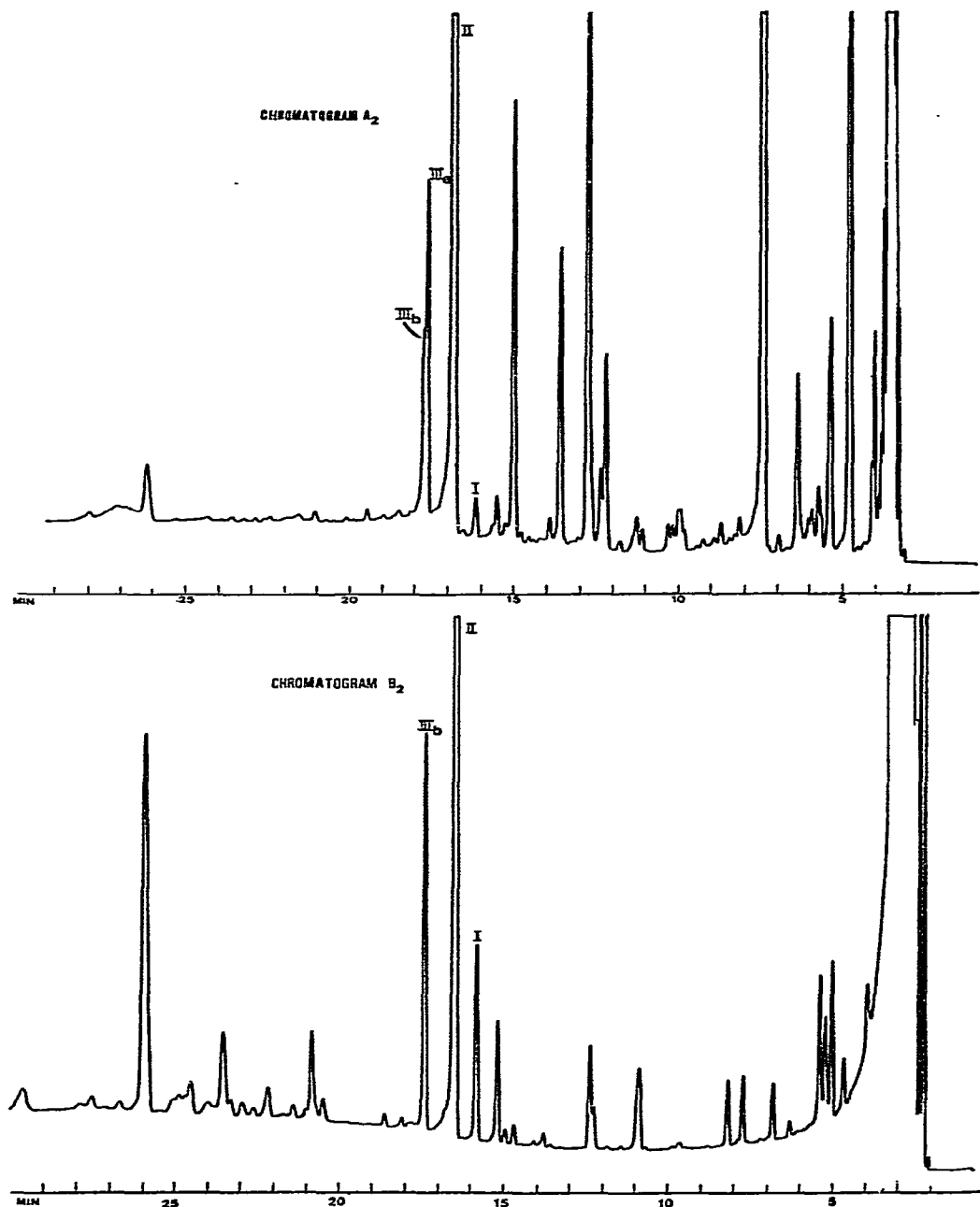


Fig. 2. Gas-liquid chromatograms of volatile extracts isolated from *Trichothecium roseum* by methods A and B on glass capillary columns (FFAP; 100 m \times 0.5 mm I.D.; helium flow-rate, 8 ml/min; pressure, 1.5 bar; oven temperature, 65–170° at 5°/min then isothermal; injector and detector temperature, 220°). Peaks as in Fig. 1.

trap hinder the gas flow and complicate the further isolation steps. On the other hand, with methods that involve liquid sample injection, the presence of highly volatile compounds is obscured by the solvent peak and a general decrease in detection limit occurs. Attempts to remove solvents by distillation result in significant losses of volatile constituents. Furthermore, steam distillation causes qualitative changes in the chromatogram owing to isomerization, oxidation and hydrolysis.

The advantages of our procedure, in addition to simplicity, are typically illustrated (Figs. 1 and 2) by the corresponding chromatograms obtained with the volatile mixtures isolated (A) following the proposed method and (B) by steam distillation of the biological material, extraction of the distillate with diethyl ether and concentration of the organic phase under a stream of nitrogen.

The peaks located in the first 5 min of chromatograms A₁ and A₂ are lost or not visible in chromatograms B₁ and B₂. Quantitative changes and degradation reactions have evidently occurred with method B and the constituents before peak III in chromatograms A₁ and A₂ are present in greater concentration when compared with chromatograms B₁ and B₂.

The sensitivity of method A is therefore considerably higher because one injection allows the chromatographic study of the equivalent of 10 g or more of fungi. Moreover, *cis*- and *trans*-isomers of octa-1,5-dien-3-ol (peaks III_a and III_b) are separated, as shown in chromatogram A₂, whereas the *cis*-isomer (peak III_a) is transformed into the *trans*-isomer (peak III_b) by method B (chromatogram B₂). On the other hand, oxidation of octa-1,5-dien-3-ol into octa-1,5-dien-3-one (peak I) is increased when method B is applied. Such conversions are very important because octa-1,5-dien-3-ol was found during our study to be the most potent attractant of the volatile compounds detected by GC in *Trichothecium roseum*.

The attractive constituents (mainly octa-1,5-dien-3-ol) are completely recovered, as confirmed by bioassays performed with a conventional trap (cryogenic procedure) connected in series after the pre-concentration trap filled with the polymer.

No significant change in the chromatogram was observed when the volatiles adsorbed on Tenax GC were stored for several weeks at 4° in a hermetically closed tube.

Finally, our method has been applied successfully to combined gas chromatography-mass spectrometry.

REFERENCES

- 1 W. Bertsch, A. Zlatkis, H. M. Liebich and H. J. Schneider, *J. Chromatogr.*, 99 (1974) 673.
- 2 G. Schomburg, H. Husmann and F. Weeke, *J. Chromatogr.*, 99 (1974) 63.
- 3 T. Yoshizawa, I. Yamamoto and R. Yamamoto, *Mem. Tokyo Univ. Agr.*, 15 (1972) 1.