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First results in trace identification of allelochemicals and pheromones by combining gas chromatography–mass spectrometry and direct deposition gas chromatography–Fourier transform infrared spectrometry

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Abstract

The advantages of a combining GC–MS and direct deposition GC–Fourier transform IR spectrometry at the same level of sensitivity are demonstrated using an apolar capillary column. This technique was applied to allelochemicals and pheromones present in minute concentrations. The identification of *Allium* volatiles and of the leek-moth male pheromones is described.

1. Introduction

In complex mixtures of volatiles, identification by gas chromatographic (GC) means alone via retention times requires a knowledge of the compounds to be identified. In many instances of extracts of environmental and food samples, the identity of the major components (priority pollutants, for instance) is known but some extracts contain unknown minor compounds at low levels. Nevertheless, identification of these minor components can be of considerable interest.

In the ecological chemistry of insects, this problem is much more widespread because semiochemicals (allelochemicals and pheromones) are often present in very low concentrations biological matrices containing numerous abundant and unknown substances. Further, these substances have a wide range of chemical diversity.

To succeed in these complex identifications, structural elucidation can be achieved through the use of coupled techniques such as GC–MS, the most commonly applied technique, MS is the method of choice owing to its high sensitivity and selectivity. However, despite its strengths, MS is not sufficient alone for univocal identification. For example, structural isomers often are not differentiated by MS and a complementary technique such as GC–Fourier transform (FT) IR spectrometry the most generally useful alternative, is needed.

Until a few years ago, it was not possible to combine the two coupled techniques GC–MS

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and GC–IR at the same level of performance owing to the relatively low sensitivity of the conventional light-pipe based interfaces. With this gas cell system, as for GC–MS interfaces, the spectrum is measured in real time as each component eluates, so that each measurement can be performed without trapping the eluates. Identified spectra can usually be obtained only from amounts in the 1–50 ng range depending on to the absorbance of the compounds. The only way to lower the detection limits to the MS range (10–100 pg) is to trap each separated compound by freezing, which is achieved through the use of matrix isolation or direct deposition techniques.

In the matrix isolation interface, argon is added to the GC eluates and the mixture is condensed on a moving metal substrate cooled to 12 K. Each solute is trapped in an argon matrix as the carrier gas is pumped off and deposited as a trace whose width can be as small as 0.3 min; this technique increases tenfold the intensity of IR spectra compared with the intensity obtained with a light-pipe (1 mm I.D.) interface. The low temperature also decreases IR band widths, which leads to an enhancement of sensitivity and specificity relative to the gas-phase interface. Further, as each component is retained on the substrate, the signal-to-noise ratio of the spectra can be increased by subsequent scanning of the eluates [1].

In the new direct deposition interface from Bio-Rad, pure column effluent is condensed on a moving ZnSe window transparent to IR radiation and cooled to 77 K. The width of the spots can be reduced to about 0.1 mm and the eluates can be scanned immediately by means of FT-IR microscopy. With this interface, the detection limits of routine real-time GC–IR have been reported to be less than 100 pg [2] and in practice the potential applicability of direct deposition GC–IR has been evaluated in environmental analysis [3,4].

In view of these advantages, it might be very useful to combine direct deposition GC–IR and GC–MS for (i) the characterization of allelochemicals responsible for insect–plant relationships (possible biopesticides) and of insect pheromones and (ii) confirmatory analyses of xenobiotics in minute concentrations. We describe here the example of *Allium* volatiles attractive to the leek-moth, a specific pest of *Allium* species, and we have identified and determined leek-moth male pheromones.

2. Experimental

2.1. Instrumentation

Gas chromatography–mass spectrometry

Chromatographic separations were performed using a Hewlett-Packard (Palo Alto, CA, USA) HP5890 II instrument with a split–splitless injector and a fused-silica capillary column (20 m × 0.22 mm I.D.) with an HP1 methylsilicone film (film thickness 0.33 μm). The conditions were as follows: injector temperature, 200°C; oven temperature programme, 70°C for 5 min, then increased at 2°C/min to 280°C; carrier gas, helium; column flow-rate, 1.0 ml/min; splitting ratio, 1:30, or splitless; injection volume, 1 μl in all experiments.

Total ion chromatograms (TIC) and mass spectra were recorded using a Hewlett-Packard HP 5898 "mass engine" with an HP UX workstation in the electron impact ionization mode at 70 eV. The transfer line was maintained at 100°C, the source temperature at 200°C and the quadrupole temperature at 100°C.

Gas chromatography–IR spectrometry

GC separations were performed using exactly the same instrument and the conditions as for GC–MS, except for the flow-rate, which can be lowered to optimize the deposition, and sometimes a delay was added before temperature programme to better the solvent separate.

The Gram–Schmidt reconstructed chromatograms (GSC), the functional group chromatograms (FGC) and the IR spectra were recorded using a Bio-Rad Digilab (Cambridge, MA, USA) FTS 45A spectrometer equipped with a Digilab Tracer direct deposition interface con-

taining its own nitrogen-cooled MCT detector and with an SPC 3200 data system for data acquisition and instrument control. The transfer line was maintained at 250°C and real-time spectra were obtained by addition of four scans, with a resolution of 8 cm^{-1}.

These two coupled techniques were used here in parallel fashion and usually the runs were not simultaneous.

2.2. Sample preparation

Pheromonal extracts

The leek moth, *Acrolepiopsis assectella* (Acrolepiidae), possesses coremata or hair-pencils that are concealed when at rest in an abdominal pocket, which opens to the exterior laterally between the eight and ninth abdominal segments. These organs are extruded during sexual behaviour when the male, attracted by the sexual pheromones of calling females, approaches and flutters its wings a few millimetres from the female. Male pheromones emitted in this fashion provoke the adoption of the acceptance posture by the calling female and thus have an aphrodisiac role.

Through pressure on the abdomen of virgin males aged from 4 to 6 days, the opening of the pocket containing the hair-pencil becomes visible. With fine forceps the hair-pencils were removed, still retracted within the epithelium of the pocket. Using this method 300 hair-pencils were removed in the middle of the photophase and immediately immersed in 0.5 ml of hexane and kept at −20°C.

Allium volatiles

Allium vineale odour was emitted by cutting green leaves in a closed glass vessel (1 l) at room temperature. Headspace volatiles were trapped for 1 hr (flow-rate 250 ml/min) on a glass cartridge (20 mm × 4 mm I.D.) containing 30 mg of Tenax GC (Alltech, Deerfield, IL, USA) (60–80 mesh) directly connected to a Gilian (Wayne, NJ, USA) LFS 113 pump [5]. The trapped volatiles were eluted from the cartridge with 1 ml of peroxide-free diethyl ether (analytical-reagent grade) and immediately analysed.

Materials

Hydrocarbons used as reference compounds were obtained from Aldrich (Milwaukee, WI, USA).

3. Results and discussion

3.1. Pheromonal extracts

For the leek-moth hair-pencil extract, the TIC and the FGC between 2800 and 3000 cm^{-1} both show nine peaks (Fig. 1). The IR and mass spectra indicate a structure based on a linear saturated hydrocarbon from $C_{16}H_{34}$ to $C_{23}H_{48}$ for the peaks 1, 2, 3, 4, 5, 6, 8 and 9 (see, e.g. the spectra of peak 2, Fig. 2). Any doubt remaining as to the existence of branched isomers is lifted by the observation of the regular decrease in the ratio of the intensity of the IR asymmetric stretch bands CH_3 at 2950 cm^{-1} versus CH_2 at 2930 cm^{-1}. The identification of peak 7 is in progress.

Each injection corresponds approximately to a male equivalent and the abundance of these *n*-alkanes can be estimated as hexadecane = 4 ng, heptadecane = 400 ng, octadecane = 10 ng, nonadecane = 200 ng, eicosane = 4 ng, heneicosane = 200 ng, docosane = 4 ng and tricosane = 30 ng.

Among the compounds of this series, only four *n*-alkanes provoked as many responses of a sexual nature in virgin females as did the male extract. These are the three *n*-alkanes with the odd number of carbon atoms, heptadecane, nonadecane and heneicosane, which are the most abundant, and also hexadecane, which is less abundant, as are the other compounds with an even number of carbon atoms. This is the first case of *n*-alkanes in which the male pheromonal role has been clearly demonstrated [6].

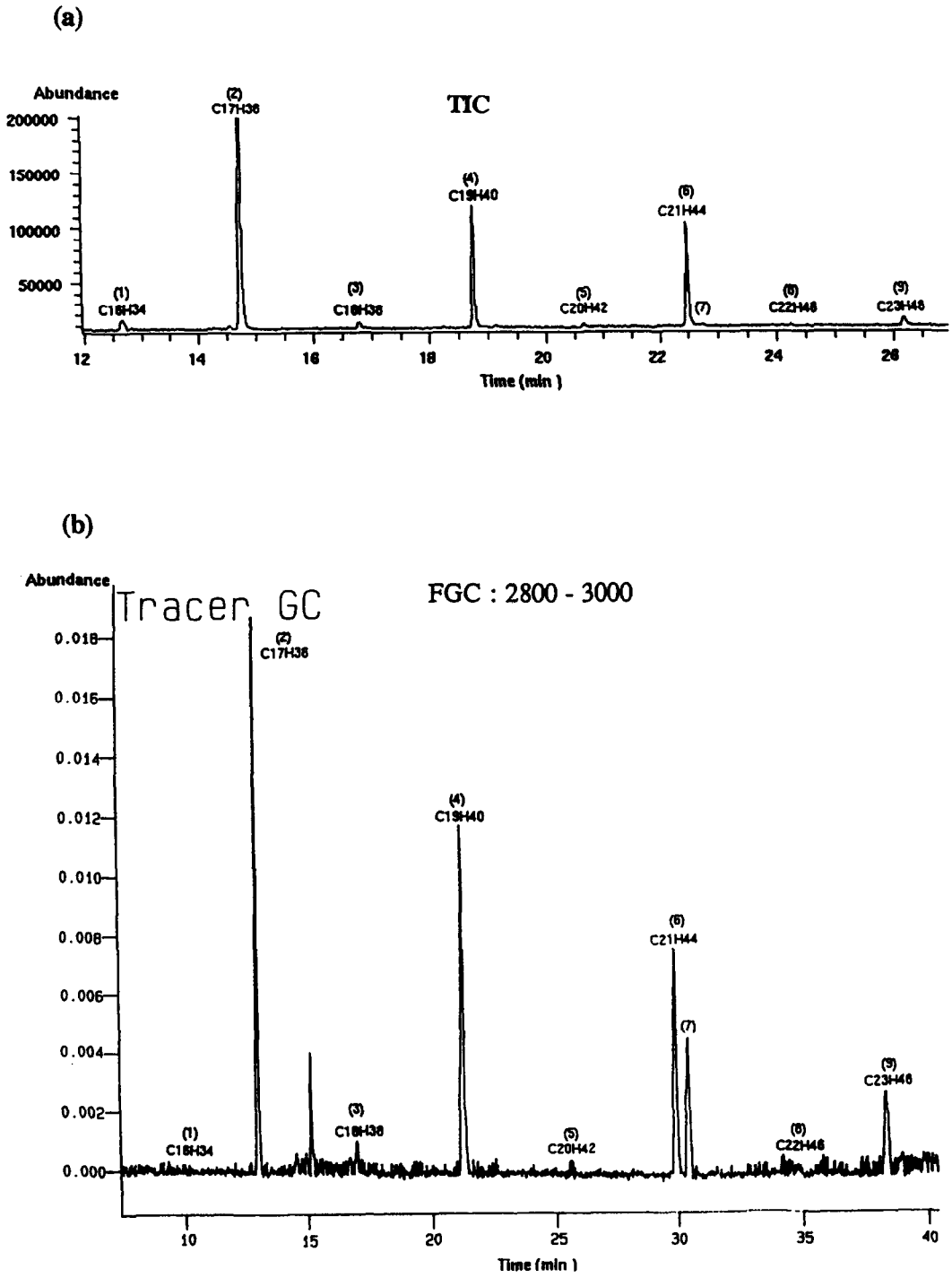

Fig. 1. (a) Total ion chromatogram and (b) functional group chromatogram (2800–3000 cm^{-1}) of pheromonal extract of the leek-moth male. For conditions, see Experimental.

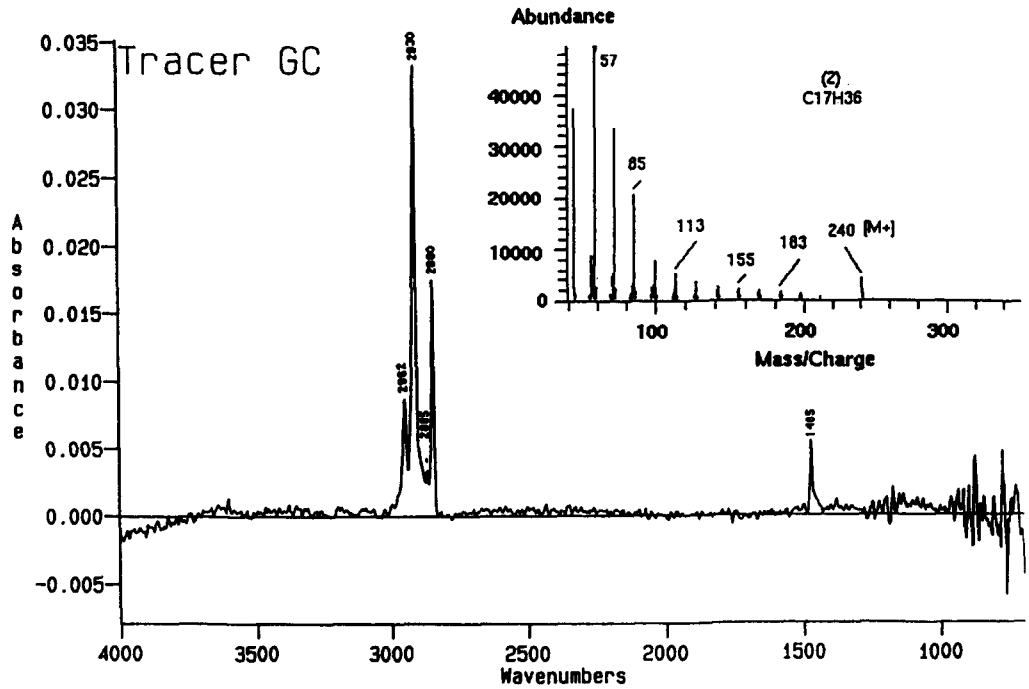

Fig. 2. IR and mass spectra of compound 2 in the pheromonal extract of the leek-moth. See Fig. 1.

3.2. *Allium* volatiles

The *Allium* odours are all attractive for the leek-moth and they contain mainly sulfur volatiles with four moieties, methyl, propyl (specified to leek, *A. Porrum*), allyl (specific to garlic, *A. sativum*) and 1-propenyl (specific to onion, *A. cepta*) [7]. Wild *Allium* species contain all these thio moieties in various proportions, depending on the species, and were screened in our laboratory.

For example, the TIC and GSC of *A. vineale* volatiles show mainly disulfides (Fig. 3). These disulfides exist as different structural isomers, e.g., compounds 1 and 3. Their mass spectra are not very different (same M^+ at 146) and are compatible with dipropenyl disulfides (diallyl disulfide according to an MS library search). Their IR spectra show the same characteristic allylic bands but the spectrum of 3 has some others that can be attributed to $-CH_3$ (deformation) for 2850 and 2940 cm^{-1}, to $C-CH_3$ (deformation) for 1340 and 1445 cm^{-1} and to —CH=CH— (out-of-plane deformation) for 925 and 950 cm^{-1} (Fig. 4). It is concluded that 1 is diallyl disulfide and 3 is allyl-1-propenyl disulfide.

All the disulfides identified in *A. vineale* are more or less attractive for the leek-moth, tested in an olfactometer [7].

From these examples it can be seen that direct deposition GC–IR is very useful for qualitative analysis of compounds at trace levels. This technique produces excellent spectroscopic information and is best suited for non-routine analysis where positive identification of compounds is required. The information obtained is complementary to the information obtained from GC–MS.

The linking of GC–MS with GC–IR at the same level of sensitivity appears to be the way to the future in chemical ecology and the combination of GC–MS and direct deposition GC–FT-IR is especially promising.

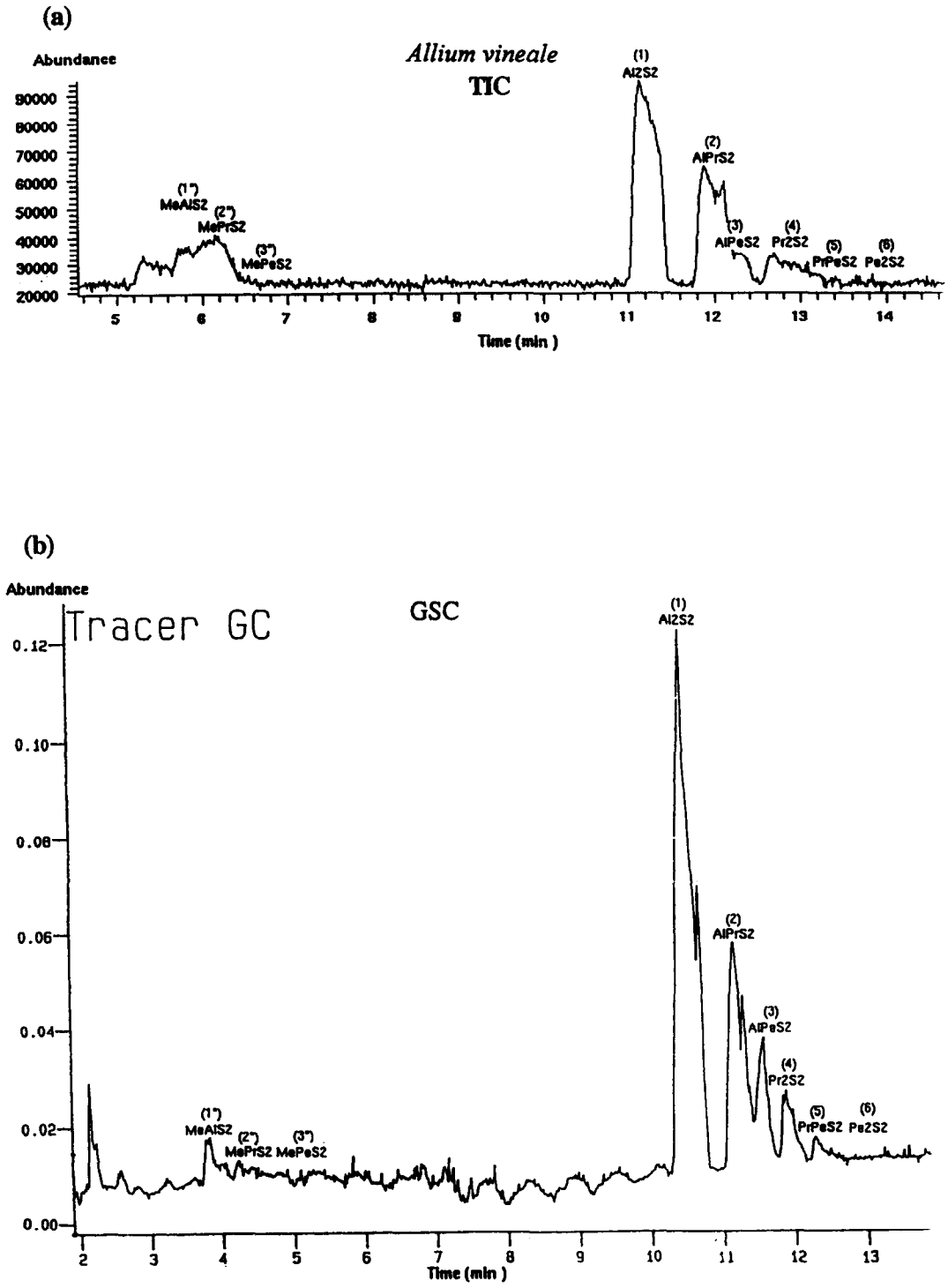

Fig. 3. (a) Total ion chromatogram and (b) Gram–Schmidt reconstructed chromatogram of *Allium vineale* trapped volatiles. For conditions, see Experimental.

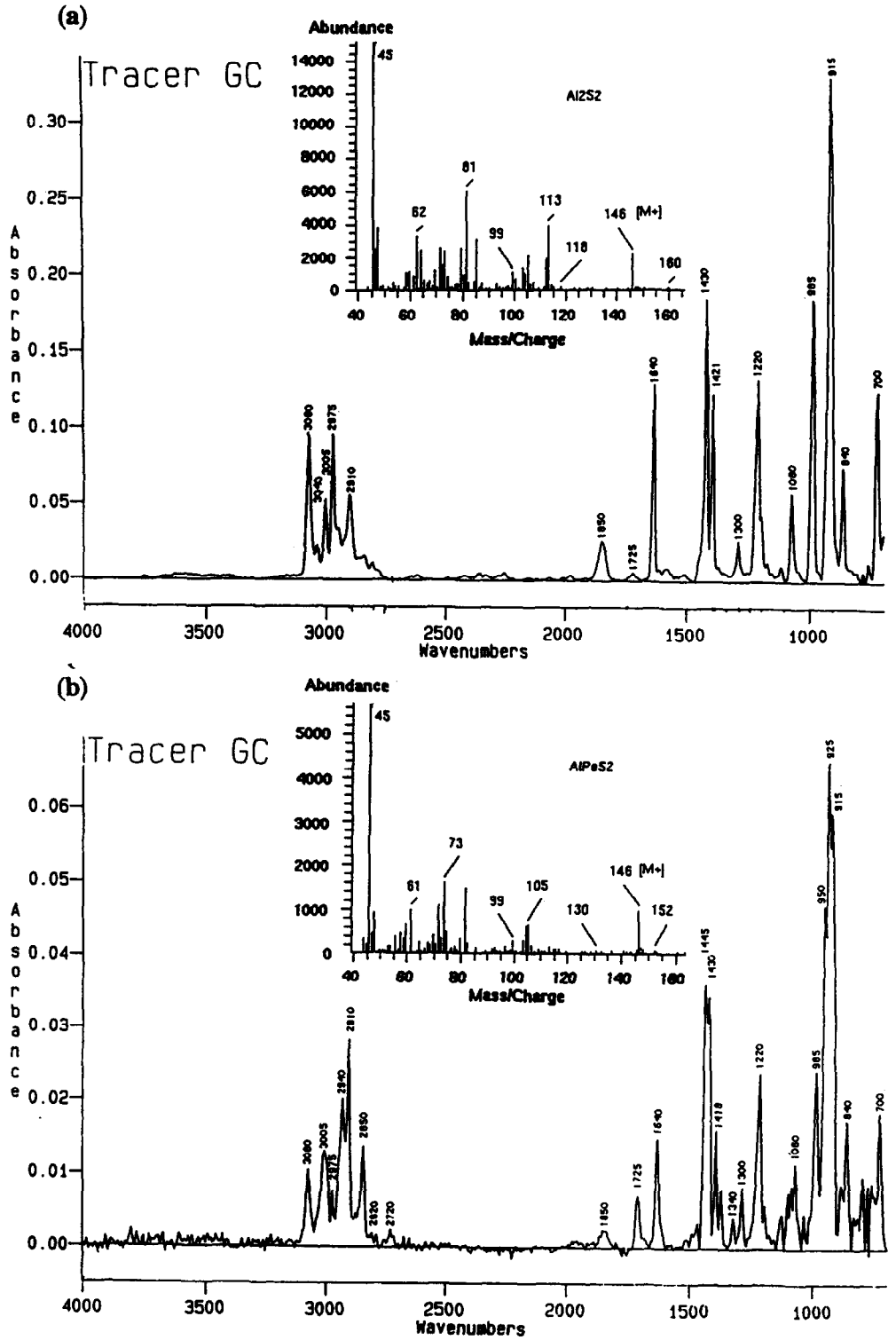

Fig. 4. IR and mass spectra of examples of *Allium* volatiles: (a) diallyl disulfide; (b) allyl 1-propenyl disulfide.

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