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What is the true odour of cut *Allium*? Complementarity of various hyphenated methods: gas chromatography–mass spectrometry and high-performance liquid chromatography–mass spectrometry with particle beam and atmospheric pressure ionization interfaces in sulphenic acids rearrangement components discrimination

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Abstract

Many sulphur volatiles were previously claimed to participate in the *Allium* odours (thiosulphinates, degradation compounds of sulphenic acids or thiosulphinates). To determine the true *Allium* odours we reexamine garlic (*Allium sativum* L.), leek (*Allium porrum* L.) and onion (*Allium cepa* L.) by different methods of isolation (extraction, trapping on adsorbent, cold trapping), transfer techniques (liquid samples, headspace SPME) and chromatographic procedures (various HPLC–MS (–MS) systems: thermabeam, electrospray and APCI, SPME–GC–MS and GC–MS). Analysis of *Allium* odours show only thiopropanal S-oxide, thiosulphinates and related compounds (zwiebelanes, cepaenes) in minor quantities and no disulphides or other rearrangement products.

Keywords: *Allium*; Extraction methods; Hyphenated techniques; Thiosulphinates; Thiopropanal S-oxide; Hexenal; Sulphur compounds, volatile

1. Introduction

When *Allium* are cut or crushed enzymatic cleavage of S-alk(en)yl cysteine sulphoxides releases volatiles sulphenic acids: R–S–OH where R represents the groups: methyl (Me), *n*-propyl (Pr), 1-propenyl (Pe) and 2-propenyl (Al) [1–3]. These highly reactive compounds rearrange immediately with various path-ways according to the nature of the R group and physico-chemical conditions:

— in the normal route they combine to form the thiosulphinates (Ti) R–SO–S–R′ (25 structures are possible according to R,R′) (Fig. 1),

— Pe sulphenic acid can also rearrange to thiopropanal S-oxide (the lachrymatory factor) [4] and zwiebelanes (identified by Bayer et al. [5] and corresponding to thiosulphinate isomers with R and R′=Pe) (Fig. 1),

— Pe and Al sulphenic acids in equilibrium with the related thiosulphinates [6] can lead to rearrangement products with three sulphur atoms, respectively cepaenes and ajoenes [7–9] (Fig. 1), and in presence of compounds, with R=Pr, the Pe sulphenic acid can give cepaene homologues [8,10] (Fig. 1).

All these compounds in liquid phase continue to

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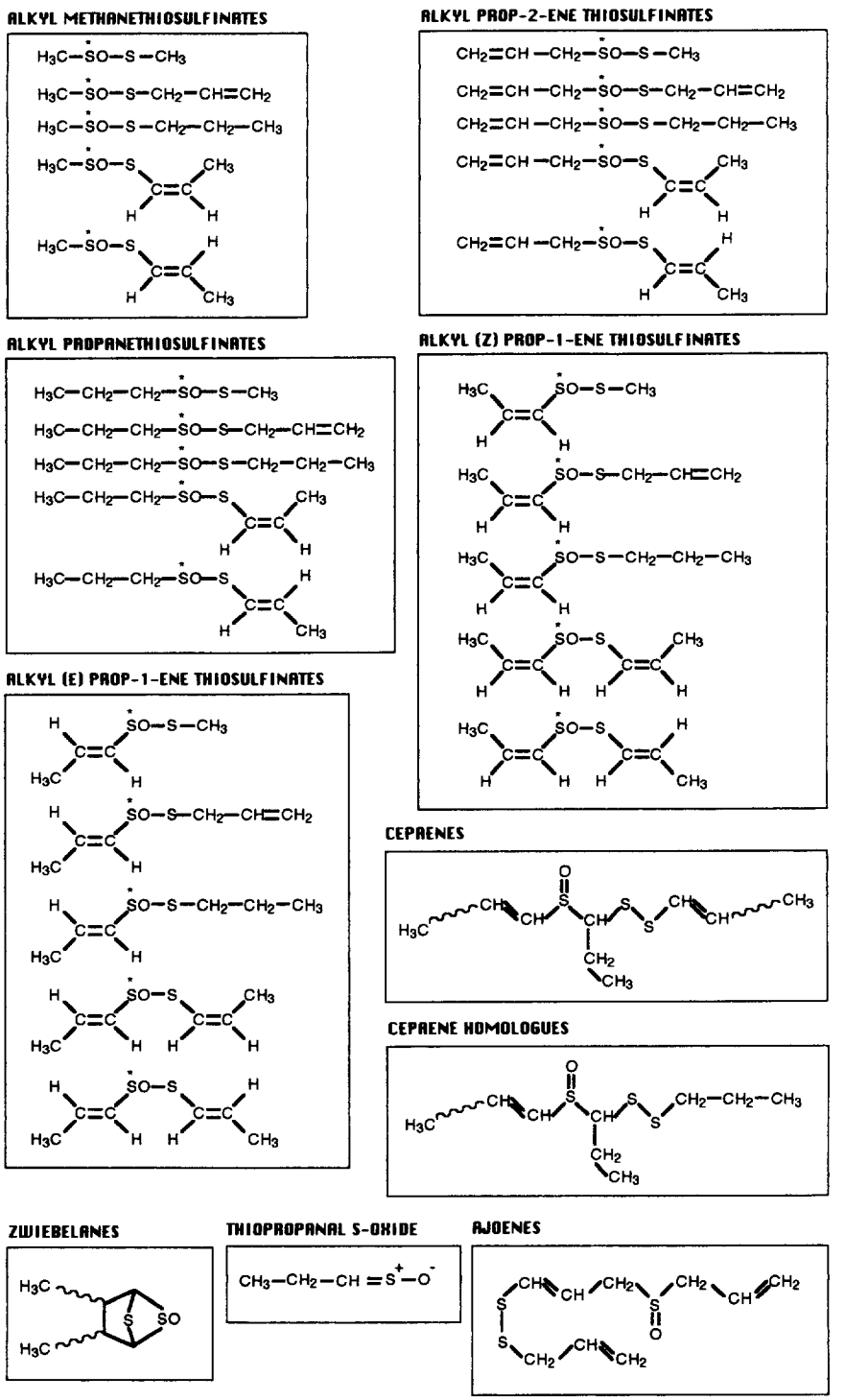

Fig. 1. Sulphur volatiles which can participate in *Allium* odours.

Table 1
Abbreviations and structures of compounds analysed

Thiosulphinates abbreviations	Structures	Abbreviations	Structures
$TiMe_2$	CH_3–SO–S–CH_3	ToMePr	CH_3–SO_2–S–CH_2–CH_2–CH_3
TiMeAl	CH_3–SO–S–CH_2–CH=CH_2	ToMePe	Ch_3–SO_2–S–CH=CH–CH_3
TiAlMe	CH_2=CH–CH_2–SO–S–CH_3	ToPrPe	CH_3–CH_2–CH_2–SO_2–S–CH=CH–CH_3
TiMePe	CH_3–SO–S–CH=CH–CH_3	$ToPr_2$	CH_3–CH_2–CH_2–SO_2–S–CH_2–CH_2–CH_3
TiPeMe	CH_3–CH=CH–SO–S–CH_3	$MePeS_2$	CH_3–S–S–CH=CH–CH_3
TiMePr	CH_3–SO–S–CH_2–CH_2–CH_3	$MePrS_2$	CH_3–S–S–CH_2–CH_2–CH_3
TiPrMe	CH_3–CH_2–CH_2–SO–S–CH_3	Al_2S_2	CH_2=CH_2CH–CH_2–S–S–CH_2–CH=CH_2
$TiAl_2$	CH_2=CH–CH_2–SO–S–CH_2–CH=CH_2	$PrPeS_2$	CH_3–CH_2–CH_2–S–S–CH=CH–CH_3
TiAlPe	CH_2=CH–CH_2–SO–S–CH=CH–CH_3	Pr_2S_2	CH_3–CH_2–CH_2–S–S–CH_2–CH_2–CH_3
TiPeAl	CH_3–CH=CH–SO–S–CH_2–CH=CH_2		
$TiPe_2$	CH_3–CH=CH–SO–S–CH=CH–CH_3		
TiPrPe	CH_3–CH_2–CH_2–SO–S–CH=CH–CH_3		
TiPePr	CH_3–CH=CH–SO–S–CH_2–CH_2–CH_3		
$TiPr_2$	CH_3–CH_2–CH_2–SO–S–CH_2–CH_2–CH3		

rearrange to more stable components like polysulphides and thiosulphonates (R–SO_2–S–R') [11,12].

In previous work we demonstrated that thiosulphinates are stable in the gas phase [13] and it is well known that the lachrymatory factor is present in onion (*Allium cepa* L.) and leek (*Allium porrum* L.) odours [4,14,15] and that 2-propenyl 2-propenethiosulphinate (allicin) is the predominant compound of garlic (*Allium sativum* L.) odour [16,17]. But we are not sure that other rearrangement products (cepaenes, cepaene homologues, zwiebelanes, ajoenes) are present in *Allium* odours as they were not detected in trappings: either their levels are significantly reduced compared to extracts, or they are artifacts of extraction.

In this study we tested different methods of isolation: extraction, trapping on adsorbent, cold trapping (room temperature steam distillation), transfer techniques: liquid samples, headspace solid-phase microextraction (SPME) and hyphenated methods: HPLC–MS, GC–MS and SPME–GC–MS to analyse the true *Allium* odours.

2. Experimental

2.1. Instrumentation

2.1.1. Gas chromatography–mass spectrometry

Chromatographic separations were performed using a Hewlett-Packard (Palo Alto, CA, USA) HP5890 II instrument with on-column injector and fused-silica capillary column (20 m×0.32 mm I.D.) with an HP1 methylsilicone film (film thickness 0.52 μm). The conditions were as follows: carrier gas helium; column flow-rate 1.0 ml/min; injection volume 1 μl in the splitless mode; oven and on-column injector temperature programme: 28°C increased at 5°C/min to 200°C.

Total ion chromatograms (TIC) and mass spectra were recorded using a Hewlett-Packard HP 5989A "Mass Engine" with an HP UX workstation in electron ionization mode (EI) at 70 eV. The transfer line was maintained at 150°C, the source temperature at 200°C and the quadrupole temperature at 100°C.

2.1.2. SPME headspace–gas chromatography–mass spectrometry

For the trapping transferred by headspace SPME the chromatographic separations were performed using a Varian 3600CX instrument with an 8200CX autosampler modified for SPME (Varian, Walnut Creek, CA, USA), a split-splitless injector, cryogenic cooling (CO_2) and a fused-silica capillary column (15 m×0.2 mm I.D.) with an HP1 methylsilicone film (film thickness 0.32 μm). The conditions were as follows: carrier gas helium; column flow-rate 1.0 ml/min; SPME fibre desorption during 2 min at 100°C; oven temperature programme: initially 0°C for 1 min increased at 5°C/min to 200°C.

Total ion chromatograms (TIC) and mass spectra were recorded using a Varian Saturn ion Trap in electron ionization mode.

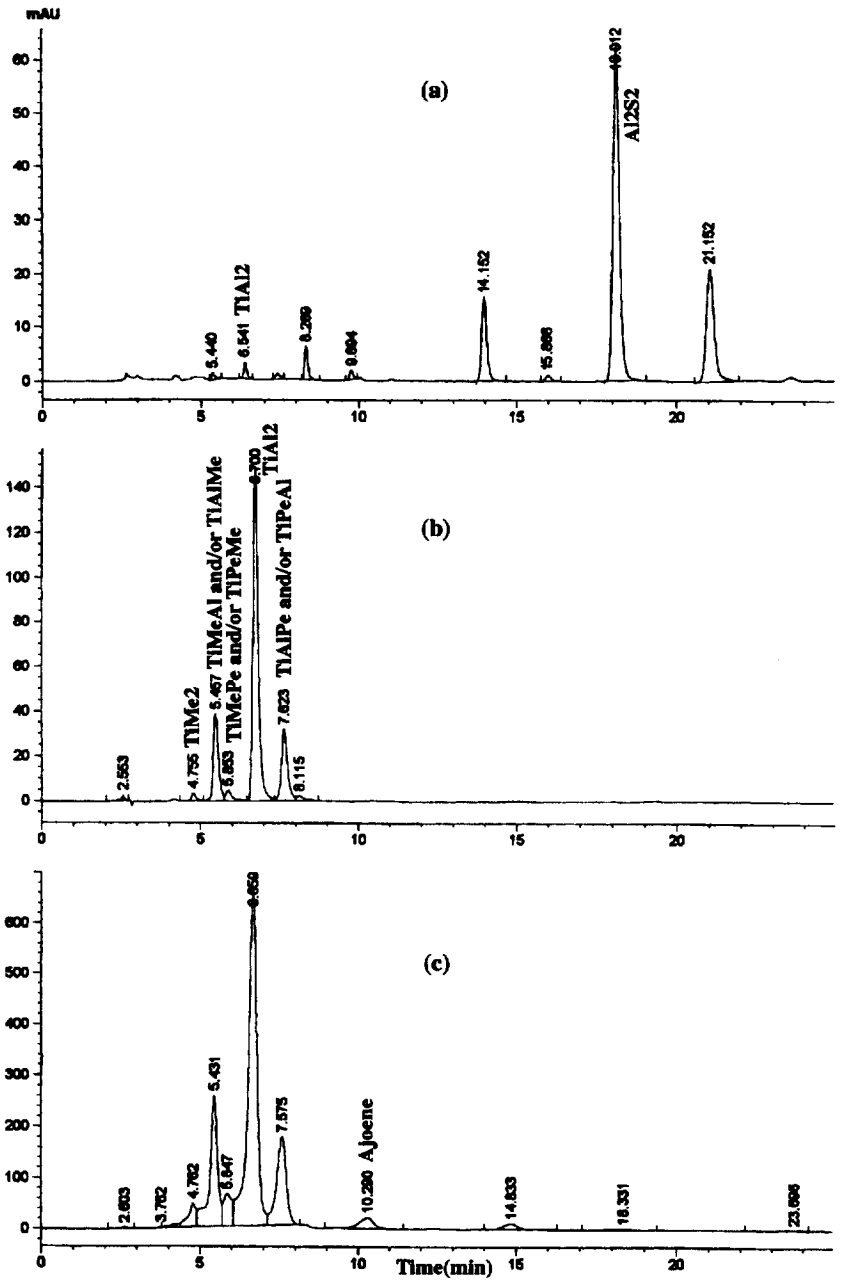

Fig. 2. HPLC–UV chromatograms of garlic odour: (a) trapped on adsorbent, (b) cold trapped and (c) extracted.

2.1.3. HPLC separation and UV detection

The samples were injected into a column connected to a Hewlett Packard 1040M diode array UV detector. The detection was at 240 nm.

2.1.4. High-performance liquid chromatography–mass spectrometry

Analysis were performed using different HPLC–MS systems with three ionization modes: electron

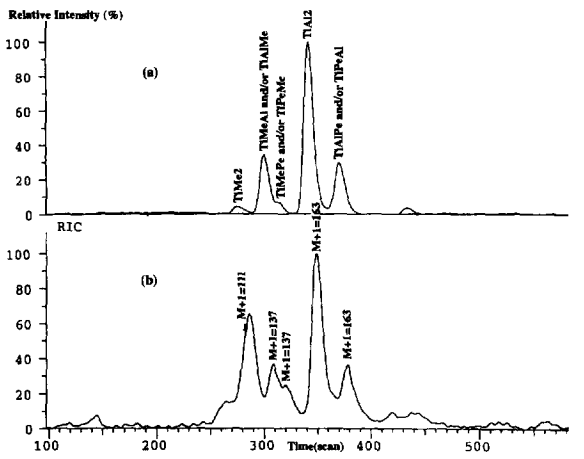

Fig. 3. Chromatograms of garlic cold trapped odour: (a) by HPLC–UV and (b) by HPLC–MS (Finnigan APCI interface).

ionization (EI), atmospheric pressure chemical ionization (APCI) and electrospray ionization (ES) in combination with HPLC–UV. In general, the ionization was made in positive mode.

Thermabeam (TB) mass spectra were obtained using a Waters "Integrity" system (Millipore Waters, Milford, MA, USA). It uses an evolution of the particle beam interface where a thermic effect assists the nebulization and the desolvation.

Electrospray (ES) mass spectra were obtained using a Finnigan MAT TSQ 7000 mass spectrometer with a Finnigan ES interface and source (Finnigan MAT, Bremen, Germany) or a API III Perkin-Elmer Sciex mass spectrometer with a Perkin-Elmer Sciex ES interface and source (Perkin-Elmer, Norwalk, CT, USA). The final desolvation is assisted by a curtain gas in the Perkin Elmer Sciex ES and by a heated capillary in the Finnigan ES.

Atmospheric pressure chemical ionization (APCI) mass spectra were obtained using a Finnigan MAT TSQ 7000 mass spectrometer with a Finnigan APCI interface and source or a API III Perkin-Elmer Sciex mass spectrometer with a Perkin-Elmer Sciex APCI interface and source. The final desolvation is assisted as for the electrospray.

With the Finnigan and Perkin-Elmer Sciex MS systems, the RP-HPLC analysis were performed on a Hewlett-Packard C_{18} column (Lichrospher 100RP18e 250×4 mm, 5 μm). The eluent was CH_3OH–water (70:30) at a flow-rate of 0.5 ml/min.

With the Waters "Integrity" system the separations were performed on a Waters column (Novapak C_{18} 100×2 mm, 5 μm). The eluent was CH_3OH–water (60:40) at a flow-rate of 0.3 ml/min.

2.2. Sample preparation and transfer

2.2.1. Trapping on adsorbent

Allium odour was emitted by cutting green leaves (leek) or bulbs (garlic and onion) in a closed glass vessel (1 l), at room temperature. Headspace volatiles were trapped: (flow-rate 250 ml/min) on a glass cartridge (20×4 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 [18] during 1 h at room temperature or 15 min at 10°C. The trapped volatiles were eluted from the cartridge with 1 ml of methanol (analytical grade) and immediately analysed by HPLC.

2.2.2. Cold trapping

Samples (50 g) of *Allium* green leaves (leek) or bulbs (garlic or onion) were rapidly cut in a flask. This flask was fitted to an other flask immersed in liquid nitrogen and connected to a vacuum pump. Volatiles and water emitted at room temperature were trapped during 20 min. This frozen sample was allowed to warm and just at melting, was injected in HPLC without concentration after 0.4 μm filtration and, if necessary, addition of a minimum amount of methanol.

2.2.3. Cold trapping transferred by headspace SPME

This sample technique based on adsorption is used to preconcentrate trace compounds and can separate analytes from the sample matrix. SPME has been developed by Arthur and Pawliszyn [19] and headspace SPME is a new variation of this adsorption technique used by Zhang and Pawliszyn for volatiles compounds analysis. [20]

During the headspace transfer, the fused-silica fibre coated with poly(dimethylsiloxane) (100 μm) was introduced into the septum closed vial con-

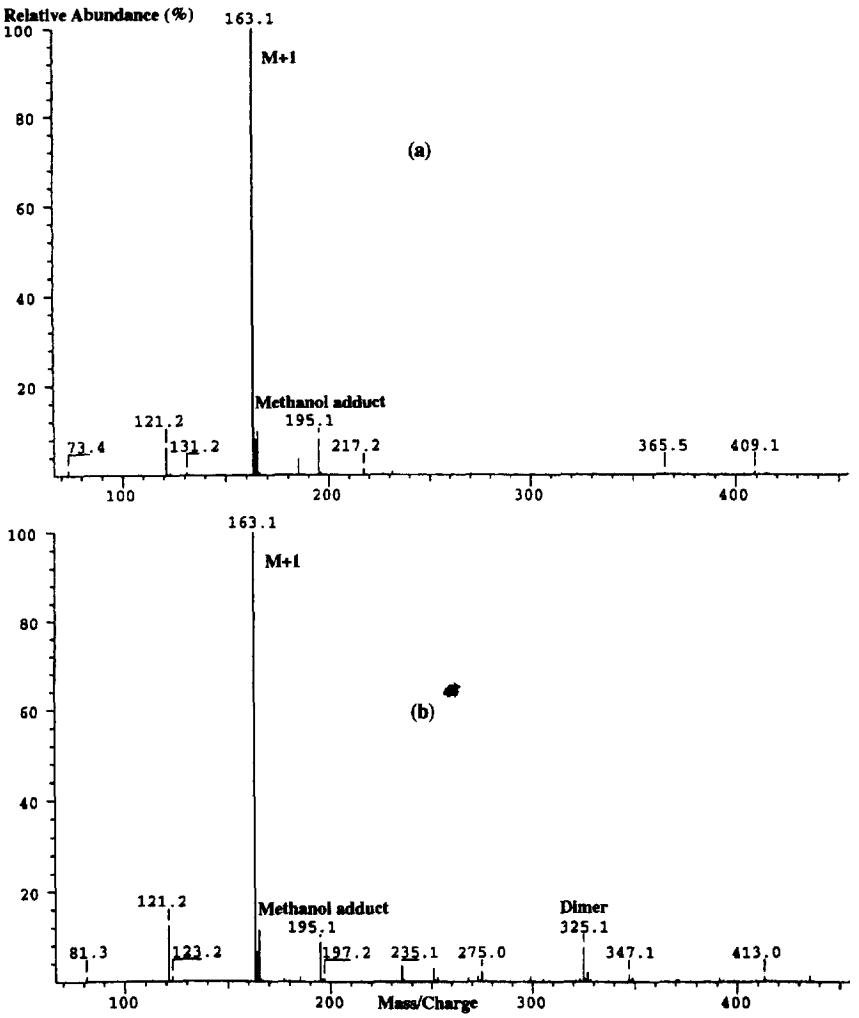

Fig. 4. Mass spectra of: (a) $TiAl_2$ and (b) unresolved isomers: TiAlPe and TiPeAl (Finnigan electrospray interface). Observation of dimers characteristic of 1-propenyl thiosulphinates.

taining trapping sample and stayed 2 min above the aqueous phase to equilibrate. Then the fibre was immediately inserted in the GC injector for desorption.

2.2.4. Extract

Samples (80 g) of cloves or bulbs rapidly cut were homogenized in 80 ml of water. The homogenate was allowed to stand at room temperature for 30 min. Then the homogenate was saturated with sodium chloride salt and extracted by dichloromethane. The organic extract was evaporated at room temperature to dryness and diluted in 400 μl of methanol for HPLC and GC analysis.

2.3. Compounds characterization

All compounds were identified by their characteristic ions according to Refs. [5,8,21–26]. Some of them were synthesized as previously described:

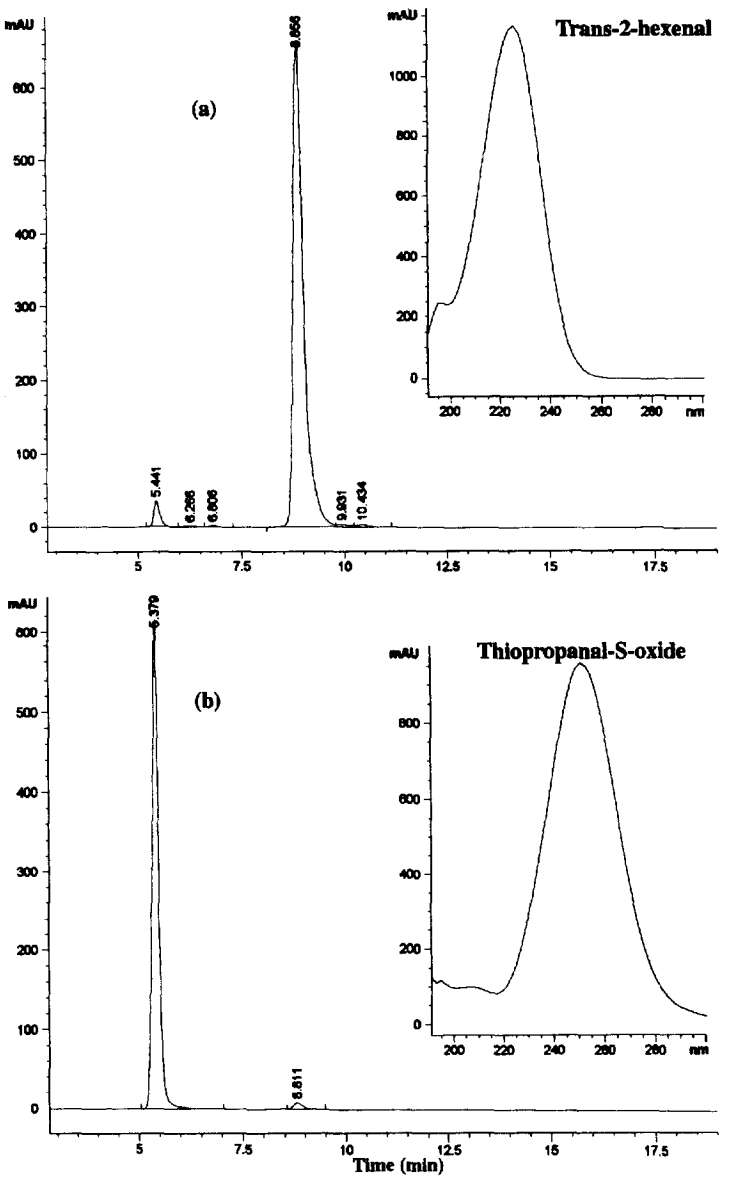

Fig. 5. HPLC–UV chromatograms of: (a) leek trapping with the spectrum of 2-hexenal and (b) onion trapping with the spectrum of thiopropanal S-oxide.

symmetric thiosulphinates (R=Me, Pr, Al) by oxidation of corresponding disulphides [27] (method derived from Small et al. [28]) and ajoene by degradation of allicin [29]. Abbreviations used and structures of compounds investigated are summarized in Table 1.

3. Results and discussion

The HPLC and GC studies of the different *Allium* species demonstrated that the predominant odour principles are thiosulphinates for garlic bulbs, thiopropanal S-oxide for onion bulbs and *trans*-2-hexen-

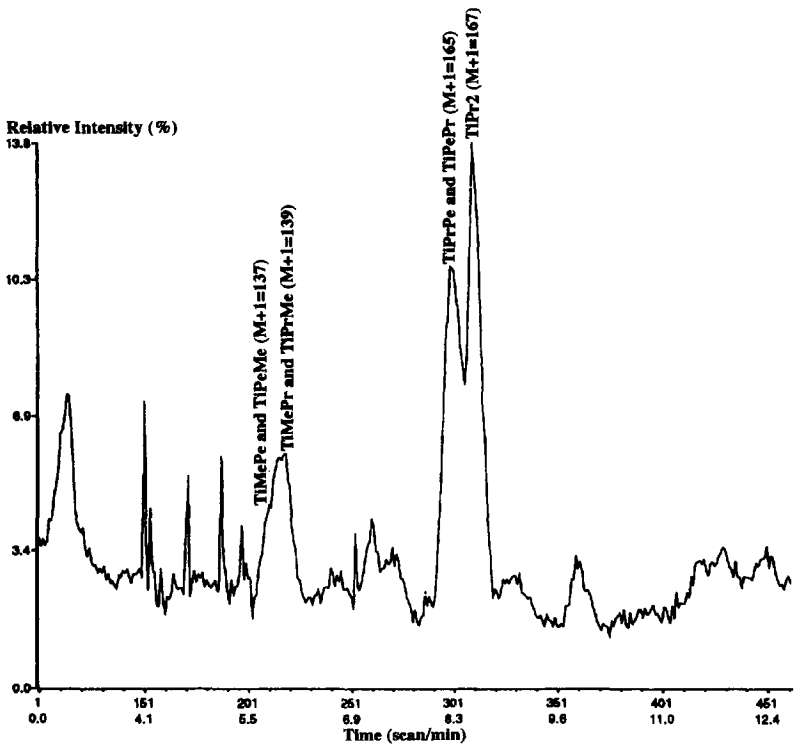

Fig. 6. HPLC–MS chromatogram of leek trapping (Perkin-Elmer APCI interface).

al for leek leaves, with no disulphides, polysulphides and thiosulphonates.

3.1. Garlic

HPLC–UV analysis of garlic trapping on Tenax showed mainly the presence of disulphides (characterized by GC–MS and GC–IR [30]) except for the short trapping time and low temperature treatment where there was a small quantity of $TiAl_2$ (Fig. 2a). For garlic cold trapping and extract, HPLC–UV showed five peaks with the characteristic maxima of thiosulphinates (between 240 and 260 nm), no disulphides and a small peak of ajoene in the extract (Fig. 2b and 2c). Thiosulphinates could be identified by specific maxima of absorption or shoulders: components with methyl group present a maximum of absorption at 250 nm, those with allyl group a maximum at 245 nm and the components with 1-propenyl group a shoulder at 206 nm.

In cold trapping, eight compounds were detected and identified by ES and APCI systems as thiosulphinates (Fig. 3) (the resolution of the chromatogram was better with the APCI interface than with the ES interface: the nebulisation was faster and the compounds stayed a shorter time in the ionization source [21]). They were characterized by their M+1 ions and differentiated by their fragment ions and their capacity of dimerization in the MS source (thiosulphinates with Pe group present more dimers than the other thiosulphinates) (Fig. 4). Depending on the geometry, the type of interface and the concentration, thiosulphinates formed dimers in variable quantities: the Perkin-Elmer Sciex HPLC–MS system created less dimers because curtain gas breaks clusters and the thiosulphinates with Pe group presented more dimers in ES Finnigan system.

GC–MS analysis of extract and cold trapping transferred by SPME showed in majority the degradation products of allicine (vinyldithiins), two

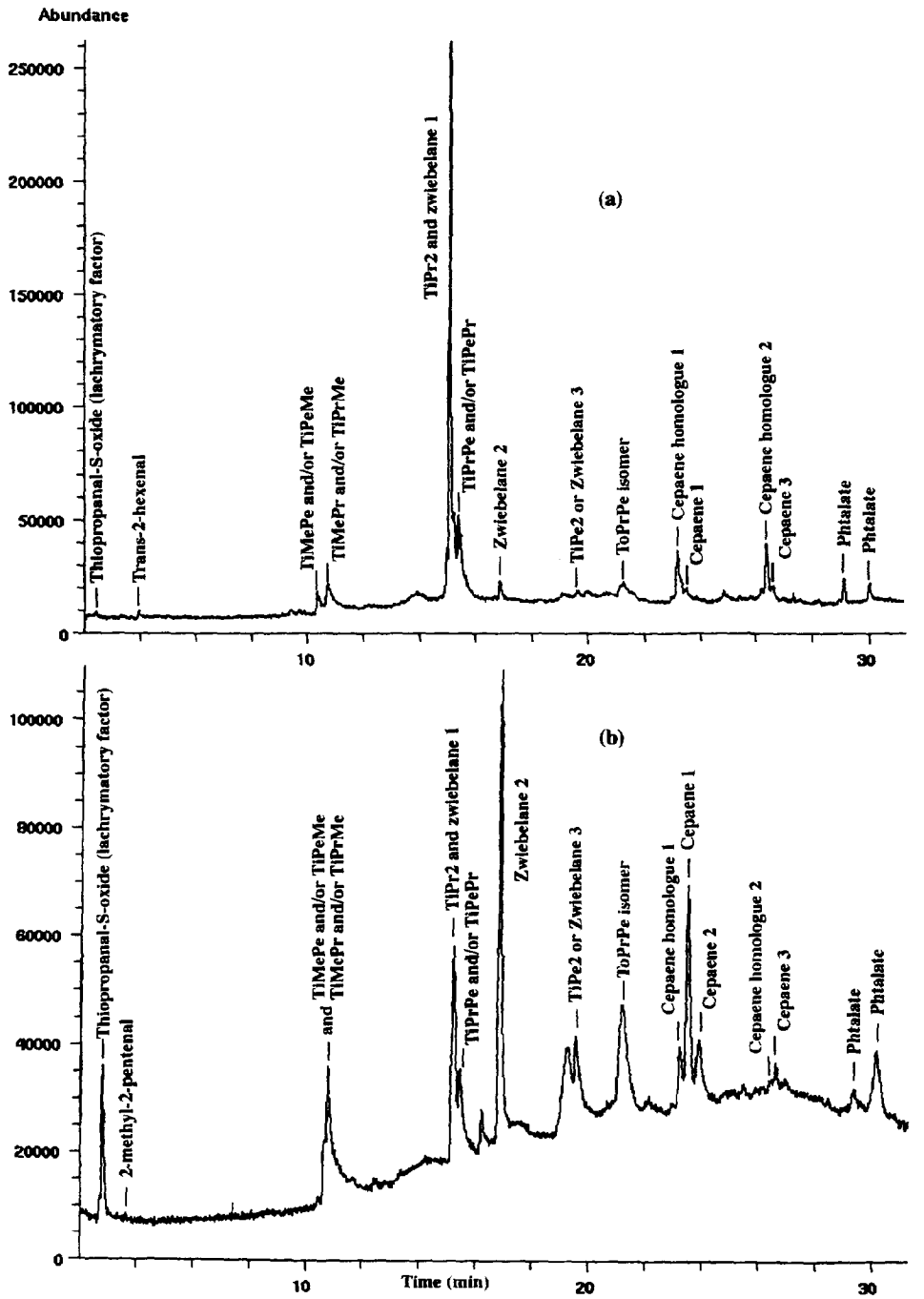

Fig. 7. GC–MS chromatograms of (a) leek extract and (b) onion extract (HP "Mass Engine" quadrupole).

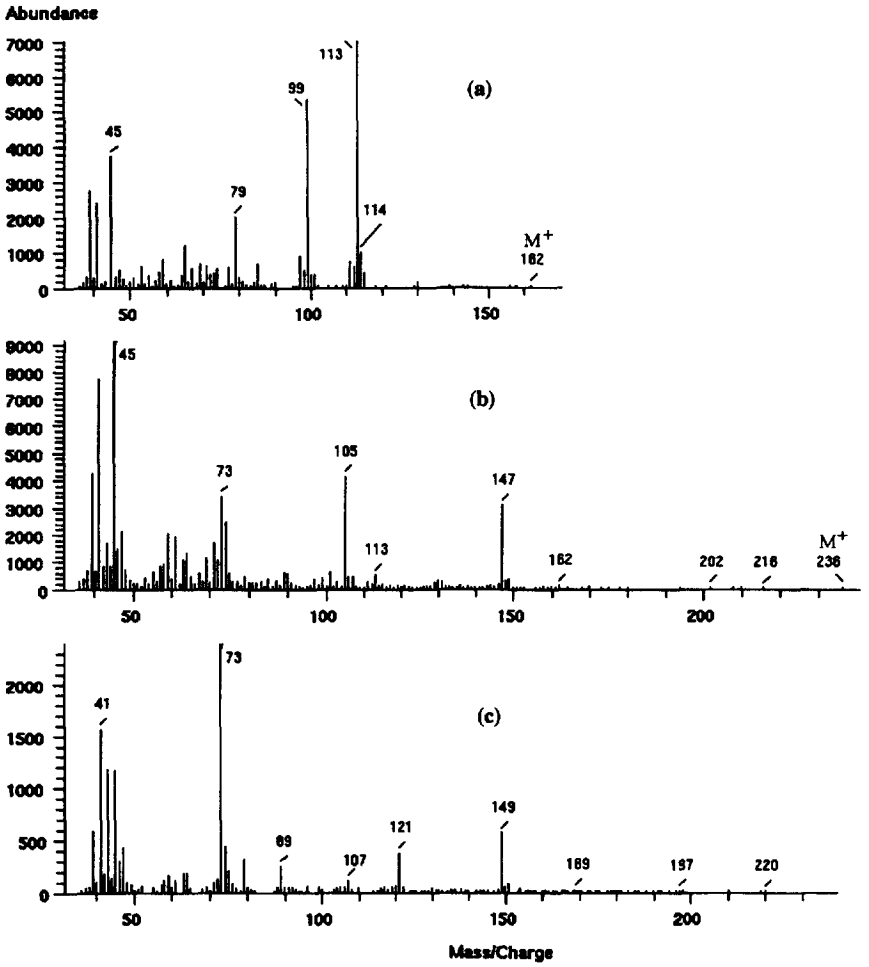

Fig. 8. Mass spectra of: (a) zwiebelane 2, (b) cepaene 1 and (c) cepaene homologue 1 (HP "Mass Engine" quadrupole).

thiosulphinate peaks (TiMePe isomers) and many disulphides. Thus GC–MS does not seem to be an appropriate method for the analysis of true garlic odour as it gives many artifacts.

In consequence true garlic odour contains eight thiosulphinates and no disulphides or other rearrangement compounds at all, except maybe a little quantity of ajoene detected in extract, the origin of which is not determined.

3.2. Leek and onion

3.2.1. Trappings

The chromatogram in HPLC–UV of leek and onion trappings presented very different profiles from garlic with one predominant peak corresponding to 2-hexenal ("green" leaf volatile) in leek and thiopropanal S-oxide in onion (with a little quantity of its degradation product, 2-methyl 2-pentenal). The maxima of absorption were 250 nm (possible S=O compound) for thiopropanal S-oxide and 225 nm for *trans*-2-hexenal (Fig. 5). These two substances having a high UV absorption, thiosulphinates and derivatives (zwiebelanes, cepaenes) appeared as minor compounds. Complementary to UV detection we used MS detection to analyse the compounds which present weak UV absorption but can give strong MS signals.

With the Thermabeam interface the ion and leek TIC showed the two predominant peaks with $M^{+\cdot}$ =

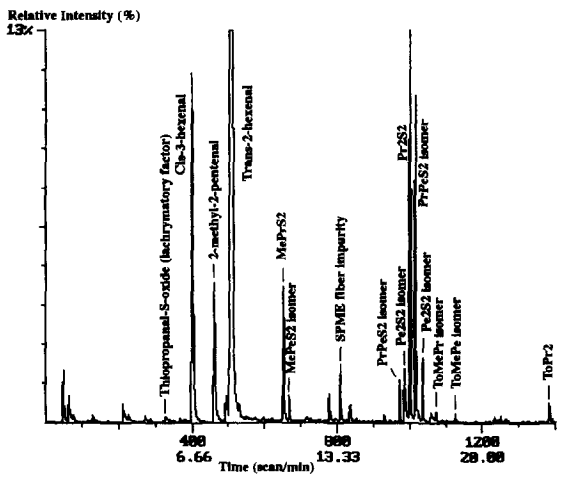

Fig. 9. Chromatogram of leek trapping transferred by headspace SPME (Varian "Saturn" ion trap).

90 and fragment ions at m/z=43, 45, 73 for onion and M^{+}=98 and fragment ions at m/z=40, 69, 83, 97 for leek. These two components were identified respectively as thiopropanl S-oxide and 2-hexenal, their mass spectra were in agreement with other reports [14,15].

In HPLC–MS (ES and APCI), these two components were not so easily detected, only in the negative chemical ionization mode. But in the positive chemical ionization mode seven other compounds were identified as thiosulphinates characterized by their M+1 ions in the two trappings (Fig. 6).

To identify the minor sulphur compounds which are difficult to detect by HPLC, we investigated the more concentrated extracts by GC–MS, which should be a more sensitive technique.

3.2.2. Extracts

Extracts GC–MS (EI) chromatograms of leek and onion showed the same sulphur components (the seven thiosulphinates, zwiebelanes, cepaenes and homologues) in different quantities: e.g. the thiosulphinates with propyl group and consequently the rearrangement products of these components (cepaene homologues) were more present in leek extract than onion extract (Fig. 7). Zwiebelanes, cepaenes and homologues were identified by their fragment ions (99 and 113 for zwiebelanes, 105 and 147 for cepaenes, 73 and 149 for homologues) (Fig. 8).

3.3. Trapping transferred by headspace SPME

Headspace SPME is developing rapidly in natural substances analysis. This technique is supposed to minimise artifact formation. In fact, GC–MS analysis of trapping with sample transfer by SPME at 100°C showed thiopropanal S-oxide, no thiosulphinates but only their degradation products (disulphides, trisulphides and thiosulphonates). Unfortunately it is impossible to use headspace SPME for concentrated extracts (organic solvants are adsorbed on SPME fibre) and the minor cepaenes and zwiebelanes are not detected (Fig. 9).

In conclusion, true leek and onion odours contain as sulphur compounds: thiopropanal S-oxide, the lachrymatory factor, seven thiosulphinates and maybe cepaenes and zwiebelanes.

4. Conclusion

All the sampling methods can produce artifacts, specially in *Allium* odours analysis where numerous rearrangements can appear. It seems that cryotrapping of *Allium* volatiles followed by direct injection is the best sampling method to observe the real components of *Allium* odours. But the samples obtained with this technique for leek and onion odours were not concentrated enough and we were obliged to work on extracts for the identification of minor rearrangement products hypothetically present in trapping (some compounds can be more or less transferred during cold trapping in comparison to extraction, nevertheless sampling methods do not affect qualitatively the thiopropanal S-oxide and thiosulphinates distribution).

We are working now on the possibility of trapping HPLC analysis as made on extracts by increasing the sensitivity using newly optimized interfaces, GC is unfortunately unusable on trapping (water sample).

For these very reactive and labile substances, only the use of various HPLC–MS methods can ascertain the absence of cepaenes and zwiebelanes in leek and onion odours, as we proved for ajoene in the more concentrated garlic odour.

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