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Determination of monocyclic aromatic hydrocarbons in plant cuticles by gas chromatography–mass spectrometry

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ABSTRACT

A method for the determination of benzene, toluene, ethylbenzene and xylenes in plant foliage was developed. Using a gas chromatography–quadrupole mass spectrometer in the selected-ion monitoring mode, calibration graphs and detection limits for these hydrocarbons were determined. *Pseudotsuga menziesii* (Mirb.) Franco needles and *Cotoneaster dammeri* Schn. "Skogholm" leaves were extracted with dichloromethane; the optimum extraction time was determined to be 6 h. Differences in the amounts of the hydrocarbons absorbed could be measured.

INTRODUCTION

Monocyclic aromatic hydrocarbons (MAHs) constitute an important fraction of the volatile organic compounds (VOCs) in ambient air. They are emitted by industrial processes and automobile exhausts. Average concentrations of benzene, toluene, ethylbenzene and xylenes in urban air vary from 1 to 70 $\mu g\ m^{-3}$, with peaks for benzene and toluene above 100 $\mu g\ m^{-3}$ [1–3]. As an increase in the content of benzene and other aromatics compensates for the decrease in octane number in unleaded fuel, increases in emission may be expected.

Exposure to aromatic hydrocarbons can cause serious health problems. Benzene is known to be a leukaemic agent in humans. The toxic properties of toluene, ethylbenzene and xylenes have also been frequently studied [4,5]. In addition, these hydrocarbons are photochemically reactive and contribute to smog.

An important feature of MAHs is their high lipophilicity [6]. They may be enriched in plant cuticles by their partitioning between the vapour state and the lipophilic cuticle. Recently, there has been some interest in the absorption of organic compounds by plant foliage. Frank and Frank [7] measured the uptake of tetrachloroethene by spruce needles and determined the partition ratios between air and

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needles. In several studies, the accumulation of mainly persistent semi-volatile chlorinated hydrocarbons in coniferous needles was investigated [8–11].

The aim of this study was to develop a simple but sensitive method for measuring amounts of benzene, toluene, ethylbenzene and xylenes absorbed in plant cuticles, in order to evaluate whether this is an important mechanism for the elimination of these pollutants from air.

EXPERIMENTAL

Sample preparation

Samples of *Pseudotsuga menziesii* (Mirb.) Franco needles were collected along a highway in the north of Belgium, running from the southeast to the northwest, which is approximately perpendicular to the direction of the prevailing winds in this area. Needles were sampled at 1.5 m above the ground on the side exposed to the highway by cutting them from the twigs with a pair of scissors. They were dropped into glass tubes provided with screw-caps without touching with the fingers. Approximately 1 g of needles (fresh weight), corresponding to 90–110 needles, were sampled per tube.

Leaves of *Cotoneaster dammeri* Schn. "Skogholm", a 0.5-m high shrub, were collected on the side of a road with dense traffic in the city of Ghent, Belgium, in the same way as the *Pseudotsuga* needles.

Dichloromethane, containing an internal standard (5 ng μl^{-1}), was added to the tubes to extract MAHs from the cuticle [1.2 ml of dichloromethane per gram of needles (fresh weight)]. The tubes were tightly closed with screw-caps and Teflon tape and placed in a slowly rotating drum (to improve contact between the solvent and the needles) for a certain period of time (see Results). The extracts were then filtered (Millex-HV 0.45 μm) and aliquots of 1 μl of filtrate were injected into the gas chromatograph

Analysis

A Hewlett-Packard Model 5890 gas chromatograph equipped with a Model 5970A quadrupole mass spectrometer and a Model 200 computer system was used to analyse the leaf extracts. A 30 m × 0.258 mm I.D. fused-silica capillary column coated with a 0.25-μm thick layer of DB-5 stationary phase was used with splitless injection. The carrier gas was helium at a linear velocity of 0.48 m s^{-1}. The injector temperature was 250°C and the gas chromatograph–mass spectrometer interface temperature 260°C. The initial oven temperature was 20°C, increased at 2°C min^{-1} for 14 min to 48°C, then at 15°C min^{-1} to 230°C. The sampling rate was five selected ion monitoring cycles per second.

RESULTS AND DISCUSSION

Instrumental parameters

The HP quadrupole mass spectrometer was used in the selected-ion monitoring mode. In Table I, the selected masses used for data acquisition and the time interval for each group of ions sampled are given.

As the leaf extracts also contained large amounts of terpenes, exhibiting retention times of 15 min and more, data acquisition had to be terminated at 14 min,

TABLE I

PARAMETERS FOR DATA ACQUISITION IN SELECTED-ION MONITORING

Group	Time interval for sampling (min)	*m/z*	Compound
1	2.50–4.00	77, 78	Benzene
2	4.00–6.60	91, 92	Toluene
3	6.60–9.00	66, 98	Perdeuterooctane
4	9.00–14.00	91, 106	Ethylbenzene, *m*-/*p*-xylene, *o*-xylene

immediately after elution of the *o*-xylene peak. At this moment, the rate of column temperature increase was changed in order to elute the terpenes quickly from the column.

To avoid peak tailing, which easily occurs at the low concentrations used, the internal standard in the extracts should be an apolar compound. As it should also be completely absent from ambient air, perdeuterooctane was chosen.

Calibration graphs

To obtain calibration graphs, eight standard solutions of mixture of 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5 and 10 ng μl^{-1} benzene, toluene, ethylbenzene, *m*-/*p*-xylene and *o*-xylene and a constant concentration of 5 ng μl^{-1} internal standard (perdeuterooctane) were injected. Then the ratios of the peak area of the MAH to that of the internal standard were plotted against corresponding concentrations for each aromatic compound. In this way, five linear eight-point calibration graphs with

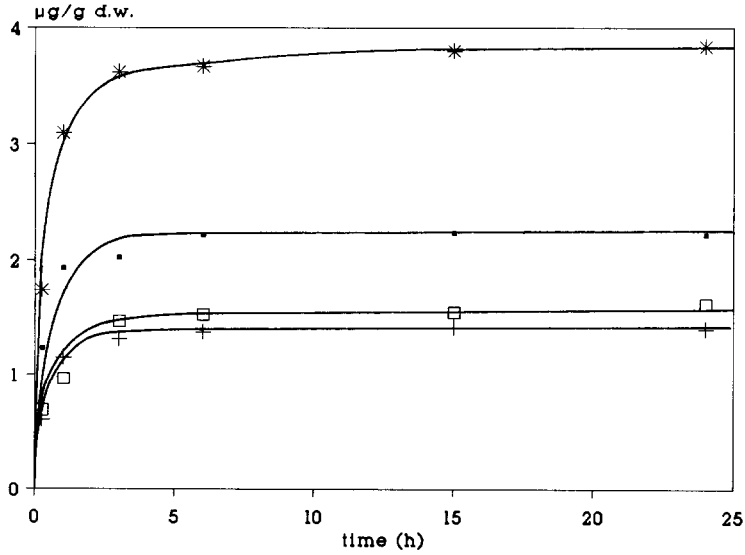

Fig. 1. Concentrations of (■) toluene, (+) ethylbenzene, (*) *m*-/*p*-xylene and (□) *o*-xylene in *Pseudotsuga menziesii* (Mirb.) Franco needles ($\mu g\ g^{-1}$ dry weight) as a function of the extraction time.

TABLE II

CONCENTRATIONS OF MAH IN LEAVES OF SIX *PSEUDOTSUGA MENZIESII* (MIRB.) FRANCO TREES AND SIX *COTONEASTER DAMMERI* SCHN. "SKOGHOLM" SHRUBS ($\mu g\ g^{-1}$ DRY WEIGHT)

Compound	*Pseudotsuga menziesii* (Mirb.) Franco												*Cotoneaster dammeri* Schn. "Skogholm"					
	1-year-old needles						2-year-old needles						1	2	3	4	5	6
	1	2	3	4	5	6	1	2	3	4	5	6						
Benzene	–	–	–	–	–	–	–	–	–	–	–	–	0.30	0.44	0.48	0.60	0.38	0.74
Toluene	3.26	3.24	4.46	3.27	3.56	3.58	3.48	2.05	4.48	2.51	3.06	2.92	0.03	0.04	0.03	0.06	0.05	0.08
Ethylbenzene	2.15	1.75	2.45	1.80	1.78	1.86	4.67	2.37	4.95	2.47	4.22	2.39	–	–	–	–	–	–
m-p-Xylene	4.69	3.73	5.25	4.35	3.89	4.06	10.15	5.46	10.95	5.31	9.06	5.64	–	–	–	–	–	–
o-Xylene	2.36	1.86	2.77	2.19	1.69	1.78	5.38	2.92	5.96	2.75	4.14	2.47	–	–	–	–	–	–

correlation coefficients of 0.999 were obtained. The relative standard deviations of the ratios for 1 ng μl^{-1} were determined to vary from 1.6 to 3% for five injections.

Detection limits

Detection limits of the MAHs were determined by diluting the standard solution until signal-to-noise ratio of 3 was reached. In this way, detection limits of 50 pg μl^{-1} for ethylbenzene, *m*-/*p*-xylene and *o*-xylene and 10 pg μl^{-1} for benzene and toluene were obtained (the relative standard deviations varied from 10.2 to 13.9% for five injections).

Analysis of plant extracts

Extraction efficiency. The amount of aromatic compounds that is extracted from the leaves is dependent on the extraction time. The most efficient extraction time was determined by analysing samples of dichloromethane that had been in contact with *Pseudotsuga menziesii* (Mirb.) Franco needles for 0.25, 1, 3, 6, 15 and 24 h. Fig. 1 shows that the extraction is completed after 6 h.

To understand why this long extraction time is necessary, the morphological structure of the plant cuticle should be considered. The plant cuticle is composed of different layers: an outermost epicuticular wax layer, soluble in organic solvents, and the cuticle proper and cuticular layers, which are insoluble in organic solvents [12]. As 10–30 s are sufficient to extract the epicuticular wax layer from leaves [12], and as in these experiments 6 h are necessary to extract MAHs, it seems that only a very small fraction of the MAHs can be absorbed in the epicuticular wax layer. The largest fraction of the MAHs can be assumed to be absorbed in the cuticle proper and the cuticular layers. The cuticle proper consists of insoluble polymeric cutin, whereas the cuticular layers are composed of a polymeric structure of cutin and cellulose, in which wax and pectin are embedded [12,13]. The extraction time of 6 h can thus be rationalized as the time necessary for the MAHs to migrate out of this polymeric structure into the dichloromethane solution.

Concentrations. With the method described, concentrations of MAHs in 1- and 2-year-old needles of six different *Pseudotsuga menziesii* (Mirb.) Franco trees and in the leaves of six different *Cotoneaster dammeri* Schn. "Skogholm" shrubs were determined (Table II).

In needle extracts of *Pseudotsuga menziesii* (Mirb.) Franco, benzene could not be detected. The xylenes were found in the highest concentrations (up to 10 μg/g of needle dry weight). Differences in concentrations between the needles of six *Pseudotsuga menziesii* (Mirb.) Franco trees and between 1- and 2-year-old needles are observed. Especially the concentrations of ethylbenzene, *m*-/*p*-xylene and *o*-xylene are higher in 2- than in 1-year-old needles from the same tree. In leaves of *Cotoneaster dammeri* Schn. "Skogholm", only benzene (0.3–0.7 $\mu g\ g^{-1}$ dry weight) and toluene (0.03–0.08 $\mu g\ g^{-1}$ dry weight) could be detected. Differences in MAH content between the leaves from six *Cotoneaster* shrubs can also be noticed.

CONCLUSION

Using the proposed method, differences in the concentrations of benzene, toluene, ethylbenzene, *m*-/*p*-xylene and *o*-xylene, absorbed in 1- and 2-year-old needles

of different *Pseudotsuga menziesii* (Mirb.) Franco trees and in leaves of different *Cotoneaster dammeri* Schn. "Skogholm" shrubs could be determined.

Apparently, the absorption of monocyclic aromatic compounds is dependent on the plant species, the individual plant and the age of the leaves. Further investigations are necessary to find the causes of these differences. Also, experiments on any possible degradation of MAHs in plants are necessary in order to determine elimination rates of MAHs from air by plants.

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