ELSEVIER

Journal of Chromatography A, 774 (1997) 213–221

JOURNAL OF CHROMATOGRAPHY A

Headspace gas chromatographic determination of the plant cuticle–air partition coefficients for monocyclic aromatic hydrocarbons as environmental compartment

Regine Keymeulen*, Gert De Bruyn, Herman Van Langenhove

Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, University of Ghent, Coupure Links 653, B-9000 Ghent, Belgium

Abstract

The plant cuticle is considered to be an important environmental compartment for gaseous air pollutants. Therefore, a method was developed to determine the partition coefficients of monocyclic aromatic hydrocarbons, such as benzene, toluene, ethylbenzene and xylene isomers, between isolated plant cuticles and air ($K_{C/A}$), using equilibrium partitioning in closed vials. Dimensionless $K_{C/A}$ [mol l^{-1} (mol l^{-1})$^{-1}$] values in the range from 130 to 4000 for isolated cuticles of *Hedera helix* L. and *Buxus sempervirens* L. were obtained. Up to an air concentration of 3500 μg l^{-1}, the $K_{C/A}$ was found to be constant. The equilibrium between air and cuticles was reached within 30 min. Temperature dependence of $K_{C/A}$ was studied in the range from −5°C to 55°C. Ln $K_{C/A}$ was found to vary with $1/T$ according to a polynomial of second order. Also, a linear regression line between log $K_{O/A}$ (octanol–air partition coefficient) and log $K_{C/A}$ could be calculated.

Keywords: Partition coefficients; Plant uptake; Air analysis; Monocyclic aromatic hydrocarbons; Benzene; Toluene; Ethylbenzene; Xylenes; Volatile organic compounds

1. Introduction

Justified attention has been given to the distribution of chemicals in the environment: once released, chemicals are distributed over the different environmental compartments (air, water, soil and biota), where they can be degraded, transformed or accumulated. Plant biomass, belonging to the compartment "biota", is believed to play a significant role in the environmental fate of pollutants, considering that 80% of the earth's land surface is covered by vegetation [1]. In fact, the three main pathways for uptake of chemicals in leaves involves (i) translocation from contaminated soils via roots, (ii) atmospheric deposition of contaminated particulates and (iii) vapour intake from the air [2]. With regard to the volatile compounds, the direct air-to-leaf transfer can be considered the key process of sorption. In earlier reported research, the concentrations of an important group of volatile organic compounds, namely the monocyclic aromatic hydrocarbons (MAHs), were determined in plant leaves that were sampled in an area polluted by traffic exhaust [3,4]. The leaf concentrations were found to be proportional with the air concentrations, suggesting that plant leaves could be used as biomonitors for air pollution [4].

All above ground parts of plants are covered by an extracellular lipophilic layer, the plant cuticle, which constitutes a barrier between the plant part and its surrounding environment [5]. It is estimated that cuticle material makes up 180–1500 kg/hectare of

*Corresponding author.

PII S0021-9673(97)00168-4

temperate forests and agricultural plant communities [6]. As the plant cuticle is the leaf constituent that is in direct contact with ambient air, volatile chemicals can directly diffuse into this lipophilic layer. A few authors already stated that the plant cuticle is an important sorption compartment for lipophilic atmospheric pollutants [1,6]. However, the contribution of the plant cuticle to the sorption of organic pollutants from air into the plant leaves has never been quantified up to now. A quantification of the importance and efficiency of the plant cuticle as a sorption compartment for volatile organic compounds could be derived from the knowledge of the partition coefficient between cuticle and air. Such a partition coefficient expresses the partitioning of a population of molecules of a substance between two phases and is calculated as the ratio of the concentration of the compound in two phases at equilibrium:

$$K_{C/A} = \frac{C_C}{C_A} \quad (1)$$

with $K_{C/A}$ the dimensionless partition coefficient [mol l^{-1} (mol l^{-1})$^{-1}$] between cuticles and air, C_C the concentration of the pollutant in the cuticle (mol l^{-1}) and C_A the concentration of the pollutant in the air (mol l^{-1}). Partition coefficients depend on physicochemical properties of both compound and phase and on physical parameters such as temperature.

It must be remarked that for the system plant cuticle–aqueous solution, partition coefficients of organic chemicals with phytopharmaceutical applications (pesticides) have already been determined [7–9].

In this paper, a method is described to determine the partition coefficients between plant cuticles and air for the monocyclic aromatic hydrocarbons (MAHs) (benzene, toluene, ethylbenzene, *m*-, *p*- and *o*-xylene), an important group of volatile organic pollutants. This method is based on gas phase equilibrium of target compounds in closed vials and their analysis by headspace gas chromatography.

Cuticle–air partition coefficients ($K_{C/A}$) were determined using isolated cuticles of two plant species: *Hedera helix* L. and *Buxus sempervirens* L. The dependence of $K_{C/A}$ on the concentration was investigated and the rate of equilibrium establishment between air and cuticle was determined. Also, the temperature dependence was investigated over the range from −5°C to 55°C. The $K_{C/A}$ values were also compared with the *n*-octanol–air partition coefficients ($K_{O/A}$) for the same compounds, so as to confirm *n*-octanol as a suitable reference phase for the plant cuticle behaviour and properties.

2. Experimental

2.1. Isolation of plant cuticles

Two plant species, namely *Hedera helix* L. and *Buxus sempervirens* L., were used for cuticle isolation. The cuticles of these species are relatively easy to separate enzymatically from the underlying cell material. Leaf discs, with a diameter of 1.8 cm for *Hedera* and 0.9 cm for *Buxus*, were punched out of the leaves with a cork borer. They were infiltrated under vacuum and incubated in a buffer solution (phthalic acid–KOH) at pH 3.6, containing 4% pectinase and 0.4% cellulase. The solution also contained 1 m*M* NaN_3 to avoid microbiological growth in the solution. In this way, cell structures of the underlying tissue of the cuticles were enzymatically degraded. The incubation lasted for ten days and the enzymatic solution was regularly stirred. Then the cuticles with rests of underlying cell material were transferred into a beaker with distilled water. A magnetic stirrer was used to stir the solution vigorously so as to separate the cell material from the cuticles. The pieces of cell material were removed from the solution by filtrating it over a coarse sieve and after a few repetitions of this rinsing procedure, clean cuticles were obtained. They were air dried, weighed and stored under a continuous flow of pure air to avoid any sorption of chemicals that were present in the laboratory air.

2.2. Preparation of closed vials

Penicillium bottles with a volume of 118 ml were filled with 200 cuticles of *Hedera helix* (isolated from 100 leaf discs) or with 600 cuticles of *Buxus sempervirens* (isolated from 300 leaf discs). The bottles were closed with Mininert stoppers (Alltech Ass.) and covered with aluminium foil to avoid

photochemical degradation reactions of the pollutants in the bottles. The gaseous pollutants were added to the bottles by injecting a real volume of saturated vapour (at 25°C) of each MAH with a gastight syringe (series A syringe with valve, Alltech Ass.) through the Mininert stopper. In the case of *m*- and *p*-xylene, approximately half of the amount of the other MAHs was injected, since these two compounds were co-eluting in the GC analysis. The saturated vapour of each MAH was generated in a closed vial provided with a septum and containing a few ml of the pure compound in the liquid phase. The vials were incubated in a thermostatic waterbath at 25°C.

Next to the bottles filled with cuticles, the same volume of saturated vapour of each MAH was injected into empty penicillium bottles provided with Mininert stoppers and covered with aluminium foil. For each determination of the partition coefficient of the MAHs, a series of five bottles with cuticles and a series of five empty bottles were identically prepared. By combining the five measurements using the bottles with cuticles and the five measurements using the empty bottles, 25 values for $K_{C/A}$ could be directly calculated (see Section 2.4).

To determine the $K_{C/A}$ at various MAH concentrations, a reference temperature of 25°C was chosen. Therefore, the vials were placed in a thermostatic waterbath at 25°C (±0.1°C).

The temperature dependence of $K_{C/A}$ was studied using a waterbath provided with a heating unit for the measurements at 25, 35, 46 and 55°C. To reach a temperature of 15, 5 and −5°C, the thermostatic waterbath was filled with ethyleneglycol and a cooling unit was provided.

2.3. Analytical procedure and parameters

To determine the MAH concentrations in the headspace of the penicillium bottles, 500 μl of air was withdrawn with a 500 μl gastight syringe (series A syringe with valve, Alltech Ass.) and immediately injected into a Varian type 3700 GC equipped with a flame ionization detector and connected to a HP 3388A integration unit. The GC was provided with a 30 m×0.53 mm I.D. fused-silica capillary column with a 5 μm thick layer of a 100% dimethylpolysiloxane stationary phase (DB-1, J and W Scientific). Carrier gas was helium at a flow-rate of 4 ml min^{-1}. Injector and detector temperature were 220°C and 250°C, respectively. Splitless injection was used. The temperature of the GC oven was programmed as follows: initial temperature 50°C, rate 4°C min^{-1}, final temperature 200°C.

2.4. Calculation of $K_{C/A}$

The mass balance in the closed vial with cuticles and air is given by

$$M_{tot} = C_C V_C + C_A V_A \quad (2)$$

with M_{tot} the total amount of MAH in the closed vial (mol), C_C the concentration of MAH in the cuticles (mol l^{-1}), V_C the volume of cuticles (l) (see Section 2.5), C_A the concentration of MAH in the air (mol l^{-1}) and V_A the volume of air in the vial (l). In the closed vial without cuticles, the same amount of compound was added, so in this case the mass balance is given by

$$M_{tot} = C_{A,tot} V_{tot} \quad (3)$$

with $C_{A,tot}$ the MAH concentration in the empty penicillium bottle (mol l^{-1}) and V_{tot} the total volume of the penicillium bottle (l).

Combining Eqs. (2) and (3) results in

$$C_{A,tot} V_{tot} = C_C V_C + C_A V_A \quad (4)$$

Substituting Eq. (1) in Eq. (4) gives

$$C_{A,tot} V_{tot} = K_{C/A} C_A V_C + C_A V_A \quad (5)$$

or, after rearranging,

$$K_{C/A} = \frac{C_{A,tot} V_{tot}}{C_A V_C} - \frac{V_A}{V_C} \quad (6)$$

Since C_A as well as $C_{A,tot}$ are both determined by injecting 500 μl of air into the GC, the ratio of $C_{A,tot}/C_A$ is equal to the ratio of the integration units obtained for the respective analyses.

This means that $K_{C/A}$ can be directly calculated without having the disposal of a calibration graph for the MAHs using the equation:

$$K_{C/A} = \frac{IU_{A,tot} V_{tot}}{IU_A V_C} - \frac{V_A}{V_C} \quad (7)$$

with $IU_{A,\,tot}$ and IU_A the integration units of MAHs obtained for the analysis of 500 μl air that was withdrawn from the vial without cuticles and from the vial containing the isolated plant cuticles, respectively. By combining the five measurements using the bottles with cuticles and the five measurements using the empty bottles two by two, 25 values for $K_{C/A}$ can be calculated.

However, in order to plot the values of C_C versus the values of C_A at different concentrations, the absolute values of the concentrations in the cuticles and those of the concentrations in the air (C_A and $C_{A,\,tot}$) have to be known. In this case, the conversion of integration units ($IU_{A,\,tot}$ and IU_A) into concentrations for both vials with and without cuticles was performed using the calibration graphs of MAHs obtained by liquid injecting of a series of standard solutions of MAHs in dichloromethane. The value for the concentration of MAHs in the cuticles (C_C) can then be calculated from the knowledge of the total amount of MAH injected in the vial ($M_{tot} = C_{A,tot}V_{tot}$), the volume of the cuticles (V_C) and the air concentration in the vial with cuticles (C_A), by rearranging Eq. (4) into:

$$C_C = \frac{C_{A,tot}V_{tot} - C_A V_A}{V_C} \tag{8}$$

To get a reliable value for C_C, the measurements were carried out using five bottles with cuticles and five bottles without cuticles, which were identically prepared and analysed. By combining the value obtained for $C_{A,\,tot}$ from one particular empty bottle with the value obtained for C_A from one particular bottle filled with cuticles, a value for C_C can be calculated. The combination of the results for the air concentrations in five bottles without cuticles and five bottles with cuticles (two by two) gives 25 values for C_C, of which the mean value and the standard deviation can be calculated.

This procedure of C_C determination was repeated for different amounts of MAHs injected into the bottles, in order to plot C_C versus C_A. In this plot, the error bars ($x \pm$S.D.) for C_A are based on five repetitions and the error bars for C_C are based on 25 repetitions.

2.5. Determination of cuticle volume (V_C)

The volume of the cuticles is calculated by

$$V_C = \frac{m_C}{\rho_C} \tag{9}$$

with V_C the cuticle volume (l), m_C the mass of the cuticles (kg) and ρ_C the density of the cuticles (kg l^{-1}). The density of both *Hedera* and *Buxus* cuticles was determined using a pycnometer. This determination is based on the gravimetric measurement of the mass of the cuticles and the calculation of the volume from the mass and density of the water that was displaced by the cuticles in the pycnometer.

For each plant species, three measurements were carried out. The mean density for *Hedera helix* was found to be 0.442 ± 0.143 (kg l^{-1}) and for *Buxus sempervirens* 1.041 ± 0.191 (kg l^{-1}).

2.6. Desorption of cuticles used in sorption experiments

Since the isolation of cuticles is very time-consuming, a method was developed to desorb or regenerate the cuticles that were used in the sorption experiments, so that they could be used for another sorption experiment. Therefore, the cuticles from each penicillium bottle were transferred into stripping vessels, through which a flow of pure air was passed at a flow-rate of 100 to 200 ml min^{-1} during 24 h at room temperature. This desorption process was found to be sufficient to remove all sorbed MAHs from the cuticles.

3. Results and discussion

3.1. Equilibrium establishment of MAHs between cuticles and air

In a series of preliminary sorption experiments of MAHs in *Hedera* cuticles, the MAH air concentrations in five closed vials containing cuticles and air, were measured at 13, 20 and 72 h after the injection of the MAHs into the closed vials. It was found that the MAH air concentrations were remarkably lower than those in the vials without cuticles, but that the air concentrations did not change significantly during the considered time interval. From this it was concluded that the sorption of pollutants in isolated plant cuticles must be a fast

process and that equilibrium is established at least within 13 h after addition of the chemicals to the two phase system.

Based on these results, a second series of experiments with one bottle containing *Hedera* cuticles was set up. In each experiment, only one MAH was injected into the vial in order to allow that the first measurement of the remaining air concentration in the vial was carried out as shortly after the addition of the MAH as possible. For the same reason, the vial was not placed in the thermostatic waterbath at 25°C, but it was manipulated at room temperature (±22°C). Even 60 s after injection, the MAH air concentration in the vial with the cuticles was already lower than in the corresponding reference empty vial. This means that immediately after addition of the MAH to the vial, the cuticles already start sorbing the chemical. The variation of the MAH air concentrations in the vial with cuticles as a function of time is shown in Fig. 1. For benzene and toluene, the equilibrium between isolated cuticles and air is reached within a period of 15 min, whereas for ethylbenzene, *m,p*-xylene and *o*-xylene, this takes around 30 min.

It must be remarked that as the cuticles had been isolated, they can be entered by the chemicals from both sides. It can therefore be assumed that the equilibrium establishment between non-isolated cuticles and air will last longer, but that it still must be in the order of a few hours.

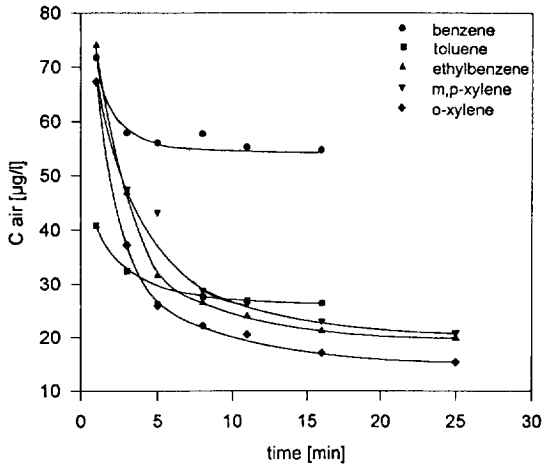

Fig. 1. MAH residue concentrations in air in a closed vial containing *Hedera helix* cuticles, as function of the equilibration time.

3.2. Cuticle–air partition coefficients at 25°C

The cuticle–air partition coefficients of benzene, toluene, ethylbenzene, *m,p*-xylene and *o*-xylene were determined at a constant temperature of 25°C for isolated cuticles of *Hedera helix* and *Buxus sempervirens*. This was done at various concentrations in order to investigate in which concentration interval of C_A the partition coefficient $K_{C/A}$ remained a constant value. Indeed, it may be presumed that by the injection of an increasing total amount of MAH in the closed two phase system, at a certain moment the cuticles will get saturated, resulting in a maximum concentration $C_{C,sat}$ of MAH in the cuticles.

In Figs. 2 and 3, the concentration of MAHs in the isolated cuticles (C_C) of *Hedera* and *Buxus*, respectively, is plotted against the corresponding air concentration (C_A) in the vials. The range of C_A for benzene, toluene, ethylbenzene, *m,p*-xylene and *o*-xylene, respectively is 18–293 µg l^{-1}, 60–120 µg l^{-1}, 21–93 µg l^{-1}, 22–87 µg l^{-1}, 14–60 µg l^{-1} for *Hedera helix* cuticles and 13–159 µg l^{-1}, 14–111 µg l^{-1}, 8.5–37 µg l^{-1}, 8.1–32 µg l^{-1}, 5–22 µg l^{-1} for *Buxus sempervirens* cuticles.

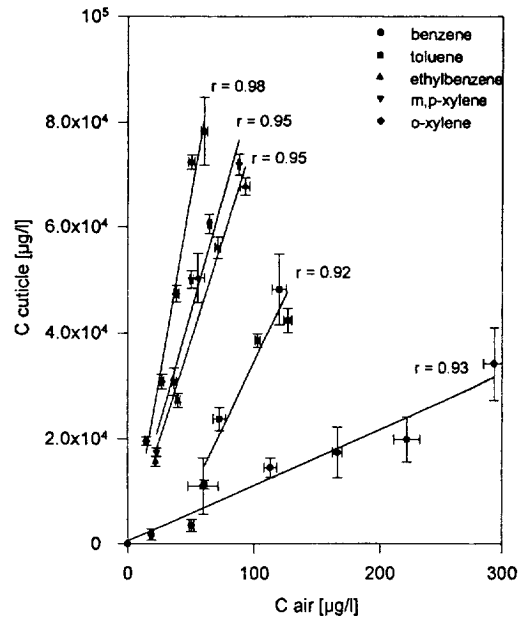

Fig. 2. MAH concentrations in *Hedera helix* cuticles versus corresponding MAH air concentrations in closed vials at equilibrium (error bars from $x-$S.D. to $x+$S.D.) with regression lines and correlation coefficients.

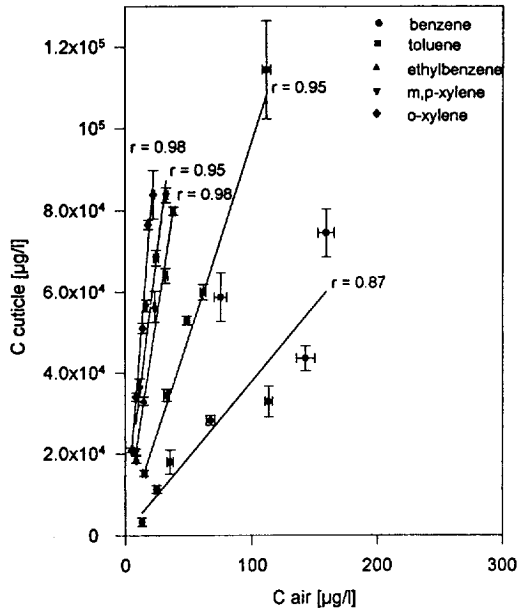

Fig. 3. MAH concentrations in *Buxus sempervirens* cuticles versus corresponding MAH air concentrations in closed vials at equilibrium (error bars from $x-$S.D. to $x+$S.D.) with regression lines and correlation coefficients.

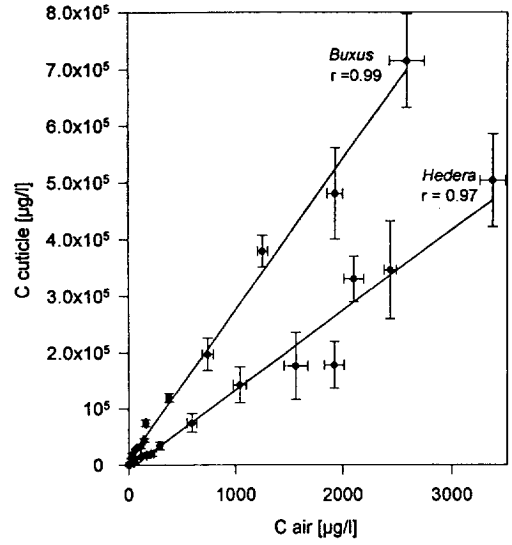

Fig. 4. Benzene concentrations in *Hedera helix* and *Buxus sempervirens* cuticles versus corresponding air concentrations of benzene in closed vials at equilibrium (error bars from $x-$S.D. to $x+$S.D.) with regression lines and correlation coefficients.

It can be noticed that for none of the compounds saturation of the cuticles has been reached. Therefore, for the most volatile of the MAHs (benzene) the concentration range was increased up to the tenfold (Fig. 4). Still, correlation coefficients of the regression lines between C_C and C_A remained high (0.97 and 0.99 for *Hedera* and *Buxus*, respectively), so no evidence was found that in the concentration range used the partition coefficient started to deviate from a constant value.

The partition coefficients $K_{C/A}$ of the MAHs, calculated as the mean of the values obtained at different MAH air concentrations for the isolated cuticles of *Hedera helix* and *Buxus sempervirens*, are given in Table 1. When the dimensionless values of $K_{C/A}$ are compared, it can be noticed that $K_{C/A}$ for *Buxus* is about 2.7 to 3.3 times larger than $K_{C/A}$ for *Hedera*, which means that $K_{C/A}$ is dependent on the plant species. The partition coefficients can also be expressed per cuticle mass instead of cuticle volume [(mol kg^{-1}) (mol l^{-1})$^{-1}$], which means that the $K_{C/A}$ value is divided by the density of the cuticles. If these values for $K'_{C/A}$ in Table 1 (expressed in [(mol kg^{-1}) (mol l^{-1})$^{-1}$]) are compared, the partition coefficients for both species closely approach each other. This is an indication that the mass of cuticle present in the vials is determining the partitioning, rather than the cuticle volume. It can therefore be assumed that the uptake of pollutants in cuticles is rather a sorption process into the cuticle tissue than an adsorption process onto the internal surface of the cuticles.

3.3. Temperature dependence of $K_{C/A}$

The temperature dependence of $K_{C/A}$ was investigated for cuticles of *Hedera helix* in the temperature range from -5°C to 55°C.

In accordance with the temperature dependence of other partition coefficients such as $K_{A/W}$ (air–water partition coefficient) [10] or $K_{O/A}$ (*n*-octanol–air partition coefficient) [11], the relation between $K_{C/A}$ and the temperature could be expected to be given by

$$\ln K_{C/A} = a \cdot \frac{1}{T} + b \qquad (10)$$

with $1/T$ the inverse of the absolute temperature and a and b respectively the slope and the intercept of

Table 1
Partition coefficients $K_{C/A}$ [mol l^{-1} (mol l^{-1})$^{-1}$] and $K'_{C/A}$ [mol kg^{-1} (mol l^{-1})$^{-1}$] between cuticles and air for *Hedera helix* and *Buxus sempervirens* [mean with standard deviation (S.D.)] at 25°C

	$K_{C/A}$ [mol l^{-1} (mol l^{-1})$^{-1}$]				$K'_{C/A}$ [mol kg^{-1} (mol l^{-1})$^{-1}$]			
	Hedera		*Buxus*		*Hedera*		*Buxus*	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Benzene	128	40	343	50	282	88	325	48
Toluene	369	38	1002	89	823	84	877	107
Ethylbenzene	764	66	2214	162	1752	152	2135	157
m,p-Xylene	880	58	2941	269	1990	115	2826	250
o-Xylene	1317	107	3978	296	2977	250	3816	288

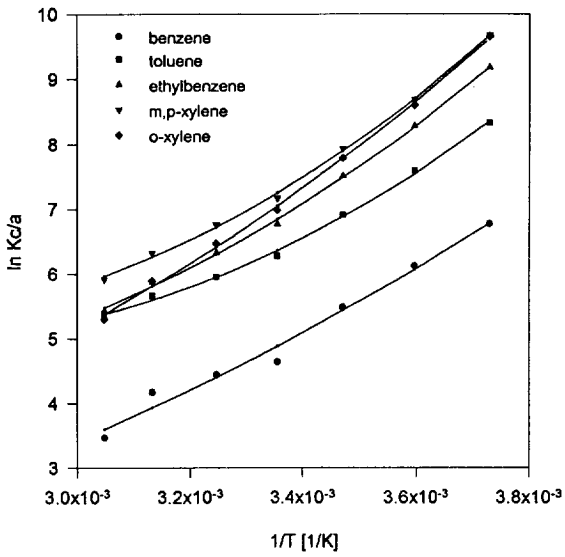

Fig. 5. Ln $K_{C/A}$ of *Hedera helix* cuticles as a function of the inverse absolute temperature ($1/T$) for the five MAHs with regression of polynomial of second order according to Eq. (11).

the linear regression line between ln $K_{C/A}$ and $1/T$. Within small temperature intervals, the slope a is equal to $-\Delta H/R$, with ΔH the enthalpy of phase change between air and cuticle and R the gas constant [12].

The variation of ln $K_{C/A}$ for *Hedera helix* cuticles as a function of $1/T$ is plotted in Fig. 5. It can be noticed that ln $K_{C/A}$ is increasing to a larger extent than could be expected from the linear relation expressed by Eq. (10). To describe the variation of ln $K_{C/A}$ mathematically, a linear regression line (i) was compared with (ii) two crossing regression lines, (iii) a polynomial of second order and (iv) a polynomial of third order. In order to evaluate which of these mathematical functions gave the best fit, the sum of squares of the residuals was calculated for each function and is reported in Table 2. It can be seen that SSE for the polynomial of second order is lower than for both the linear regression with one line or with two crossing lines. Moreover, the fit of a polynomial of third order is not resulting in an

Table 2
Sum of squares of residuals (SSE) for (i) a linear regression line, (ii) two crossing linear regression lines, (iii) a polynomial of second order and (iv) a polynomial of third order to describe the relation between ln $K_{C/A}$ and $1/T$ and the values for parameters A, B and C in Eq. (11)

	SSE				Parameters in Eq. (11)		
	Lin. regr.	2 Cr. lines	Polynom. 2nd order	Polynom. 3rd order	A ($\times 10^6$)	B ($\times 10^4$)	C
Benzene	0.17	0.13	0.13	0.13	1.282	−4.031	3.98
Toluene	0.15	0.12	0.02	0.02	3.071	−1.645	26.99
Ethylbenzene	0.11	0.08	0.01	0.01	2.584	−1.208	18.27
m,p-Xylene	0.17	0.14	0.01	0.01	3.316	−1.705	27.12
o-Xylene	0.08	0.07	0.02	0.01	2.044	−7.626	9.63

additional decrease of SSE. It can therefore be concluded that a polynomial of second order gives the best description of the relation between ln $K_{C/A}$ and $1/T$, which can be expressed by the equation

$$\ln K_{C/A} = A\left(\frac{1}{T}\right)^2 + B\left(\frac{1}{T}\right) + C \quad (11)$$

The values for the parameters A, B and C for each compound are also given in Table 2.

This can be thermodynamically interpreted as follows: when the relation between ln K and $1/T$ is described by a linear regression line, ΔH is a constant value over the entire temperature interval. In this case the increase of ln K with decreasing temperature is a consequence of the decrease in thermic agitation energy of the molecules in the two phase system. A faster increase of ln K than determined by a linear relation with $1/T$ implies that ΔH is no longer constant over the considered temperature interval. As a reason for this variation of the enthalpy of phase change between air and cuticles, a change in cuticle properties and thus in sorption behaviour as a function of temperature can be suggested.

3.4. Comparison of $K_{C/A}$ with $K_{O/A}$

In accumulation studies of non-polar organic chemicals into living organisms, *n*-octanol is often used as a surrogate for the organisms. The uptake of non-polar chemicals into *n*-octanol is considered to be proportional to that into organisms, with the consequence for a series of compounds a high uptake in *n*-octanol should correspond to a more favorable partitioning into the organisms.

In the case of partitioning of non-polar chemicals between the lipophilic cuticle and air, it can be presumed that the *n*-octanol–air partition coefficient ($K_{O/A}$) must be proportional to $K_{C/A}$. According to Schwarzenbach et al. [12], there is a relation between the partition coefficients of two different organic solvents for a given series of compounds that undergo the same type of interactions with both organic solvents. In the case that *n*-octanol is chosen as a reference solvent, the partition coefficient $K_{S/A}$, which expresses the equilibrium partitioning of a chemical between a solvent S and air, is related with the partition coefficient between *n*-octanol and air according to

$$\log K_{S/A} = c\log K_{O/A} + d \quad (12)$$

The parameters (slope, intercept and correlation coefficient) of this linear regression between the cuticle–air partition coefficient for *Hedera helix* and *Buxus sempervirens* cuticles and the *n*-octanol–air partition coefficient (as reported in literature [12]) for benzene, toluene, ethylbenzene, *m,p*-xylene and *o*-xylene is given in Table 3. The slope c is in both cases very close to 1. From theoretical thermodynamic principles [12], the slope of this linear regression line is proportional to the ratio of the partial molar excess free energies in the solvent and that in *n*-octanol ($\Delta G^x_{S/A}/\Delta G^x_{O/A}$) and the intercept is proportional to $\Delta G^C_{S/A}$. $\Delta G^C_{S/A}$ and $\Delta G^x_{S/A}$ respectively refer to the free energy of transfer of the central structure C of the molecule (the stem of the compound) on which the moiety x is attached and to the free energy of transfer of the moiety x itself. In the case of the monocyclic aromatic hydrocarbons, x corresponds to a methyl group. A value of $c=1$ means that substitution of a hydrogen atom on the benzene ring by a methyl group has the same effect on the partial molar excess free energies of the compound in both organic solvents. A value of $c<1$ means that the methyl group substitution renders the molecule more incompatible with the solvent as compared with the reference solvent *n*-octanol. In the case of the plant cuticles, a slope of 1.01 and 0.96 for *Hedera* and *Buxus*, respectively, were found, which means that for both cuticles a methyl group substitution on the chemicals has the same effect on the partial molar excess free energies as compared with *n*-octanol. It can thus be concluded that there is a good correspondence between the properties of *n*-octanol and the plant cuticle.

Table 3
Parameters of the linear regression line between log $K_{C/A}$ and log $K_{O/A}$ for *Hedera helix* and *Buxus sempervirens* according to Eq. (12)

	Linear regression line of log $K_{C/A}$ vs. log $K_{O/A}$		
	Slope (c)	Intercept (d)	Correlation (r)
Hedera helix	0.96	−0.57	0.997
Buxus sempervirens	1.01	−0.27	0.997

4. Conclusions

A method to determine the partition coefficients of monocyclic aromatic hydrocarbons between isolated plant cuticles of *Hedera helix* and *Buxus sempervirens* and air was developed. This method is based on equilibrium partitioning between cuticles and air of the compounds in closed vials, and successive determination of target compounds by headspace gas chromatographic analysis. The establishment of the dynamic equilibrium between cuticles and air was found to take approximately 15 min for benzene and toluene and 30 min for ethylbenzene, *m,p*-xylene and *o*-xylene. The dimensionless $K_{C/A}$ values of MAHs [(mol l^{-1}) (mol $l^{-1})^{-1}$)] at 25°C were determined to range from 130 to 4000 depending on the compound and on the plant species. Experiments on the temperature dependence of $K_{C/A}$ for *Hedera* cuticles showed that a temperature drop of 60°C increased the $K_{C/A}$ by a factor of 30 to 40. The relation between ln $K_{C/A}$ and $1/T$ was found to be described best by a polynomial of second order. This implies that the enthalpy of phase change ΔH varies with temperature. A linear regression line between log $K_{C/A}$ and log $K_{O/A}$ could be calculated for both plant species. The slope of these lines approximated 1, indicating that within the series of compounds from benzene to the xylenes, the substitution of a methyl group has the same effect on the partial molar excess free energies in the cuticle as compared to *n*-octanol.

Acknowledgments

The National Fund for Scientific Research Belgium is gratefully acknowledged for providing a senior research assistant fellowship for R.K.

References

[1] S.L. Simonich and R.A. Hites, Environ. Sci. Technol., 28 (1994) 939.
[2] S.L. Simonich and R.A. Hites, Environ. Sci. Technol., 29 (1995) 2905.
[3] R. Keymeulen, H. Van Langenhove and N. Schamp, J. Chromatogr., 541 (1991) 83.
[4] R. Keymeulen, N. Schamp and H. Van Langenhove, Atmos. Environ., 27A (1993) 175.
[5] P.J. Holloway, in D.F. Cutler, K.L. Alvin and C.E. Price (Editors), The Plant Cuticle, Academic Press, New York, 1982, pp. 1–32.
[6] M. Riederer, Environ. Sci. Technol., 24 (1990) 829.
[7] F. Kerler and J. Schönherr, Arch. Environ. Contam. Toxicol., 17 (1988) 1.
[8] E. Chaumat and A. Chamel, Plant Physiol. Biochem., 28 (1990) 719.
[9] M. Riederer and J. Schönherr, Ecotoxicol. Environ. Saf., 8 (1984) 236.
[10] J. Dewulf, D. Drijvers and H. Van Langenhove, Atmos. Environ., 29 (1995) 323.
[11] T. Harner and D. Mackay, Environ. Sci. Technol., 29 (1995) 1599.
[12] R.P. Schwarzenbach, P. Gschwend and D.M. Imboden, Environmental Organic Chemistry, Wiley, New York, 1993.