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Determination of partition coefficients of monocyclic aromatic hydrocarbons between leaf essential oil and air by headspace gas chromatography

Regine Keymeulen[a,*], Bart Parewijck[a], Anna Górna-Binkul[b], Herman Van Langenhove[a]

[a]*Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, University of Ghent, Coupure 653, B-9000 Ghent, Belgium*

[b]*Department of Environmental Chemistry, Faculty of Chemistry, Nicholas Copernicus University, Gagarin Str 7, PL-87-100 Torun, Poland*

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Abstract

Partition coefficients of monocyclic aromatic hydrocarbons (MAHs) between leaf essential oil of nine plant species and air ($K_{EO/A}$) were determined using equilibrium partitioning in closed vials and headspace gas chromatography. $K_{EO/A}$ values [mol l^{-1} (mol l^{-1})$^{-1}$] ranged from 68 for benzene in *Taxus baccata* L. to 6038 for *o*-xylene in *Juniperus x media* Van Melle essential oil. Linear regression lines between log $K_{EO/A}$ and log $K_{O/A}$ (*n*-octanol/air partition coefficient) of the MAHs for the nine plant species were calculated. Temperature dependence of $K_{EO/A}$ was investigated in the temperature range from 5°C to 45°C and a linear relation between ln $K_{EO/A}$ and $1/T$ was found.

Keywords: Partition coefficients; Plant uptake; Monocyclic aromatic hydrocarbons

1. Introduction

Partition coefficients are used to describe the distribution of a substance among different media. They express the equilibrium partitioning of a population of molecules between two phases and are calculated as the ratio of the concentration of a compound in one phase to that in the other phase at equilibrium. They can be considered to be independent of concentration, as long as the activity coefficient γ_i of a compound i in the considered phase is not substantially changed [1]. According to Prausnitz [2], this is the case in a liquid phase when the dissolved concentration of the solute remains lower than 3 mole%. Above this concentration, the solute:solute interactions can no longer be neglected.

Partition coefficients depend on physical-chemical properties of both compound and phase and on physical parameters such as temperature. Partitioning of a vapour between water and air for instance, is described by the Henry's Law Constant or air/water partition coefficient ($K_{A/W}$). Since the $K_{A/W}$ is an important parameter in the study of transfer processes of various pollutants in the environment, it has been studied extensively [3–7]. Within the framework of distribution studies of pollutants among the different environmental compartments (air, water, soil, biota), the partition coefficient between leaf essential oil and air ($K_{EO/A}$) can be of interest to estimate the importance of essential oil

*Corresponding author.

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containing plants as sink for air pollutants. Such a partition coefficient has not been reported up to now. Sato and Nakajima [8] measured partition coefficients between air and olive oil at 37°C in order to find correlations with blood/air partition coefficients.

In the present paper a method is described to determine partition coefficients of the monocyclic aromatic hydrocarbons (MAHs: benzene, toluene, ethylbenzene, *p*-xylene and *o*-xylene) between leaf essential oil and air ($K_{EO/A}$), using a closed vial-equilibration technique in combination with headspace gas chromatography. This method was applied to determine $K_{EO/A}$ of nine different plant species, from which the essential oil had been isolated. The $K_{EO/A}$ values obtained for the five compounds and for each plant species were compared with $K_{O/A}$ (octanol/air partition coefficient). Since the equilibrium partitioning of MAHs between air and essential oil can be expected to be a temperature-dependent process, the $K_{EO/A}$ was measured in the temperature range from 5°C to 45°C for the essential oil of one plant species.

2. Experimental

2.1. Procedure of partition coefficient determination

To find a suitable method for the determination of the essential oil/air partition coefficient, methods described in literature to determine $K_{A/W}$ were examined. Since it is very difficult to determine the concentrations of a substance in both phases simultaneously, various methods have been developed to determine $K_{A/W}$ based on the measurement of the gas phase concentration [6,9]. However, the methods used for $K_{A/W}$ determination are not directly applicable to determine the essential oil/air partition coefficient. The EPICS (Equilibrium Partitioning in Closed Systems) method for instance, which was firstly described by Lincoff and Gosset [4] and which was applied successfully for $K_{A/W}$ determination of MAHs and volatile chlorinated hydrocarbons [7,9], only gives a good estimation for K (standard deviation less than 10%) if K is larger than 0.1. Using the formula to calculate the standard deviation of K in the EPICS method, which was proposed by Gossett [9] and adapting it to the vials that were used, resulted in S.D. values of over 100% if K is smaller than 0.001. Inverting the essential oil/air partition coefficient ($K^{-1}_{EO/A}$) gives values between 1×10^{-4} and 10×10^{-4}, which would indeed result in an S.D. with the EPICS method of over 100%.

The method presented in this paper, which was found most suitable for $K_{EO/A}$ determination, is based on equilibrium partitioning in closed vials with direct measurement of the gas phase concentration using gas chromatography.

2.2. Extraction of essential oil

Twigs of nine plant species (*Cedrus atlantica* Man. 'Glauca', *Chamaecyparis lawsoniana* (Murr.) Parl 'Lanae', *Juniperus virginiana* L., *Juniperus x media* Van Melle 'Pfitzeriana aurea', *Lavandula angustifolia* Mill. 'Dwarf Blue', *Pinus nigra* Arnold, *Pinus sylvestris* L., *Pseudotsuga menziesii* (Mirb.) Franco, *Taxus baccata* L. 'Semper Aurea') were collected between September and December 1994. Only the leaves were used for essential oil extraction, so they were picked from the twigs and their fresh weight was determined up to 0.01 g precisely. Immediately after sampling, combined steam distillation-solvent extraction was carried out by means of a modified Likens-Nickerson extraction apparatus. Dichloromethane was used as extraction solvent. About 50 ml of dichloromethane was used to extract samples of 250 g fresh plant material. Depending on the yield of essential oil, the extraction was repeated three, four, or in the case of *Taxus baccata* even five times (with a total mass of 1200 g fresh needles). All dichloromethane extracts of the same species were gathered and the extraction solvent was evaporated from the essential oil in a rotavapor. Until $K_{EO/A}$ was measured, the extracted essential oils were stored in the freezer.

2.3. Preparation of closed vials

Vials of 5.5 ml which can be closed airtightly with Mininert valves (Alltech Ass.), were used as equilibration vessels. The precise volume of each vial was calculated by weighing the vessels successively empty and filled with water. Exactly 0.5 ml of

essential oil was pipetted in each vial. For most plant species, the yield of essential oil was sufficient to prepare at least 3 vials. In the case of *Taxus baccata* and *Cedrus atlantica*, which both had a low yield of essential oil, vials of 1.5 ml were used in which 50 μl (*Taxus*) or 100 μl (*Cedrus*) of essential oil was transferred by means of a liquid syringe.

Subsequently, a mixture containing equal volumes of pure benzene, toluene, ethylbenzene, *p*-xylene and *o*-xylene was prepared. From the densities of the chemicals, the mass fractions of each compound in the mixture could be calculated. Approximately 10 μl of the mixture was injected into the 0.5 ml leaf essential oil present in the vials. For the species *Taxus baccata* and *Cedrus atlantica*, the volume of MAHs injected was 1 and 2 μl, respectively, proportional to the lower volume of essential oil in the vials. The exact injected mass was determined by weighing the syringe before and after injection with an accuracy of 0.0001 g. To establish equilibrium of MAHs between oil and air, the vials were kept overnight in a thermostatic waterbath at 25°C (±0.1°C).

The temperature dependence of $K_{EO/A}$ was studied using a waterbath provided with a heating unit (for measurements at 25, 35, 40 and 45°C) or a cooling unit (for measurements at 15°C). To reach a temperature of 5°C, the thermostatic bath was filled with ethyleneglycol instead of water.

2.4. Analytical procedure and parameters

After equilibrium was reached (overnight), 50 μl of the air in the vial was withdrawn into a 100 μl gastight syringe (series A syringe with valve, Alltech Ass.) and injected immediately into a Varian type 3700 GC equipped with a FID detector and connected to a HP3388A integrator. Of each vial, two injections of the headspace were carried out.

The GC was provided with a 30 m×0.53 mm ID fused-silica capillary column with a 5 μm thick layer of a 100% dimethylpolysiloxane stationary phase (DB-1, J&W Scientific). The carrier gas was helium at a flow-rate of 6.4 ml min^{-1}. Splitless injection was used. The temperature program of the GC oven was as follows: initial temperature 36°C, rate 4°C min^{-1}, final temperature 200°C, final time 10 min.

2.5. Calibration procedure

The mass of MAHs present in the aliquot of 50 μl of air that was withdrawn from the vials, was calculated from the peak areas using calibration graphs that were obtained by direct injection of standard solutions of MAHs in dichloromethane. These direct liquid injections of 1 μl solution into the GC were performed with a microliter syringe, reaching reproducibilities of less than 3%. This calibration technique based on direct liquid injection was shown to be well correlated with the vapour phase calibration technique generated by a closed two-phase system [10].

2.6. Calculation of $K_{EO/A}$

The calculation of $K_{EO/A}$ is based on the mass balance in the closed vial:

$$m_{tot} = C^*_{EO}V_{EO} + C^*_A V_A \quad (1)$$

with m_{tot} the total amount of MAH in the closed vial (mol), C_{EO} the concentration of MAH in essential oil (mol l^{-1}), V_{EO} the volume of essential oil (l), C_A the concentration of MAH in air (mol l^{-1}) and V_A the volume of air (l).

According to the definition of the essential oil/air partition coefficient, the concentration of the substance in the essential oil can be described as:

$$C_{EO} = K^*_{EO/A}C_A \quad (2)$$

with $K_{EO/A}$ the dimensionless partition coefficient [mol l^{-1} (mol l^{-1})$^{-1}$].

Substituting Eq. (2) in Eq. (1), gives:

$$K_{EO/A} = \frac{m_{tot} - C^*_A V_A}{C^*_A V_{EO}} \quad (3)$$

3. Results and discussion

3.1. Partition coefficients $K_{EO/A}$ of MAHs at 25°C

The dimensionless partition coefficients [mol l^{-1} (mol l^{-1})$^{-1}$] of benzene, toluene, ethylbenzene, *p*-xylene and *o*-xylene between essential oil and air for nine plant species are listed in Table 1. The number of vials used for each species is also indicated. By

Table 1
Partition coefficients between essential oil and air [mol l^{-1} (mol l^{-1})$^{-1}$] for nine plant species at 25°C

Plant species	No. of vials	Essential oil/air partition coefficient					
			B	T	EB	*p*-X	*o*-X
Cedrus atlantica	2	mean	**150**	**325**	**615**		**770**
'Glauca'		S.D.	11	31	88		125
		% S.D.	7.5	9.7	14.4		16.2
Chamaecyparis lawsoniana	3	mean	**789**	**1868**	**3613**	**3909**	**4619**
'Lanae'		S.D.	88	236	419	457	523
		% S.D.	11.1	12.6	11.6	11.7	11.3
Juniperus virginiana	2	mean	**747**	**1630**	**3038**	**3117**	**3156**
		S.D.	98	210	348	383	397
		% S.D.	13.1	12.9	11.4	12.3	12.6
Juniperus x media	5	mean	**974**	**2555**	**4899**	**5057**	**6038**
'Pfitzeriana aurea'		S.D.	61	303	582	532	673
		% S.D.	6.2	11.9	11.9	10.5	11.2
Lavandula angustifolia	3	mean	**804**	**1723**	**3439**	**3653**	**4342**
'Dwarf Blue'		S.D.	80	207	325	366	449
		% S.D.	10.0	12.0	9.4	10.0	10.3
Pinus nigra	3	mean	**596**	**1365**	**2652**	**2842**	**3342**
		S.D.	63	202	290	315	268
		% S.D.	10.6	14.8	10.9	11.1	8.0
Pinus sylvestris	4	mean	**806**	**1824**	**3369**	**3553**	**4101**
		S.D.	138	182	294	302	325
		% S.D.	17.2	10.0	8.7	8.5	7.9
Pseudotsuga menziesii	3	mean	**989**	**2423**	**4446**	**4462**	
		S.D.	138	382	394	341	
		% S.D.	13.9	15.7	8.9	7.6	
Taxus baccata	1	mean	**68**	**104**	**171**		**227**
'Semper aurea'		S.D.	4.7	2.9	2.7		3.6
		% S.D.	6.9	2.8	1.6		1.6

B: benzene; T: toluene; EB: ethylbenzene, *p*-X: *p*-xylene; *o*-X: *o*-xylene; S.D.: standard deviation.

multiplying the number of vials by two (two injections of the headspace per vial), the number of repetitions of each $K_{EO/A}$ determination is found. The missing values in Table 1 are due to the fact that in some plant species a component of the essential oil headspace co-eluted with one of the MAHs and consequently no value for $K_{EO/A}$ could be calculated.

The $K_{EO/A}$ values in the nine species vary over two orders of magnitude, from 68 for benzene in *Taxus baccata* up to 6038 for *o*-xylene in *Juniperus x media* essential oil.

The $K_{EO/A}$ values agree well with the olive oil/air partition coefficients reported by Sato and Nakajima [8], who found values of 492, 1471, 3791, 3694 and 4360 for benzene, toluene, ethylbenzene, *p*-xylene and *o*-xylene, respectively, at a temperature of 37°C.

When the $K_{EO/A}$ of the respective compounds are compared among the plant species investigated, *Taxus baccata* and *Cedrus atlantica* show much lower values than the seven other species. The difference in $K_{EO/A}$ between two species belonging to the same genus (e.g., *Pinus sylvestris* and *P. nigra* or *Juniperus virginiana* and *J. x media*) is comparable to the difference in $K_{EO/A}$ between this species and a species of another genus. So no particular similarity in $K_{EO/A}$ is found among species of the same genus.

3.2. Correlation between log $K_{EO/A}$ and log $K_{O/A}$

When accumulation of non-polar organic molecules from water into living organisms is considered, water-immiscible organic solvents like *n*-octanol are often used as a surrogate for organisms or parts of organisms. Although the extent of uptake from water into *n*-octanol is in most cases not identical to that

into organisms, it is directly proportional, which means that within a series of compounds, higher accumulation into organisms corresponds to more favourable partitioning into *n*-octanol [1]. Also, in the case of pollutant partitioning between a biological lipophilic matrix, such as the leaf essential oil, and air, proportionality between this partition coefficient ($K_{EO/A}$) and the *n*-octanol/air partition coefficient ($K_{O/A}$) can be presumed.

According to Schwarzenbach et al. [1], the partition coefficient between air and two different organic solvents of a given set of compounds, which undergo the same type of intermolecular interactions with both organic solvents, are related with each other. This relation, e.g., between the $K_{S/A}$ of an organic solvent S and the $K_{O/A}$ of the reference solvent *n*-octanol, for a particular set of organic compounds, is given by

$$\log K_{S/A} = a \log K_{O/A} + b \quad (4)$$

The slope a of this linear regression line is proportional to the ratio of the partial molar excess free energies in the solvent and in *n*-octanol ($\Delta G^{x}_{S/A}/\Delta G^{x}_{O/A}$) and the intercept b 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 MAHs, x corresponds to a methyl group. A value of $a = 1$ means that the 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 $a < 1$ means that the methyl group substitution makes the molecule more incompatible with the solvent as compared with the reference solvent *n*-octanol [1].

In order to calculate the regression line between log $K_{EO/A}$ and log $K_{O/A}$, values of the partition coefficient $K_{O/A}$ between *n*-octanol and air for the five MAHs can be derived from

$$K_{O/A} = \frac{K_{O/W}}{K_{A/W}} \quad (5)$$

with $K_{O/W}$ and $K_{A/W}$ the octanol/water and the air/water partition coefficients, respectively [11,12]. Since partition coefficients are dependent on temperature, a reference temperature of 25°C is chosen. The values of $K_{O/W}$ and $K_{A/W}$ at this temperature for the five MAHs can be found in the literature [1].

The regression line between log $K_{EO/A}$ and log $K_{O/A}$ was calculated for all investigated plant species and is shown in Fig. 1 for five out of the nine investigated plant species. Since the lines for seven species (*P. menziesii*, *J. x media*, *J. virginiana*, *P. nigra*, *P. sylvestris*, *C. lawsoniana* and *L. angustifolia*) were situated very close to each other, only three of these seven species are represented in Fig. 1 for the sake of clarity. The regression lines for *Taxus baccata* and *Cedrus atlantica* are found below those of the other species, which is a consequence of the lower $K_{EO/A}$ values measured for these two species (cf., Table 1).

Slope, intercept and correlation coefficient of the regression lines between log $K_{EO/A}$ and log $K_{O/A}$ are given in Table 2 for all plant species investigated. The slope appears to be dependent on the plant species: it varies from 0.504 for *Taxus baccata* to 0.766 for *Juniperus x media*. All slopes are below 1, indicating that the substitution of a methyl group in the series from benzene to the xylenes renders the molecule less compatible with the essential oil than with *n*-octanol. This implies that at a constant concentration of a pollutant in the air, less molecules will be sorbed in the essential oil than in an equal volume of *n*-octanol. Calculating the uptake of chemicals in plants on a lipid basis using the $K_{O/A}$

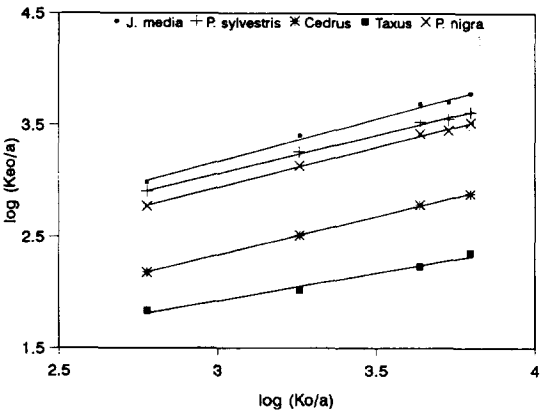

Fig. 1. Log $K_{EO/A}$ versus log $K_{O/A}$ for five plant species (*Juniperus x media*, *Pinus sylvestris*, *Cedrus atlantica*, *Taxus baccata* and *Pinus nigra*), with linear regression lines.

Table 2
Parameters of the regression line between log $K_{EO/A}$ and log $K_{O/A}$ for nine plant species according to Eq. (4)

	Linear regression line of log $K_{EO/A}$ vs. log $K_{O/A}$		
	Slope (a)	Intercept (b)	Correlation (r)
Cedrus atlantica	0.701	0.230	0.9998
Chamaecyparis lawsoniana	0.745	0.834	0.999
Juniperus virginiana	0.639	1.112	0.993
Juniperus x media	0.766	0.880	0.997
Lavandula angustifolia	0.716	0.914	0.999
Pinus nigra	0.729	0.754	0.999
Pinus sylvestris	0.688	1.003	0.998
Pseudotsuga menziesii	0.710	1.040	0.994
Taxus baccata	0.504	0.414	0.990

values, as is proposed in the literature [12–14], can thus lead to systematic errors. It can be concluded that there is a need to measure partition coefficients between lipophilic matrices of living organisms and air directly, rather than to calculate the uptake of chemicals by using the $K_{O/A}$ value.

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

In accordance with the temperature dependence of $K_{O/A}$ [12], the relation between $K_{EO/A}$ and the temperature can be expected to be given by

$$\ln K_{EO/A} = c\left(\frac{1}{T}\right) + d \qquad (6)$$

with c and d constants. The increase of ln $K_{EO/A}$ of *Pinus nigra* essential oil as a function of the inverse absolute temperature ($1/T$) is shown in Fig. 2. Taking into account that over small temperature ranges the temperature dependence of the partition coefficient of a liquid compound can be expressed as a function of the enthalpy change ΔH by

Table 3
Parameters of the regression line between ln $K_{EO/A}$ and $1/T$ for the five MAHs according to Eq. (6)

	Linear regression line of ln $K_{EO/A}$ vs. $1/T$		
	Slope (c)	Intercept (d)	Correlation (r)
Benzene	3074	−3.81	0.986
Toluene	2769	−1.93	0.985
Ethylbenzene	2975	−1.94	0.986
p-Xylene	2918	−1.68	0.986
o-Xylene	3340	−2.94	0.988

$$\ln K = -\frac{\Delta H}{R}\frac{1}{T} + \text{constant} \qquad (7)$$

[1], the enthalpy of phase change $\Delta H_{EO/A}$ between air and essential oil is given by $-c \times R$, where R is the gas constant and c is the slope of the regression line between ln $K_{EO/A}$ and $1/T$. The slope, intercept and correlation coefficient of the regression lines for the five MAHs are given in Table 3.

$\Delta H_{EO/A}$ values of *Pinus nigra* essential oil for the five MAHs are around −25 kJ/mol.

A temperature drop of 30°C increases $K_{EO/A}$ by a factor of 4 to 5, which is a much lower increase compared to the factor of 30 that was found by Harner and Mackay [12] for the $K_{O/A}$ of hexachlorobenzene over a temperature interval from −10 to 20°C.

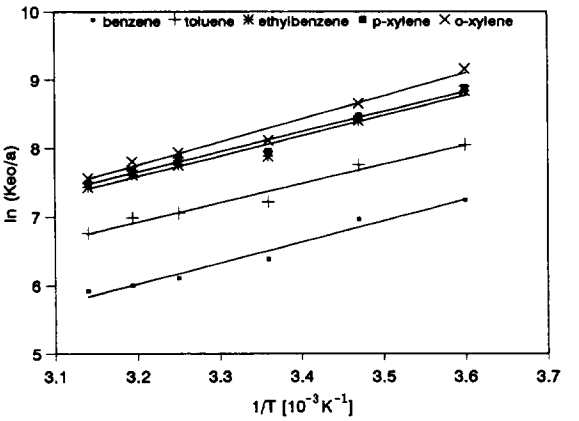

Fig. 2. Ln $K_{EO/A}$ of *Pinus nigra* essential oil as a function of the inverse absolute temperature ($1/T$) for the five MAHs, with linear regression lines.

When the temperature dependence of the essential oil/air partition coefficient is compared with that of the air/water partition coefficient [7], the absolute value of the slope is in the same order of magnitude. Essential oil/air partitioning is thus as temperature sensitive as air/water partitioning.

4. Conclusions

A method based on equilibration in closed vials and headspace gas chromatography for the measurement of the partition coefficients of monocyclic aromatic hydrocarbons between leaf essential oil and air was developed. The method was applied for the $K_{EO/A}$ determination of MAHs in isolated essential oil of nine plant species.

A linear regression line between log $K_{EO/A}$ and log $K_{O/A}$ could be calculated for the nine plant species. The slope of these lines indicated that within the series of compounds from benzene to the xylenes the substitution of another methyl group renders the molecule less compatible with the essential oil than with *n*-octanol.

From the temperature-dependence measurements, it was found that a temperature drop of 30°C increased the $K_{EO/A}$ by a factor of 4 to 5.

As it is believed that the octanol/air partition coefficient $K_{O/A}$ is an important parameter which measures the partitioning of chemicals between atmosphere and biota, it seems from the results of this study that the real partition coefficient between a lipophilic matrix and the air can substantially differ from this $K_{O/A}$. There is, thus, a need to measure partition coefficients between lipophilic compartments of living organisms and air directly, rather than to calculate the uptake of pollutants by estimation from $K_{O/A}$.

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