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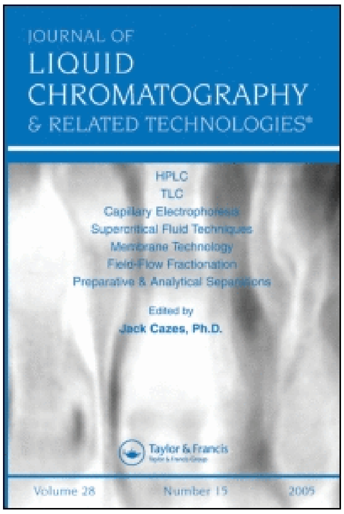

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DETERMINATION OF LIPOPHILICITY OF CHLORINATED ALICYCLIC COMPOUNDS BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A RP-HPLC procedure has been developed for measuring the capacity factor (k') of a series of chlorinated alicyclic compounds. The chromatographic behavior measured on a 4.5 mm i.d. x 3.3 cm C-18 column with methanol / water as the mobile phase was related to the volume fraction of methanol (ϕ). A linear relationship was found between log k' and ϕ, showing the correlation coefficient $\gamma \rangle 0.99$, for each of the 15 chlorinated alicyclic compounds tested. The log k_w , the capacity factor obtained by extrapolation of the retention data from binary eluents to 100 % water, was chosen as a measure of the solute lipophilicity. Since log k_w is considered as a valuble index of the lipophilicity of a compound, the determined values will be used for the quantitative structure-activity relationship studies of the chlorinated alicyclic compounds.

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INTRODUCTION

The lipophilicity of a bioactive molecule is one of the most important physicochemical properties which influences its capacity to move through biological compartments. It is generally defined as the tendency of a chemical to distribute between an immiscible nonpolar solvent and water. The logarithm of the partition coefficient of a chemical in the *n*-octanol/water system (log K_{ow}), which is usually measured by 'shake-flask' method, is widely used because of its simplicity and some similarity between *n*-octanol and biological membranes. The 'shake-flask' method works in most cases, but it results in large errors for chemicals with log K_{ow}'s larger than 4, and it is time-consuming and requires considerable amounts of pure stable compounds [1]. It has been proven that the retention capacity factor (k') of a compound in a reversed-phase high performance liquid chromatography (RP-HPLC) system is a reliable indirect descriptor of the lipophilicity of a compound [1-5, 10-16]. Moreover, the recent studies have shown that log k_w, the retention capacity factor which is extrapolated from a binary phase to 100% water in a RP-HPLC system, is an even better descriptor of lipophilicity than the isocratic factor [4-5,10-11].

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The chlorinated alicyclic compounds, which were widely insecticides used in the past including aldrin, dieldrin, heptachlor and their structural analogs, constitute a large group of compounds which are environmentally and toxicologically important [6-7]. Their neurochemical action occurs through their binding to the γ-aminobutyric acid (GABA) receptor at the chloride channel [6]. These compounds are generally very non-polar; for example, aldrin has a log K_{ow} as high as 5.9 [9]. But unfortunately, few log K_{ow} 's have been documented for this class of compounds. Direct measurement of their *n*-octanol/water partition coefficients by the conventional 'shake-flask' method is difficult because of their highly lipophilic chracteristics and the availability of adequate amounts for the measurement. In this paper, we are reporting a systematic study of the lipophilicity of these compounds by using a RP-HPLC method, and the measured data will be used in the on-going reseach of quantitative structure-activity relationships (QSAR) for the compounds.

MATERIAL AND METHODS

Chemicals. The purity for each of the 15 chlorinated alicyclic compounds is greater than 98%. The structures of the compounds were further confirmed by proton-NMR spectra. A stock solution of each compound was made at a concentration of 1mg/ml in methanol and stored at -20 °C. All other chemicals and solvents were of analytical reagent or of HPLC grade.

Apparatus and Chromatographic Conditions. The RP-HPLC system consisted of a Waters 6000A pump coupled with a U-6K injector, a 4.5 mm i.d. x 3.3 cm C-18 analytical column with a particle size of 3 microns, which was manufactured by Perkin-Elmer Corp., Norwalk, Connecticut, a variable-wave-length ultraviolet detector (Spectroflow 757, ABI Analytical Kratos Division, Ramsey, New Jersey), which was set at 210 nm or 220 nm, and a recorder (Cole-Parmer Instrument Company, Chicago, Illinois).

Measurement of log k'. The dead volume of the system was measured by injecting a 10% $NaNO_3$ solution. The stock solutions of the tested compounds were diluted with methanol to the final injection concentration around 100 μg/ml. A 15-μl injection was made in triplicate. According to their chromatographic behavior, the retention times were determined at five different methanol/water eluent ranges from 60% to 80% of methanol by volume. At each mobile phase composition, the capacity factor was calculated according to $k' = (t_R - t_0)/t_0$, where t_R and t_0 were the retention times of the analyte and of the non-retained compounds respectively. The log k_w values, were obtained

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from y-intercept of the plots of log k' *versus* volume fraction of methanol in the mobile phase.

RESULTS AND DISCUSSIONS

The structural information of the 15 tested compounds is given in Figure. 1.

The chromatographs of all tested compounds were accomplished under a variety of conditions in which the volume fraction of methanol (ϕ) in the mobile phase varied from 0.60 to 0.80, since smaller fractions of this component led to unreliable and long retention times. Most of the chlorinated alicyclic compounds are very hydrophobic. This attribute results in unreliably long retention times and trailing of separations by using the commonly used C-18 columns (10 cm or 25 cm in length). However these obstacles were eliminated by introducing a 4.5 mm i.d. x 3.3 cm C-18 analytical column packed with 3-micron support, and this allowed the tested compounds to be eluted at a reasonable time even in the case of the most polar mobile phase. Separations were improved by adding a trace amount of phosphoric acid at a concentration of 0.01% by volume to the mobile phases, and the reproducibility of retention behavior was not affected for the tested compounds (see Figure 2.).

Retention capacity factors (k') at each methanol fraction are given in Table 1 for the 15 tested compounds. Although the monocratic log k's are possibly correlated to other lipophilic descriptors, the established log k' - lipophilicity correlation for a given class of compounds cannot be extrapolated either to different solutes or to other similar or even identical separation systems, and it may result in misleading data owing to solute-solvent interactions [4].

The log k_w, the retention capacity factor of a compound when 100% water is employed as mobile phase in a RP-HPLC system, was used for evaluating the lipophilicity of the compounds because it eliminated

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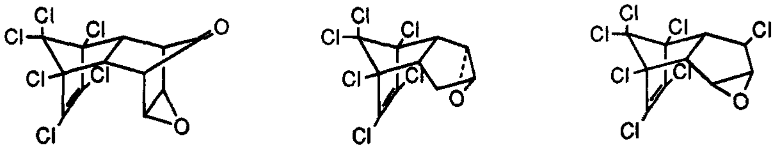
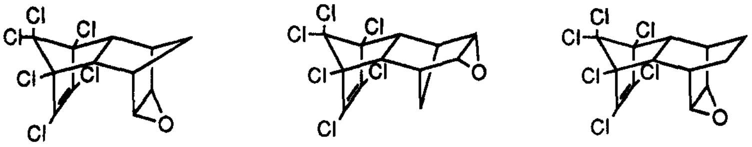


1. 12-Ketoendrin

2. Chlordene epoxide

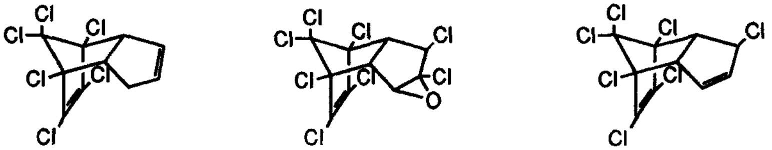
3. Heptachlor epoxide



4. Endrin

5. Dieldrin

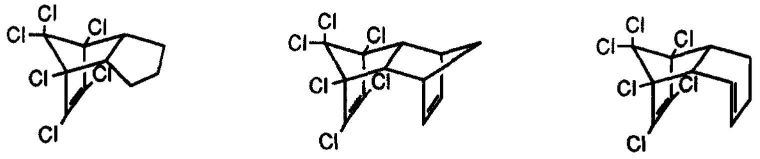
6. Epoxide of 14



7. Chlordene

8. Oxychlordane

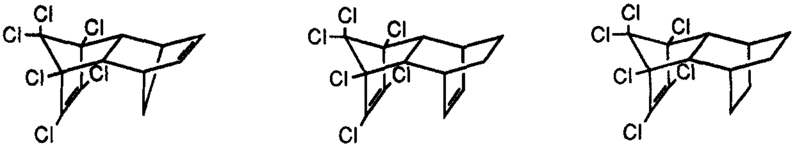
9. Heptachlor



10. Dihydrochlordene

11. Isodrin

12. @



13. Aldrin

14. @@

15. Octahydro-derivative of 14

FIGURE 1. Structures of the Tested Compounds

@: Hexachlorocyclopentadiene/cyclohexa-1,3-diene adduct
@@: Hexachloronorbornadiene/cyclohexa-1,3-diene adduct

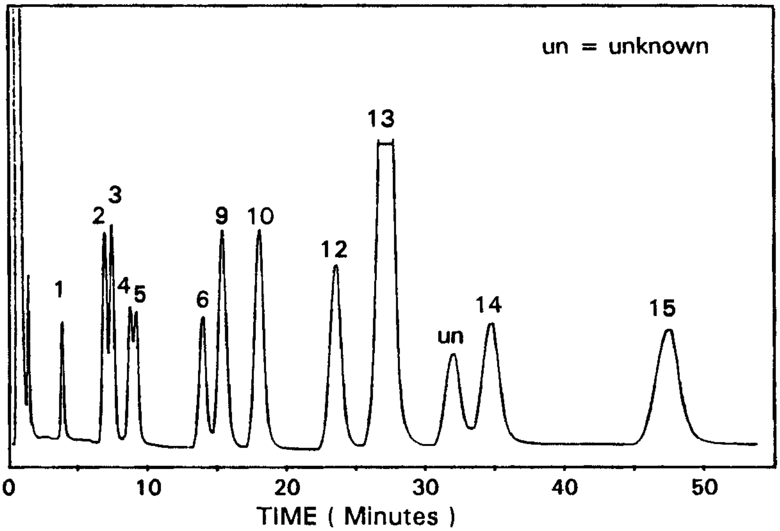


FIGURE 2. HPLC Profile of the Tested Compounds. Column: 4.5 mm i.d. x 3.3 cm C-18 cartridge pack; Mobile phase: 70/30 methanol/water + 0.01% H_3PO_4; Flow rate: 1.0 ml/min; Detector: UV-210 nm; Temp.: 25 °C.

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selective solute-solvent interactions and is more closely related to log K_{ow} than isocratic capacity factors [4, 12-16].The log k_w' was determined by extrapolating the polycratic retention capacity factors (log k's) from binary eluents to 100% water. It was found that for the 15 tested compounds, the relationship between solute retention and the composition of methanol in the mobile phase can be described by the equation:

$$\log k' = \log k_w - S\,\phi \qquad (1)$$

where S refers to the slope of log k' *vs.* ϕ plots. The corrlation coefficients ($\gamma > 0.99$) showed that log k' and ϕ were highly linearly correlated for the 15 compounds. The slopes for the equations were in the scope of 4.84 to 6.93. The extrapolated log k_w's are given in Table 2.

TABLE. 1 *Isocratic Retention Capacity Factors (k') of the Tested Compound*

Compound	ϕ: Methanol/water (v/v)				
Number	0.60	0.65	0.70	0.75	0.80
1	22.737	11.562	7.342	3.991	2.388
2	46.286	23.366	14.081	6.887	4.253
3	53.571	26.158	15.211	7.695	4.141
4	63.395	30.921	18.363	9.296	5.285
5	71.150	33.406	19.443	9.620	5.252
6	112.514	52.522	30.467	14.946	8.019
7	120.974	54.229	28.844	14.345	7.594
8	136.601	59.478	30.662	14.495	7.215
9	136.601	60.777	33.650	14.956	7.722
10	161.867	71.660	39.641	18.238	10.103
11	220.067	87.790	50.001	20.690	11.278
12	222.032	96.616	52.047	21.391	12.224
13	267.860	113.063	60.476	24.074	13.832
14	357.519	149.764	78.426	29.466	17.502
15	517.449	210.398	108.462	43.807	20.961

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TABLE 2. Linear Relationship between Log k' and ϕ: $\log k' = \log k_w - S\phi$

Compound	S	log k_w	γ^2 (n = 5)*
1	4.84	4.24	0.9970
2	5.21	4.78	0.9965
3	5.51	5.02	0.9987
4	5.36	5.00	0.9979
5	5.61	5.20	0.9980
6	5.68	5.44	0.9980
7	5.96	5.64	0.9983
8	6.34	5.92	0.9989
9	6.21	5.85	0.9980
10	6.01	5.79	0.9971
11	6.42	6.16	0.9945
12	6.35	6.14	0.9956
13	6.49	6.30	0.9948
14	6.70	6.55	0.9939
15	6.93	6.86	0.9989

* γ = Correlation coefficient.

The relationship between the slope S and the intercept values (log k_w) was investigated for the tested compounds. A good linear correlation was observed: correlation coefficient = 0.9937. Slope S depends on the size of the solute molecule and the structure of polar functional groups. The high linear correlation coefficient may be a reflection of the uniqueness and suitability of the methanol-water system for estimating the lipophilicity of the compounds [4].

CONCLUSION

The retention capacity factor (log k') of a compound in a RP-HPLC system can be used as a descriptor of its lipophilicity. The isocratic log k' was measured at five different compositions of the eluent, and the log k_w was extrapolated from the linear relationship between log k' and the fraction of methanol in the mobile phase for each of the 15 chlorinated alicyclic compounds. The log k_w's may be advantageous in describing the lipophilic properties of the structurally related, very nonpolar chlorinated alicyclic compounds, which for use in QSAR studies.

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