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## POSSIBLE RELATIONSHIP BETWEEN LIPOPHILICITY AND BEHAVIOUR IN REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY AND GAS-LIQUID CHROMATOGRAPHY

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### SUMMARY

Gas-liquid chromatographic (GLC) retention indices of 31 aniline derivatives were measured on six columns of different polarity. Relationships between reversed-phase thin-layer chromatographic (TLC)  $R_M$  values,  $\log P$  values and GLC retention data (retention indices extrapolated to 0°C and retention index differences measured on different columns) were investigated by stepwise regression analysis. Significant relationships were found in only a few instances but the regression coefficients were not high enough for predicting lipophilicity. A dependence of the relationship between lipophilicity and retention data on the type of compounds was observed and difficulties with GLC as a technique for determining lipophilicity are emphasized. The influence of TLC systems on  $R_M$  values, the influence of organic solvents on the partition coefficients and the influence of temperature and stationary phases on GLC retention indices are discussed.

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### INTRODUCTION

Lipophilicity is a useful and important parameter in quantitative structure-activity relationship (QSAR) investigations. Using lipophilicity as an independent variable, one can design new biologically active molecules by QSAR methods. Lipophilicity can be measured in many ways, *e.g.*, by partition in the 1-octanol-water system<sup>1,2</sup>, giving  $\log P$  values, by reversed-phase thin-layer chromatography (RP-TLC) and reversed-phase over-pressured layer chromatography<sup>3-5</sup>, giving  $R_M$  values, by reversed-phase high-performance liquid chromatography (RP-HPLC)<sup>6,7</sup> and gas-liquid chromatography (GLC)<sup>8-12</sup>. Clifford and Watkins<sup>8</sup> used GLC relative retention ( $\pi_{GLC} = r_x/r_h$ , where  $r_x$  and  $r_h$  are the retentions of a compound  $x$  and a parent compound  $h$ , respectively) with success for chemical structure-biological activity investigations. Steurbaut *et al.*<sup>9</sup> and Rittich and Dubsy<sup>11</sup> found no correlation between GLC relative retention and linear free energy parameters.

Boček<sup>10</sup> used oleyl alcohol and water as GLC stationary phases for measuring partition coefficients. This method is suitable only for highly volatile solutes. In certain instances highly significant linear relationships were found between partition

TABLE I  
 CHEMICAL STRUCTURE, PARTITION COEFFICIENT (A),  $R_{M(0)}$  VALUES (B, C, D) AND EXTRAPOLATED RETENTION INDICES (E, G, H, I, J, K)  
 OF THE ANILINE DERIVATIVES INVESTIGATED  
 For meaning of symbols A-K see Table II.

No.	Compound	A	B**	C**	D**	E	G	H	I	J	K
1	Aniline	0.92	50.9	18.7	46.5	947	1077	950	1587	2000	1440
2	2-Methylaniline	1.34	94.0	55.8	888.1	1116	1189	1028	1609	1739	1539
3	4-Methylaniline	1.40	105.5	67.5	103.5	1003	1212	1077	1637	1821	1595
4	2,5-Dimethylaniline	2.34	140.6	94.3	136.2	1237	1288	1131	1696	1887	1546
5	2-Methoxyaniline	1.23	83.0	41.5	71.0	1148	1368	1041	1710	1642	1692
6	4-Methoxyaniline	0.86	67.2	26.5	59.2	1328	1499	1254	1771	1898	1706
7	3-Hydroxyaniline	0.18	-15.4	-41.1	-14.3	1151	1492	1319	2475	1698	2252
8	4-Hydroxyaniline	0.04	-17.1	-38.4	-18.3	1407	1449	1478	2430	2458	2116
9	4-Chloroaniline	1.83	101.2	92.9	104.0	1179	1272	1161	1924	1986	1620
10	2,4-Dichloroaniline	2.69	177.2	136.4	171.8	1277	1407	1265	1964	1553	1730
11	3-Bromoaniline	2.10	118.1	107.8	121.0	1228	1373	1232	1682	1706	1760
12	4-Bromoaniline	2.05	128.1	103.5	132.8	1219	1278	1155	1899	2155	1767
13	2-Iodoaniline	2.98	157.6	116.6	157.0	1193	1639	1229	1541	2031	1776
14	2-Nitroaniline	1.81	99.2	73.8	101.0	1325	1545	1301	2174	2048	1883
15	2,3-Dimethylaniline	-*	133.6	87.4	134.1	1242	1316	1153	1701	1708	1705
16	2,6-Dimethylaniline	-	135.6	89.9	130.7	1210	1311	1120	1687	1850	1530
17	3,5-Dimethylaniline	-	138.9	96.1	136.0	1242	1301	1149	1744	1818	1678
18	2,4,6-Trimethylaniline	-	174.6	125.8	169.1	1294	1073	1224	1714	1795	1657
19	2-Ethyl-6-methylaniline	-	159.1	115.6	153.0	1271	1403	1206	1675	1681	1639
20	2-Isopropylaniline	-	157.9	167.5	167.5	1302	1333	1158	1676	1659	1568
21	3-Aminoaniline	-	23.2	-18.5	13.2	1367	1632	1390	2182	1977	2068
22	2-Chloroaniline	-	95.6	83.5	97.8	1054	1276	1112	1701	1450	1618
23	3,5-Dichloroaniline	-	183.7	152.3	184.6	1320	1536	1400	2303	1809	1828
24	2,4,6-Trichloroaniline	-	105.7	184.2	256.2	1190	1474	1348	1843	1503	1330
25	2-Chloro-4-nitroaniline	-	138.8	116.8	145.5	1580	1895	1606	2281	1703	2031
26	4-Chloro-3-nitroaniline	-	131.4	124.2	145.1	1569	1843	1499	2296	2200	2021
27	2,6-Dichloro-4-nitroaniline	-	213.7	174.4	296.6	1567	1976	1584	2769	1939	2044
28	2,4-Dibromoaniline	-	205.2	165.3	230.5	1380	1526	1349	2239	1927	1840
29	2,4,6-Tribromoaniline	-	240.3	213.2	323.6	1363	1739	1684	2193	1940	1816
30	2,6-Dinitroaniline	-	152.0	-96.8	151.8	1210	1777	1451	2245	2121	1978
31	2,4,6-Trinitroaniline	-	151.8	113.5	155.0	1486	1773	1519	2302	1853	2076

\* - = No data available.

\*\* Data taken from ref. 15.

parameters and GLC retention parameters by Valkó and Lopata<sup>12</sup>. They found good correlations only for a few types of compounds using partition coefficients measured in 1-octanol–water, cyclohexane–water or chloroform–water, and were unable to give a generally useful GLC method for measuring partition data.

In this paper, we consider the application of lipophilicity for 31 aniline derivatives. Earlier, we tried to use this technique for triazine herbicides<sup>13</sup> and carboxamide derivatives<sup>14</sup>. With the triazines we obtained good results for 15 derivatives. A highly significant linear relationship was found between their  $R_M$  values and retention indices; the correlation coefficient of the equation was 0.941 [ $F$  (Fischer test value) = 45.9]. The results were not as good for 28 carboxamide derivatives, the correlation coefficient for the best equation being 0.773 ( $F = 38.65$ ). No significant correlation was found between  $R_M$  values and GLC retention data determined by some other workers<sup>9,11</sup>, but satisfactory correlations were found in other instances<sup>8,10,12</sup>.

In this work, we measured the GLC (Kováts) retention indices of 31 aniline derivatives on six stationary phases of different polarities and examined the relationships between their  $R_M$  values and the GLC retention data.

## EXPERIMENTAL

The aniline derivatives investigated are listed in Table I. The  $R_M$  values were taken from the literature<sup>15</sup> and the log  $P$  values were taken from the Hansch–Leo compilation<sup>16</sup>. The Kováts retention indices ( $I$ ) were measured with a Packard 7400 gas chromatograph with 180 cm  $\times$  2 mm I.D., glass columns equipped with a flame ionization detector. The stationary phases were 3% Apolar (AP), 3% Carbowax 20M (CW), 3% OV-1 (OV1), 3% OV-17 (OV17), 3% OV-275 (OV275) and 3% Epon-1001 (EP) on Supelcoport (80–100 mesh) and the carrier gas was nitrogen at a flow-rate of 50 ml/min. Retention indices were measured at three column temperatures and extrapolated to 0°C according to eqn. 1 (see below).

To determine the relationship between log  $P$ ,  $R_M$  and  $I$  values stepwise regression analysis was used. The analysis was carried out on a Hewlett-Packard 9845B computer.

## RESULTS AND DISCUSSION

The measured retention indices could not be used for direct investigations, as only retention indices measured at the same temperature and pressure can be compared. Retention indices of the aniline derivatives containing different substituents could not be measured at the same temperature. We therefore determined the retention indices at three different temperatures. The temperature dependence of the retention index value is a hyperbolic function, but the curve can have a significant linear portion. We calculated the linear regression equations between the retention values and temperatures:

$$I = i + bt \quad (1)$$

where  $I$  is the Kováts retention index,  $i$  is the intercept,  $b$  is the slope and  $t$  is the

temperature. For all molecules the linear regression equations were highly significant, with correlation coefficients ranging from 0.970 to 1.000. As the measurements were made at temperatures belonging to the linear part of the hyperbolic function the linear regression analysis used was justified. In order to simplify our calculations we used the  $i$  values for further investigations. An arbitrary extrapolation to 0°C does not affect the results of the following regression analyses between  $\log P$ ,  $R_M$  and  $I$  values. Relationships between  $\log P$  and  $R_{M(0)}$  values were also studied<sup>15</sup>. The  $R_M$  values were measured in methanol (m), acetone (ac) and acetonitrile (an) with different concentrations of organic solvent in the eluent system, and then the  $R_M$  values were extrapolated to zero concentration of the organic phase [ $R_{M(0)}$ ]. Highly significant linear relationships were found for  $\log P$  and  $R_{M(0)}$  in all three eluent systems, with correlation coefficients ranging from 0.975 to 0.987. The best equation was found for  $R_{M(0)an}$ :

$$\log P = 0.218 + 0.0149 R_{M(0)an} \quad (2)$$

with the number of compounds ( $n$ ) = 14 and the regression coefficient ( $r$ ) = 0.987.

As we could not find  $\log P$  values for the 31 compounds in the Hansch-Leo compilation<sup>16</sup>, in most instances we used  $R_{M(0)an}$  values as a parameter characterizing lipophilicity, which is in good agreement with  $\log P$ . Earlier, it was established<sup>12</sup> that lipophilicity correlates well not with the retention indices but with the differences between them. We therefore included in our data set not only  $i$ , but also  $\Delta i$ .

Relationships were sought between  $\log P$ ,  $R_{M(0)}$ , and  $i$ ,  $\Delta i$  values. The variables studied are listed in Table II. Stepwise regression analysis was carried out on our data set. Only a few significant equations were found:

$$D = 229.19 + 0.3085 T \quad (n = 31, r = 0.702) \quad (3)$$

$$C = 231.00 + 0.2787 W \quad (n = 31, r = 0.730) \quad (4)$$

$$C = 4.34 + 0.24 H - 0.12K \quad (5)$$

$$(n = 31, r = 0.797, s = 49.71, F = 23.36)$$

$$D = 139.48 + 0.45 H - 0.33 K \quad (6)$$

$$(n = 31, r = 0.782, s = 49.80, F = 21.99)$$

where  $s$  is the residual error and  $F$  is the Fischer test value.

Although these equations are significant above the 99.9% level ( $P < 0.1\%$ ), the relationships are not good enough for use in predicting lipophilicity values ( $R_M$  or  $\log P$  values). Considering the equations above, we found the best equations between  $R_M$  values and  $\Delta i_{OV17-EP}$ ;  $\Delta i_{AP-EP}$ ;  $i_{AP} - i_{EP}$ . We did not find any significant relationship between  $R_M$  and  $i$  values, which showed that there was no direct connection between GLC data and lipophilicity. Studying the variables obtained by stepwise regression analysis, we can establish that there is a relationship only between lipophilicity and retention index differences. The most significant equations are found for retention index differences on non-polar (AP) and polar (EP) columns. This finding is in good agreement with earlier result<sup>12</sup>.

To find some explanation for the poor correlation between retention data and lipophilicity, we chose ten compounds among the 31 with similar structures (compounds 1, 2, 3, 4, 15, 16, 17, 18, 19 and 20) and investigated the relationship between  $D$  and  $i$  values for these ten compounds separately. We found that the relationships

TABLE II  
THE VARIABLES STUDIED

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A:	$\log P^*$ (logarithm of the partition coefficient in 1-octanol-water)
B:	$R_{M(0)m}^{**}$ (retention parameter, determined by TLC in methanol-water)
C:	$R_{M(0)ac}^{**}$ (retention parameter, determined by TLC in acetone-water)
D:	$R_{M(0)an}^{**}$ (retention parameter, determined by TLC in acetonitrile-water)
E:	$i_{OV1}$ (extrapolated retention index <sup>***</sup> , measured on OV-1 column)
G:	$i_{OV-17}$ (extrapolated retention index <sup>***</sup> , measured on OV-17 column)
H:	$i_{AP}$ (extrapolated retention index <sup>***</sup> , measured on Apolan column)
I:	$i_{CW}$ (extrapolated retention index <sup>***</sup> , measured on Carbowax 20M column)
J:	$i_{OV275}$ (extrapolated retention index <sup>***</sup> , measured on OV-275 column)
K:	$i_{EP}$ (extrapolated retention index <sup>***</sup> , measured on Epon 1001 column)
L:	$\Delta i_{OV1-OV17}$ ( $i_{OV1} - i_{OV17}$ )
M:	$\Delta i_{OV1-AP}$ ( $i_{OV1} - i_{AP}$ )
N:	$\Delta i_{OV1-CW}$ ( $i_{OV1} - i_{CW}$ )
O:	$\Delta i_{OV1-OV275}$ ( $i_{OV1} - i_{OV275}$ )
P:	$\Delta i_{OV1-EP}$ ( $i_{OV1} - i_{EP}$ )
Q:	$\Delta i_{OV17-AP}$ ( $i_{OV17} - i_{AP}$ )
R:	$\Delta i_{OV17-CW}$ ( $i_{OV17} - i_{CW}$ )
S:	$\Delta i_{OV17-OV275}$ ( $i_{OV17} - i_{OV275}$ )
T:	$\Delta i_{OV17-EP}$ ( $i_{OV17} - i_{EP}$ )
U:	$\Delta i_{AP-CW}$ ( $i_{AP} - i_{CW}$ )
V:	$\Delta i_{AP-OV275}$ ( $i_{AP} - i_{OV275}$ )
W:	$\Delta i_{AP-EP}$ ( $i_{AP} - i_{EP}$ )
X:	$\Delta i_{CW-OV275}$ ( $i_{CW} - i_{OV275}$ )
Y:	$\Delta i_{CW-EP}$ ( $i_{CW} - i_{EP}$ )
Z:	$\Delta i_{OV275-EP}$ ( $i_{OV275} - i_{EP}$ )

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\* Data taken from ref. 16.

\*\* Data taken from ref. 15.

\*\*\* For the mode of extrapolation, see eqn. 1.

were basically different from those for all 31 molecules. In some instances the relationship is better for the ten alkyl derivatives, whereas in other instances the relationship is significantly poorer.

A few equations are given in Table III, which compares the correlation coefficients for the ten and all 31 compounds. Not surprisingly, our results show that GLC retention depends on the type of solute and the type of stationary phase. The volatility of a compound is determined by intermolecular forces between the sample and the stationary phase. It can be seen from the correlation data for eqns. 7-12 (Table III) that the GLC retention can be in good agreement with the lipophilicity for compounds with similar substituents (e.g.,  $r = 0.966$  for the ten alkyilanilines), but the relationship can also be very poor for compounds with different substituents ( $r = 0.4149$  for the 31 aniline derivatives in the same relationship). The opposite of this result can also be true, the significant relationship between  $D$  and  $T$  ( $r = 0.7021$  for 31 compounds) decreases dramatically ( $r = 0.0123$ ) if we calculate the relationship only for the ten alkyilanilines.

From these examples, we can see that the type of solute may influence the relationship considerably. In some instances there was a significant relationship between  $R_{M(0)an}$  and  $\Delta i$  values, e.g., extrapolated retention index differences measured

TABLE III

RELATIONSHIPS BETWEEN  $R_{M(0)an}(D)$  AND  $i, \Delta i$  VALUES FOR TEN ALKYL DERIVATIVES

$X$	$Y$	$a$	$b$	$r$	$r$ for 31 compounds	Eqn. No.
$D^*$	$O^*$	225.09	0.162	0.881	0.389	7
$D^*$	$E^*$	-212.29	0.285	0.928	0.346	8
$D^*$	$H^*$	-370.16	0.444	0.966	0.415	9
$D^*$	$T^*$	127.90	0.004	0.012	0.702	10
$D^*$	$W^*$	278.27	0.323	0.473	0.727	11

\* For meaning of symbols, see Table II.

on Apolan and Epon stationary phases correlated well with  $R_{M(0)an}$  values, but the relationship is not suitable for predicting  $R_M$  and  $\log P$  values exactly.

Summarizing our investigations on the possible relationship between lipophilicity and behaviour in GLC, we may conclude that GLC is not practical and cannot be recommended as a technique for determining lipophilicity, because in most instances the establishment of suitable experimental conditions is difficult.

In certain instances GLC could be useful but its applicability will always depend on the type of solute. For instance, earlier we obtained excellent results for *s*-triazine derivatives<sup>13</sup> but poor correlations for carboxamide derivatives<sup>14</sup>. In addition to the type of compounds, the stationary phases used are also very important. One must be very careful about the temperature at which the indices are measured, because retention indices measured at the same temperature can be compared. Several factors can influence the lipophilicity data. The  $R_M$  values will be different in different eluent systems and the concentration of the organic phase may also influence the results. The results are different if the  $\log P$  values are measured in 1-octanol-water, cyclohexane-water or other organic phase-water systems. The great variety of significant parameters make it difficult to determine lipophilicity by GLC. Hence, we recommend RP-TLC or HPLC for the determination of lipophilicity in most instances.

## REFERENCES

- 1 T. Fujita, J. Iwasa and C. Hansch, *J. Am. Chem. Soc.*, 86 (1964) 5175.
- 2 C. Hansch and S. M. Anderson, *J. Org. Chem.*, 32 (1967) 2583.
- 3 C. B. C. Boyce and B. V. Milborow, *Nature (London)*, 208 (1965) 537.
- 4 C. L. Biagi, A. M. Barbaro, M. T. Gamba and M. C. Guerra, *J. Chromatogr.*, 41 (1969) 371.
- 5 É. János, T. Cserhádi and E. Tyihák, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 633.
- 6 J. M. Call, *J. Med. Chem.*, 18 (1975) 549.
- 7 M. S. Mirrlees, S. J. Moulton, C. T. Murphy and P. J. Taylor, *J. Med. Chem.*, 19 (1976) 615.
- 8 D. R. Clifford and D. A. M. Watkins, *Pestic. Sci.*, 2 (1971) 41.
- 9 W. Steurbaut, W. Dejonckheere and R. H. Kips, *J. Chromatogr.*, 160 (1977) 37.
- 10 K. Boček, *J. Chromatogr.*, 162 (1979) 209.
- 11 B. Rittich and H. Dubsy, *J. Chromatogr.*, 209 (1981) 7.
- 12 K. Valkó and A. Lopata, *J. Chromatogr.*, 252 (1982) 77.
- 13 É. János, B. Bordás and T. Cserhádi, *Acta Phytopathol.*, 20 (1985) 343.
- 14 É. János, B. Bordás and T. Cserhádi, *J. Chromatogr.*, 286 (1984) 63.
- 15 T. Cserhádi, *Chromatographia*, 18 (1984) 318.
- 16 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.