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CORRELATION OF THE CHROMATOGRAPHIC RETENTION OF SOME PHENYLACETIC AND PHENYLPROPIONIC ACID DERIVATIVES WITH MOLECULAR STRUCTURE

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SUMMARY

Retention indexes of sixteen phenylacetic and phenylpropionic acid derivatives were determined by gas chromatography (GC) on OV-101 and OV-17 stationary phases. Capacity factors of these compounds were also measured by high-performance liquid chromatography (HPLC) on a C_{18} column. The retention behaviour was correlated with several variables expressing molecular structure. The best results were obtained with molar refraction (according to the Gladstone and Dale system) in GC and substituent parameters, π, in HPLC.

INTRODUCTION

The number of non-steroidal antirheumatic drugs has grown substantially over the last few years. Although heterogeneous by nature they can be catagorized into several classes, of which one of the most important is represented by phenylacetic and phenylpropionic acid derivatives. Some of these compounds have been analyzed by thin-layer chromatography (TLC)[1], gas chromatography (GC)[2–10], gas chromatography–mass spectrometry (GC–MS)[11–14] and high-performance liquid chromatography (HPLC)[15–21]. The published methods have been employed mostly for the determination of individual drugs in body fluids.

The prediction of retention characteristics for other derivatives is frequently associated with the correlation of retention indexes and capacity factors with molecular structure. The main problem here is to find variables which are closely correlated with the retention. Several authors[22–25] have correlated retention with connectivity indexes; Calixto and Raso[26] have shown that such indexes can be replaced by Van der Waals volumes. Jinno and Ishigaki[27] have correlated the Van der Waals volume with the retention behaviour of alkylbenzenes in reversed-phase HPLC (RP-HPLC). Good correlations between the RP-HPLC retention behaviour of some arylacetic acids and substituent parameters, π, have been found[20]. Molar refraction has also

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been proposed[28,29], because it reflects the electrostatic properties of the molecule. Haken *et al.*[30] have correlated the retention indexes of some phenylacetic esters with the number of carbon atoms.

We have studied sixteen derivatives of phenylacetic and phenylpropionic acids which are either potential antirheumatics or their metabolites. The goal was to determine their retention characteristics by GC and HPLC. The data could then be employed for identification and optimization purposes. The GC retention indexes were correlated with the Van der Waals volume and molar refraction, and the HPLC capacity factors were correlated with the same variables and with the substituent parameters, π.

THEORETICAL

Calculation of capacity factors

When capacity factors of weak acids are to be correlated with their molecular structure, then it is preferable to use capacity factors of the undissociated molecules. Because of the pH limitations of reversed-phase columns, capacity factors cannot always be determined at low pH values, but they can be calculated as follows[31]

$$k = f_{HA}k_{HA} + (1 - f_{HA})k_{A^-} \quad (1)$$

$$f_{HA} = c_{H^+}/(c_{H^+} + K_a) \quad (2)$$

where f_{HA} is fraction of undissociated molecules, c_{H^+} is concentration of solvated protons, K_a the dissociation constant of the weak acid and k_{HA}, k_{A^-} are the capacity factors of the undissociated and dissociated molecules, respectively. Eqn. 1 can be written:

$$k = f_{HA}(k_{HA} - k_{A^-}) + k_{A^-} \quad (3)$$

If the capacity factors are determined at various pH values, then by assuming various values for K_a a straight line which more or less fits the experimental points can be obtained. The straight line which gives the maximum value for the correlation coefficient determines the best estimation of K_a and k_{HA}. For this optimization algorithm, a program in BASIC was written.

EXPERIMENTAL

Chemicals

All chemicals used were of analytical grade purity. Methanol, acetic acid and sodium hydroxide were obtained from Lachema (Brno, Czechoslovakia), trimethylsilane and hexamethyldisilazane from Merck (Darmstadt, F.R.G.). Derivatives of phenylacetic and phenylpropionic acids (as specified in Table I) were kindly supplied by Ing. M. Kuchař, Research Institute for Pharmacy and Biochemistry (Prague, Czechoslovakia). Solvents for HPLC were distilled and degassed before use. The mobile phase was prepared as follows: to 600 ml of methanol were added 380 ml of water and 0.6 ml of acetic acid; the pH was adjusted to the desired value with several

TABLE I

RETENTION INDEXES ON STATIONARY PHASES OV-101 and OV-17

Precision is expressed as the standard deviation (N = 5).

Number	*Name*	$I_{OV\text{-}101}$	$I_{OV\text{-}17}$
I	2-Phenylpropionic acid	1299.7 ± 1.2	1453.0 ± 0.9
II	2-(4-Methylphenyl)propionic acid	1385.6 ± 0.7	1547.8 ± 0.9
III	2-(4-Ethylphenyl)propionic acid	1473.1 ± 0.8	1634.2 ± 0.4
IV	2-(4-Isobutylphenyl)propionic acid	1614.3 ± 0.3	1751.9 ± 0.8
V	2-(3-Chlorophenyl)propionic acid	1455.4 ± 0.3	1567.6 ± 0.4
VI	2-(4-Bromophenyl)propionic acid	1574.0 ± 0.6	1751.3 ± 0.7
VII	2-(4-Methoxyphenyl)propionic acid	1528.6 ± 0.2	1735.2 ± 0.3
VIII	2-(4-Phenoxyphenyl)propionic acid	2066.7 ± 0.9	2270.7 ± 1.3
IX	Phenylacetic acid	1269.4 ± 0.3	1442.8 ± 0.3
X	4-Ethylphenylacetic acid	1452.2 ± 0.6	1571.2 ± 0.4
XI	4-Isopropylphenylacetic acid	1514.4 ± 0.5	1680.8 ± 0.7
XII	4-Cyclohexylphenylacetic acid	1939.5 ± 1.2	2142.9 ± 0.9
XIII	4-(*n*-Octyloxy)-3-chlorophenylacetic acid	2305.0 ± 1.7	1552.8 ± 1.4
XIV	4-Isopropoxy-3-chlorophenylacetic acid	1766.5 ± 0.5	1966.6 ± 0.9
XV	4-Benzyloxy-3-chlorophenylacetic acid	2206.8 ± 1.4	2618.7 ± 1.5
XVI	4-Allyloxy-3-chlorophenylacetic acid	1837.0 ± 1.0	2080.6 ± 0.9

drops of 1 M sodium hydroxide. The volume of the mixture was then made up to 1 l. For the determination of capacity factors, each acid was dissolved in 1 ml of methanol–water (70:30, v/v).

Apparatus and chromatographic conditions

GC measurements were made on a Sigma 2 gas chromatograph (Perkin-Elmer, Norwalk, CT, U.S.A.) connected to a Sigma 10 integrator. The instrument was equipped with 2 m × 2 mm I.D. glass columns packed with 3% OV-101 or 3% OV-17 on Chromosorb W HP (100–120 mesh). The analysis conditions were as follows: temperatures, column, 200°C, injection port, 240°C and flame ionization detector, 260°C; nitrogen carrier gas, 30 ml/min. Retention times were measured with an accuracy of ±0.01 min.

For HPLC a SP-8100 liquid chromatograph connected to a SP-4100 integrator (Spectra-Physics, Santa Clara, U.S.A.) was used. The eluate was monitored at 245 nm using a SP-8400 variable wavelength UV detector. The volume of the injection loop was 10 μl. The glass column (15 cm × 3.3 mm I.D.) was packed with 5-μm Separon SI C_{18} (Laboratorní Přístroje, Prague, Czechoslovakia). The column temperature was 45°C, and the mobile phase flow-rate was 0.75 ml/min. The column was equilibrated for 45 min before the first sample injection. The pH values reported are corrected for the methanol–water mixture[32].

Derivatization

For GC measurements the acids were converted into their trimethylsilyl esters as follows: 0.2 mg of the acid were dissolved in 40 μl of the reaction mixture, trimethylsilane–hexamethyldisilazane (2:1, v/v). The reaction mixture was left to stand for 15 min at room temperature; 0.2 μl were then injected on the column.

RESULTS AND DISCUSSION

Measurement of retention indexes by GC

For an accurate determination of retention indexes there is a need to evaluate correctly the dead time, t_0. The methods for dead time measurement have been reviewed by Wainwright and Haken[33]. We used the method of Kaiser[34]; here the dead time was optimized to the highest value of the correlation coefficient, r, for the log $t'_{R,n}$–n correlation, where n is the carbon number of the n-alkane and t'_R the corrected retention time. The t_0 value was accepted when r was greater than 0.99999 and the shifts in t_0 were small (less then 0.001 min). A mixture of five or six n-alkanes was injected on the column with the sample in order to minimize variations. The n-alkanes were chosen so as to be eluted before and after the sample peak. The measurements were made in a linear region of the separation isotherm, as shown by injection of concentrations ten times higher and smaller than the actual concentration. In both cases, the retention times were unchanged.

The retention indexes of the sixteen compounds of interest were measured on stationary phases OV-101 (non-polar) and OV-17 (medium polar) as shown in Table I. They can be used for identification purposes. Huber *et al.*[35] stated that an identification by means of retention indexes measured on two stationary phases of different polarities has the same reliability as one from low-resolution mass spectra.

Correlation of retention indexes with molecular structure

Due to the lack of experimental data suitable for correlation it was decided to correlate the retention behaviour with variables computed from group contributions. From tabulated values[36], the Van der Waals volume, V_W, and the molar refraction were calculated. The molar refraction was calculated for three different systems: according to Lorenc and Lorentz, MR_{LL}, Gladstone and Dale, MR_{GD}, and Vogel, MR_V (Table II).

The results of the correlation with retention indexes are presented in Table III and Figs. 1 and 2 (in Figures only the correlation with MR_{GD} is shown). Correlations with MR_{GD} and MR_{LL}, which are defined as the products of the molar volume with an expression containing the refractive index, gave approximately the same results. The results obtained with MR_V are less promising as expected from its definition as the product of the refractive index and the relative molecular weight. V_W, which expresses the only steric properties of the molecule, gives much poorer results, as seen by the shift on going from non-polar OV-101 to medium polar OV-17.

Although the values of Exner's function are not higher than the limit for "succesful correlation" (0.5)[37], the standard deviations are such, that predictions on the basis of these correlations could only be very approximate. For this reason the compounds were arranged into three groups: A, derivatives of 2-phenylpropionic acid with various alkyl substituents (I–IV), B, similar derivatives of phenylacetic acid (IX–XII) and C, various derivatives of both acids (V–VIII and XIII–XVI). Table IV contains the results for MR_{GD}. These correlations can also be seen in Figs. 1 and 2.

The correlation for group A was very good (S.D. was only 6.6 index units on OV-101). However, for group B the S.D. was ten times higher. Fig. 1 shows the anomalous behaviour of 4-cyclohexylphenylacetic acid, which was eluted *ca.* 200 index units later than expected according to the first three members of the group.

TABLE II

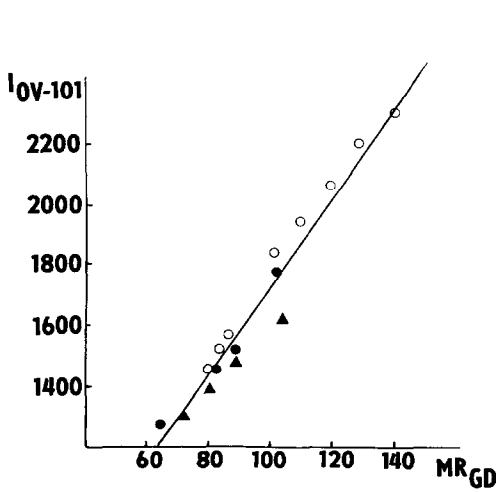
CALCULATED VALUES OF THE VAN DER WAALS VOLUME, MOLAR REFRACTIONS AND π PARAMETER

Compound	$V_W(cm^3/mol)$	$MR_{LL}(cm^3/mol)$	$MR_{GD}(cm^3/mol)$	$MR_V(g/mol)$	Σπ
I	110.03	41.98	72.24	231.04	1.80*
II	121.18	47.14	81.33	253.79	2.30
III	131.41	51.79	89.06	274.43	2.78
IV	151.86	61.06	104.59	315.48	3.70
V	119.71	47.43	82.38	287.36	2.48
VI	122.11	50.40	87.70	354.17	2.70
VII	124.88	48.73	84.19	277.64	1.81
VIII	157.05	68.60	120.00	383.49	3.40
IX	99.81	37.37	64.45	210.53	1.45*
X	121.19	47.18	81.27	253.92	2.43
XI	131.41	51.79	89.06	274.43	2.85
XII	154.08	63.58	109.57	338.28	3.91
XIII	195.95	81.97	141.24	458.04	5.41
XIV	144.79	58.68	102.05	354.71	2.71
XV	166.74	73.94	130.07	440.05	3.36
XVI	141.31	58.34	101.97	354.31	2.61

* Experimental value.

This can be explained by the fact that the bulky cyclohexyl and phenyl groups occupy a greater volume than that predicted from group contributions. Therefore a greater steric interaction with the stationary phase can be expected.

On OV-101 a good correlation was obtained even for group C. This group is formed by derivatives which differ in the nature of the heteroatoms (Cl, Br, O) at



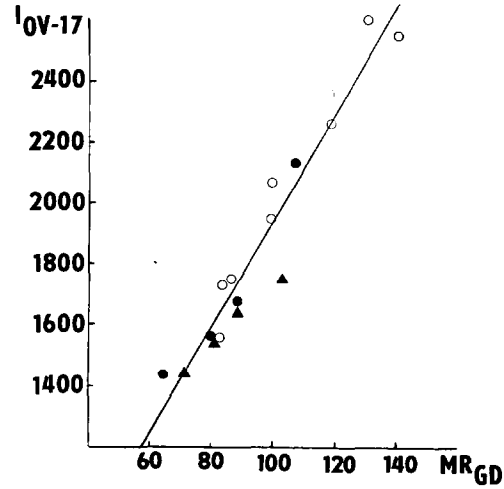


Fig. 1. Correlation between retention indexes on OV-101, $I_{OV\text{-}101}$, and molar refraction according to Gladstone and Dale, MR_{GD}. ▲, Alkyl derivatives of phenylpropionic acid; ●, alkyl derivatives of phenylacetic acid; ○, other derivatives.

Fig. 2. Correlation as in Fig. 1 except on OV-17.

TABLE III

CORRELATION OF MOLECULAR STRUCTURE WITH RETENTION INDEXES ON OV-101 AND OV-17

Values for $I_{OV\text{-}17}$ are given in parentheses.

	V_W	MR_{LL}	MR_{GD}	MR_V
r	0.940 (0.913)	0.971 (0.952)	0.977 (0.960)	0.956 (0.950)
r^2	0.883 (0.833)	0.943 (0.906)	0.954 (0.921)	0.913 (0.902)
S.D.	113 (158)	78.9 (119)	71.0 (108)	97.3 (121)
Exner's function	0.347 (0.418)	0.241 (0.311)	0.216 (0.283)	0.298 (0.316)
Slope	12.5 (14.2)	25.9 (29.7)	14.9 (17.1)	4.26 (4.96)
y intercept	−39.0 (−81.8)	226 (206)	237 (213)	321 (292)

various positions. In spite of this, the value for the correlation coefficient is very high. On OV-17 the correlation is not as good due to the electronegativity of the heteroatoms.

Multiple linear regression of the correlations did not bring about any improvements, because of a strong intercorrelation between the variables used.

Measurement of capacity factors and pK_a by HPLC

Capacity factors were measured at four pH values, 3.80, 4.80, 5.17 and 5.75. Their relative standard deviations were typically less than 0.4% ($N = 4$). Values of

TABLE IV

CORRELATION OF $I_{OV\text{-}101}$ AND $I_{OV\text{-}17}$ WITH MR_{GD} FOR INDIVIDUAL GROUPS

	A	*B*	*C*
r	0.9992 (0.995)	0.979 (0.965)	0.994 (0.974)
r^2	0.9984 (0.991)	0.958 (0.931)	0.989 (0.949)
S.D.	6.60 (15.0)	71.5 (97.8)	36.8 (94.2)
Exner's function	0.0401 (0.0961)	0.207 (0.264)	0.106 (0.226)
Slope	9.79 (9.24)	14.8 (15.7)	14.5 (17.1)
y intercept	593 (795)	268 (356)	306 (253)

k_{HA}, k_{A^-} and pK_a for each acid were calculated by the optimalization method described (Table V). The derivatives with electronegative substituents exhibited smaller values of pK_a than the underivatized ones, in agreement with theory. On the contrary, the alkyl derivatives showed the opposite effect. The influence of substituents is clearly not as important as for halogenoacetic acids, because of the distance from the carboxyl group.

TABLE V

CALCULATED pK_a VALUES AND CAPACITY FACTORS OF THE UNDISSOCIATED AND DISSOCIATED FORMS OF THE ACIDS

Compound	pK_a	k_{HA}	k_{A^-}
I	5.36	1.179	0.221
II	5.42	1.832	0.526
III	5.45	2.810	0.682
IV	5.70	7.551	2.859
V	5.11	1.885	0.262
VI	4.98	2.152	1.066
VII	5.05	0.926	0.522
VIII	5.35	4.992	0.289
IX	5.11	0.737	0.039
X	5.15	1.890	0.911
XI	5.21	2.683	1.209
XII	5.29	9.448	4.297
XIII	4.83	78.01	15.45
XIV	4.93	2.770	0.615
XV	5.04	5.696	1.040
XVI	4.93	2.273	0.489

Correlation of capacity factors with molecular structure

An additional variable, the substituent parameter π (derived from partition coefficients between 1-octanol and water), was used for the correlation of ln k_{HA} with molecular structure. The π values calculated according to refs. 1, 20 and 38 are summarized in Table II. The value for the phenoxy group includes a correction for the interaction between two aromatic rings. The difference in the π parameter between phenylacetic and phenylpropionic acids was calculated from experimental data.

The molar volume and molar refraction in HPLC gave lower values of the correlation coefficients than in GC (Table VI). The hydrophobicity for alkyl derivatives can be estimated from these group contributions; this is impossible for derivatives with heteroatoms. This was documented by excluding group C from the calculations (Table VI). The use of molar volume and refraction as variables is limited in that these variables do not differentiate between geometrical isomers.

Better correlations were obtained with the substituent parameter π which reflects closely the partition process and does differentiate between structural isomers. The results (Table VI) yield a linear relationship between ln k_{HA} and this parameter. There was no need to classify the substances into groups as in GC.

TABLE VI

CORRELATIONS OF MOLECULAR STRUCTURE WITH ln k_{HA} VALUES

Values in parentheses are for alkyl derivatives only.

	V_W	MR_{LL}	MR_{GD}	MR_V	π
r	0.93 (0.997)	0.89 (0.998)	0.88 (0.998)	0.78 (0.997)	0.992 (0.996)
r^2	0.86 (0.994)	0.79 (0.996)	0.77 (0.996)	0.61 (0.993)	0.984 (0.993)
S.D.	0.42 (0.075)	0.51 (0.050)	0.55 (0.053)	0.72 (0.076)	0.14 (0.081)
Exner's function	0.37 (0.077)	0.46 (0.063)	0.47 (0.057)	0.63 (0.084)	0.13 (0.086)
Slope	0.043 (0.046)	0.083 (0.0'97)	0.047 (0.057)	0.012 (0.021)	1.15 (1.01)
y intercept	−4.75 (−4.92)	−3.49 (−3.96)	−3.35 (−3.99)	−2.68 (−4.60)	−2.14 (−1.76)

CONCLUSIONS

The retention indexes measured here could be employed for identification purposes. On OV-101, their correlation with molecular structure gave good results for all groups, but only for alkyl derivatives on OV-17. 4-Cyclohexylphenylacetic acid showed abnormal behaviour due to the steric interactions of its two bulky groups. The variables MR_{GD} and MR_{LL} gave the best correlations.

The ln k_{HA} values are linearly related to the substituent parameter π. When values of these parameters are accesible, the results of the correlation can be used for a relatively accurate prediction of retention behaviour. For alkyl derivatives the correlation is excellent regardless of the variable used.

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