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STUDIES IN THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC BEHAVIOUR

GAS CHROMATOGRAPHIC STUDY OF MONOALKYLPYRIDINES

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SUMMARY

The additivity principle and solute–stationary phase interactions have been investigated for a series of mono-substituted alkylpyridines chromatographed on both polar and non-polar stationary phases. The increase in retention index due to electronic and steric effects arising from the position of the substituted alkyl group is discussed. It is concluded that the dominant factor controlling the chromatographic behaviour is the ring nitrogen atom and that a complex interrelationship between electronic and steric effects results in a non-linear increase in retention index on both stationary phases for a side-chain length of up to at least three carbon atoms.

INTRODUCTION

The relationship between the structure of molecules and their chromatographic behaviour was established by the theoretical work of James and Martin[1], who observed that the plot of the number of carbon atoms in the molecule against the logarithm of their retention value is linear for a given homologous series at a given temperature. This work was further extended to demonstrate the identification of compounds by the measurement of retention data of homologous series on two different columns of different polarity when it was observed that by plotting the retention volumes of aliphatic amines on two different columns each homologous series always gave a straight line[2]. It was later shown[3,4] that the plot of the logarithm of the retention values rather than the direct values is a more useful aid to identification of compounds.

However, the most useful and widely used expression for chromatographic retention behaviour is that due to Kováts[5], which, although originally postulated for the prediction of the retention behaviour of hydrocarbons (his so-called retention

TABLE I

EXPERIMENTAL CONDITIONS

	System 1	*System 2*	*System 3*	*System 4*	*System 5*	*System 6*
Stationary phase	Carbowax 20M 15%	Carbowax 20M 15%	Carbowax 20M 15%	Apiezon L 15%	Apiezon L 15%	Apiezon L 15%
Solid support	Chromosorb W AW-DCMS	Chromosorb W AW-DCMS	Chromosorb W AW-DCMS	Chromosorb W AW-DCMS	Chromosorb W AW-DCMS	Chromosorb W AW-DCMS
Mesh size	85–100	85–100	85–100	85–100	85–100	85–100
Column length (m)	2.0	2.0	2.0	2.0	2.0	2.0
Column diameter (cm, O.D.)	0.3	0.3	0.3	0.3	0.3	0.3
Carrier gas flow-rate (ml nitrogen/min)	30	30	30	30	30	30
Recorder chart speed (cm/h)	38	38	38	38	38	38
Column temperature (°C)	90	100	110	110	130	150

index system), has been extended by several workers to include a variety of compounds[6–8]. This is the basis for the application of the additivity principle to gas chromatography, and states that in any homologous series the retention index of higher members increases by 100 units per methylene group introduced.

This paper describes an investigation into the applicability of these principles to the gas chromatographic behaviour of mono-substituted alkylpyridines under carefully controlled conditions such that variations in behaviour of homologous series and of isomeric compounds can be attributed to solute–stationary phase interactions brought about by electronic and steric effects due to the substituted group.

Chromatographic conditions were chosen in which the solute–stationary phase interactions were likely to be dissimilar so that by comparing retention indices obtained by chromatographing the compounds on columns of differing polarity, solute–stationary phase interactions can more readily be studied. Thus Apiezon L, a non-polar paraffin grease, was chosen as the non-polar stationary phase, and Carbowax 20M, a polyethylene glycol, as the polar stationary phase, although it was appreciated that the terms polar and non-polar are not absolute and that Apiezon L, for example, the structure and composition of which is unknown[9], is slightly polar compared to squalane. However, it was because of the differences in polarity between Apiezon L and Carbowax 20M and not for their absolute polarities that they were selected for this study.

Relatively large percentage coatings of each stationary phase were used in order to eliminate, or at least minimise, absorption effects due to the solid support.

EXPERIMENTAL

The pyridines were chromatographed on a Pye Unicam (Cambridge, Great Britain) Series 104 gas chromatograph which was fitted with a gas flow controller and flame ionization detector. The experimental conditions employed are given in Table I.

The carrier gas flow was checked before each run with a soap-bubble flowmeter and the column temperature was checked with a thermocouple. Retention times are the average of three runs under these rigorously controlled conditions, in which the average deviation was about 2%, and were measured directly from the recorder chart. Retention times below 3 min (approx. 2 cm) were measured by means of an illuminated magnifier (×7 magnification) (Magniray, Verebes and Co., London, Great Britain) with measuring disc, others were measured by a steel rule having 0.5-mm graduations. The unit of retention time used was that corresponding to the distance on the recorder chart quoted to the nearest 0.2 mm.

The retention times thus measured were adjusted for column dead volume by subtracting the "air peak" time (t_0) calculated from the equation[10]

$$t_0 = \frac{t_{n+k}^2 - t_n \cdot t_{n+2k}}{t_n + t_{n+2k} - 2t_{n+k}}$$

where t_n, t_{n+k} and t_{n+2k} are the retention times of reference hydrocarbons with n, $n+k$, and $n+2k$ carbon atoms, respectively.

TABLE II

RETENTION DATA OF THE HOMOLOGOUS SERIES OF *n*-ALKANES

t'_R values are quoted to the nearest 0.2 units.

n-Alkane	*System 1*		*System 2*		*System 3*		*System 4*		*System 5*		*System 6*	
	t'_R	*Log* t'_R	t'_R	*Log* t'_R	t'_R	*Log* t'_R	t'_R	*Log* t'_R	t'_R	*Log* t'_R	t'_R	*Log* t'_R
Heptane	–	–	–	–	–	–	9.6	0.9823	6.0	0.7782	4.2	0.6232
Octane	–	–	–	–	–	–	19.2	1.2833	11.6	1.0645	7.6	0.8808
Nonane	5.6	0.7482	4.4	0.6435	3.4	0.5315	38.0	1.5798	21.6	1.3345	13.6	1.1335
Decane	11.0	1.0414	8.4	0.9243	6.2	0.7924	77.6	1.8899	41.6	1.6191	24.8	1.3945
Dodecane	37.4	1.5729	27.0	1.4314	19.6	1.2923	316.2	2.5000	147.6	2.1691	78.2	1.8932
Tetradecane	126.0	2.1004	85.8	1.9335	58.4	1.7664	–	–	521.8	2.7175	238.4	2.3773
Hexadecane	420.0	2.6232	267.6	2.4275	171.2	2.2335	–	–	–	–	–	–

Retention indices were calculated by the Kováts equation by substituting the adjusted retention time (t'_R) for the retention volume (V_g), thus

$$I_x = 100k \frac{[\log t'_{Rx} - \log t'_{Rn}]}{[\log t'_{Rn+k} - \log t'_{Rn}]} + 100n$$

where t'_{Rn} and t'_{Rn+k} are adjusted retention times of reference hydrocarbons having n and $n+k$ carbon atoms, respectively, and $t'_{Rn} \leqslant t'_{Rx} \leqslant t'_{Rn+k}$.

The chromatographic systems were calibrated by analysing a homologous series of n-alkanes, the retention data of which are given in Table II. In addition, certain n-alkanes were added to each sample of alkylpyridine and chromatographed simultaneously whenever retention times permitted.

RESULTS AND DISCUSSION

In a previous study in this series[6], investigating the validity of the additivity principle as applied to a series of mono-substituted alkylphenols, it was concluded (1) that a minimum chain length was required before the increase in retention index (ΔI) for each methylene group added to the side chain approximated to that of an alkane, *i.e.* 100 units, and (2) that the dominant factor controlling the chromatographic behaviour was the phenolic hydroxyl group.

In the present study, a similar series of mono-substituted alkylpyridines has been examined in order to investigate the effect the ring nitrogen atom has on the validity of the additivity principle and on solute–stationary phase interactions.

To ensure the accuracy of the retention values presented —since on these depend any hypotheses which are made regarding the correlation between chemical structure of the alkylpyridines and their chromatographic behaviour— the average retention time of each compound from three consecutive runs was taken. Also the pyridines were chromatographed at three column temperatures on each stationary phase so that the effect of any inconsistent values, for a particular pyridine on an individual system, was minimised.

The adjusted retention times and the retention indices for the series of n-alkylpyridines examined are given in Table III, from which it is evident that the 2-substituted pyridines had considerably lower retention times than the corresponding 3- and 4-substituted compounds. Also, there was only a small difference between the retentions of the 3- and 4-substituted compounds. These generalisations held for all the chromatographic systems studied, although it had been anticipated that different retention characteristics would pertain to the polar and non-polar stationary phases employed.

The increase in retention index (ΔI) for each methylene group added for the homologous series of n-alkylpyridines is given in Table IV, from which it can be seen that there was a non-linear increase in retention index for each methylene group added for the 2-, 3- and 4-substituted compounds on both stationary phases.

When using Carbowax 20M as the stationary phase, the introduction of a methylene group to the 2-position of the pyridine produced an increase in retention index only one third that of the theoretical increment of 100 units. With subsequent additions of methylene groups to the 2-position, the increase in retention index became progressively greater and, although n-alkylpyridines with a chain length above three

TABLE III

RETENTION DATA FOR THE HOMOLOGOUS SERIES OF *n*-ALKYLPYRIDINES

t'_R values are quoted to the nearest 0.2 units.

Pyridine	*System 1*		*System 2*		*System 3*		*System 4*		*System 5*		*System 6*	
	t'_R	*I*	t'_R	*I*	t'_R	*I*	t'_R	*I*	t'_R	*I*	t'_R	*I*
Pyridine	34.2	1185	25.4	1190	18.8	1193	11.6	728	7.6	736	5.6	748
2-Methyl-	41.8	1218	30.6	1222	22.4	1224	20.2	808	12.6	815	8.8	825
3-Methyl-	64.4	1289	47.2	1297	34.2	1302	29.6	863	18.0	871	12.2	881
4-Methyl-	67.4	1297	49.0	1303	35.8	1310	30.0	865	18.0	871	12.2	881
2-Ethyl-	62.2	1284	44.8	1288	32.0	1290	38.4	901	22.8	908	15.0	916
3-Ethyl-	106.6	1372	76.2	1380	53.6	1384	57.0	957	33.0	964	21.2	974
4-Ethyl-	114.2	1384	81.2	1390	57.8	1399	60.0	964	34.2	970	22.2	982
2-*n*-Propyl-	101.0	1364	70.8	1367	49.4	1369	76.4	998	43.0	1005	26.8	1013
4-*n*-Propyl-	185.2	1464	128.0	1470	88.0	1475	113.4	1054	60.6	1059	37.4	1072

TABLE IV

RETENTION INDEX (I) AND INCREASE (ΔI) FOR EACH METHYLENE GROUP ADDED FOR THE HOMOLOGOUS SERIES OF *n*-ALKYLPYRIDINES

Pyridine	*System 1*		*System 2*		*System 3*		*System 4*		*System 5*		*System 6*	
	I	ΔI	I	ΔI	I	ΔI	I	ΔI	I	ΔI	I	ΔI
Pyridine	1185	—	1190	—	1193	—	728	—	736	—	748	—
2-Methyl-	1218	33	1222	32	1224	31	808	80	815	79	825	77
2-Ethyl-	1284	66	1288	66	1290	66	901	93	908	93	916	91
2-*n*-Propyl-	1364	80	1367	79	1369	79	998	97	1005	97	1013	97
3-Methyl-	1289	104	1297	107	1302	109	863	135	871	135	881	133
3-Ethyl-	1372	83	1380	83	1384	82	957	94	964	93	974	93
4-Methyl-	1297	112	1303	113	1310	117	865	137	871	135	881	133
4-Ethyl-	1384	87	1390	87	1398	88	964	99	970	99	982	101
4-*n*-Propyl-	1464	80	1470	80	1477	79	1054	90	1059	89	1072	90

carbon atoms were not available, it would seem reasonable to postulate that the ΔI value would eventually reach 100 units when the side-chain carbon number was sufficiently large. A similar effect was obtained when the 2-*n*-alkylpyridines were chromatographed on Apiezon L although here the introduction of a methylene group into the nucleus produced a much larger ΔI value and, with subsequent additions of methylene groups to the 2-position, the ΔI value approached 100 units at 2-*n*-propylpyridine, *i.e.* side-chain carbon number equals three.

Although there was a non-linear increase in retention index on both stationary phases for each methylene group added to the 2-position, the greatest deviation from linearity was shown by the introduction of a methylene group into the pyridine nucleus, an effect which has been observed for phenols on paper chromatography[11] and gas chromatography[6]. This deviation from linearity can best be explained by considering systems 1, 2 and 3, which employ the polar Carbowax 20M as stationary phase and produce the most pronounced effects. Here there will be strong interaction between the lone pair electrons of the ring nitrogen atom and the lone pairs of electrons of the oxygen atom of the polarised hydroxyl group of the stationary phase; this interaction will tend to repel pyridine and hence encourage its rapid elution. Introduction of a methylene group at the 2-position would increase the polarity of the nitrogen atom, thus increasing the solute–stationary phase interaction, resulting in a relatively short increase in retention time, and hence a small ΔI value would be obtained. Subsequent additions of methylene groups produce only slightly stronger electron-repelling groups than methyl, whilst with increasing side-chain carbon number there would be increasing steric hindrance between the nitrogen atom and the stationary phase. Therefore, larger ΔI values would be expected with subsequent additions to the side chain, although these values will not approximate to 100 units until the approach of the nitrogen atom to the stationary phase has been completely sterically hindered. The values obtained for methyl-, ethyl-, and *n*-propylpyridines, 32, 66, and 79, respectively, are evidence of the increasing contribution of steric hindrance to the retention index for higher members of the homologous series.

A similar argument applies to Apiezon L, but the solute–stationary phase interactions are less pronounced because of the non-polar character of this phase.

For the 3-substituted alkylpyridines, the introduction of a methylene group will cause a hyperconjugative release of electrons which will have the effect of reducing the polarity of the nitrogen atom. Thus the strong solute–stationary phase interaction which occurs with pyridine will be much reduced and the retention index of 3-methylpyridine will be increased by more than the theoretical increment for the addition of a methylene group; hence the ΔI value will be greater than 100. Subsequent additions of methylene groups to the 3-position will result in only a small further deactivation of the molecule; also there will be competition between electronic and steric effects, the steric outweighing the electronic with increasing side-chain length. Thus it would be expected that the ΔI values would approach 100; however, for 3-ethylpyridine this was not the case with either stationary phase, the ΔI values being 83 for Carbowax 20M and 93 for Apiezon L. Therefore, some small solute–stationary phase interaction must occur which is at present unexplained. Unfortunately, no 3-substituted alkylpyridine with a chain length larger than two carbon atoms could be obtained for this investigation and consequently their solute–stationary phase interactions could not be pursued.

In the case of the 4-substituted alkylpyridines, the introduction of a methylene group will increase the polarity of the molecule and encourage intermolecular association due to dipole–dipole attraction, which will probably take the form of loosely associated dimers, formed by two 4-methylpyridine molecules being stacked diametrically one above the other. This will lead to a decrease in volatility and hence a greater increase in retention index than the theoretical increment. Thus the ΔI value for 4-methylpyridine would be expected to be greater than 100. That this is the case is evident from Table IV, from which ΔI for Carbowax 20M is 112–117 and for Apiezon L 133–137. This difference in ΔI between the two phases is brought about by the greater competition between the solute and the stationary phase with Carbowax 20M systems, which results in less intermolecular association and thus a shorter retention time.

The addition of a second methylene group to the 4-position does not alter the dipole–dipole attraction forces and neither will there be any appreciable steric effects. Therefore it would be expected that the ΔI value for 4-ethylpyridine would be close to 100. This is the case for Apiezon L, but not for Carbowax 20M since, in the latter case, the additional methylene group will tend to increase the solute–stationary phase interactions at the expense of intermolecular association of pyridine molecules thus resulting in a shorter retention time and a ΔI value less than 100.

With further additions of methylene groups to the 4-position, electronic effects due to the alkyl group will be more or less constant and steric effects will become increasingly prominent. Therefore intermolecular association becomes progressively less, resulting in a gradual decrease in ΔI values. Hence the ΔI values for 4-*n*-propylpyridine are less than those for 4-ethylpyridine. Again the lower ΔI value obtained for the Carbowax 20M systems can be attributed to a greater decrease in intermolecular association due to solute–stationary phase interactions.

CONCLUSION

It is concluded that the dominant factor controlling the chromatographic behaviour of mono-substituted alkylpyridines is the ring nitrogen atom, and any

increase in retention index depends on the position of substitution relative to this. There is also a complex interrelationship between electronic and steric effects with increasing side-chain carbon number which results in a non-linear increase in retention index on both polar and non-polar stationary phases.

For 2-substituted alkylpyridines the increase in polarity of the ring nitrogen atom, brought about by the introduction of the methylene group, results in greater solute–stationary phase interactions which are eventually overcome by steric effects as the side-chain length increases. When chromatographed on Apiezon L, a side-chain length of at least three carbon atoms is required before the theoretical increment of 100 units of retention index is approached, whereas for Carbowax 20M, on which the solute–stationary phase interactions are more pronounced, the side-chain length would have to be considerably more than three carbon atoms.

In the case of 3-substituted alkylpyridines, the decrease in polarity of the ring nitrogen atom brought about by hyperconjugative release of electrons will result in a decrease in solute–stationary phase interactions relative to pyridine, and hence to an increase in retention index greater than the theoretical increment.

The introduction of a methylene group into the 4-position will increase the polarity of the molecule and encourage dipole–dipole intermolecular association. This leads to a decrease in volatility and hence to a greater increase in retention index than the theoretical increment.

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