Structural regularities governing sorption and gas chromatographic retention of aromatic nitrogen-containing heterocyclic compounds

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The regularities governing differences in the gas chromatographic retention indices of aromatic nitrogen-containing heterocyclic compounds were studied during gas chromatographic analysis on a nonpolar capillary column. The retention indices of pyrrole, pyrazole, imidazole, 1,2,4-triazole, oxazole, thiazole, isoxazole, pyridine, pyridazine, pyrimidine, pyrazine, and *s*-triazine and their alkyl derivatives depend on the number, nature, and arrangement of heteroatoms and alkyl groups in the cycles. The majority of homologous series of *n*-alkyl-substituted azoles and azines and *n*-alkylbenzenes is characterized by anomalously high differences between the retention indices of ethyl and methyl homologs. A scheme of calculation of retention indices of aromatic heterocyclic compounds from increments of heteroatoms was proposed.

Key words: capillary gas chromatography, aromatic nitrogen-containing compounds, retention indices, structural sorption regularities.

Gas chromatographic (GC) identification of nitrogen-containing heterocyclic compounds in various samples is often difficult because of the absence of reference substances. In these cases, calculated retention parameters of unavailable compounds are used for identification. Various models were proposed to correlate the structure of molecules of nitrogen-containing heterocycles and their GC retention.[1–9] For example, the retention parameters of various homologs were calculated on the basis of analysis of the GC properties of homologous series of *n*-alkyl-substituted pyridines[1] and imidazoles.[5] The correlations between the retention indices of alkylpyridines and alkylquinolines and their structural parameters, such as the van der Waals volume and indices of molecular binding, were considered.[8] It has been shown that the retention indices of polyalkyl-substituted pyridines and pyrazines,[2,9] and oxazoles and thiazoles,[3] can be calculated from the increments of substituents, taking into account their position in the cycles relative to heteroatoms. The retention indices of oxazoles and thiazoles can also be calculated from the GC behavior of similar O- and S-containing compounds.[4] It has been found that dependences of the retention parameters of pyridine and quinoline bases on their boiling points are described by linear equations.[1]

The results of studying the physicochemical properties of azoles and azines were summarized in the monographs.[10,11] In this work, we report the comparative study of the GC behavior of five- and six-membered nitrogen-containing heterocyclic aromatic compounds. The purpose of this work is to study the dependence between the retention indices and the amount, nature, and arrangement of heteroatoms and alkyl substituents in aromatic heterocycles.

Experimental

Retention indices (*I*) of alkyl-substituted derivatives of aromatic nitrogen-containing compounds (Table 1) were determined on a Pye-Unicam 104 chromatograph with a flame-ionization detector, which was adapted for this work with capillary columns. A glass capillary column packed with OV-101/KF (50 m × 0.3 mm) prepared by the known procedure[12] was used. The thickness of the phase layer was 0.4 mm. Analysis was carried out at 110 °C, and the temperature of the

Table 1. Retention indices (*I*) of alkyl-substituted derivatives of nitrogen-containing heterocyclic compounds on a capillary column with OV-101 at 110 °C

Compound	*I*	Compound	*I*
N-Methylpyrrole	731	*N*-Propylimidazole	1074
N-Ethylpyrrole	803	*N*-Ethyl-1,2,4-triazole	900
N-Propylpyrrole	886	*N*-Propyl-1,2,4-triazole	984
1,2,5-Trimethylpyrrole	976	5-Methyloxazole	666
3(5)-Methylpyrazole	930	5-Methylisoxazole	702
4-Methylpyrazole	964	3,5-Dimethylisoxazole	801
3,5-Dimethylpyrazole	1010	3-Methylpyridazine	982
N-Methylpyrazole	743	4-Methylpyridazine	1045
N-Ethylpyrazole	809	3,6-Dimethylpyridazine	1052
N-Propylpyrazole	893	4-Methylpyrimidine	827
N-Butylpyrazole	988	5-Methylpyrimidine	864
N-Pentylpyrazole	1086	2-Methylpyrazine	812
N-Ethylimidazoles	991		

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Table 2. Increments of methyl units ($\delta I(CH_2)$) in retention indices in homologous series of *n*-alkyl-substituted nitrogen-containing heterocyclic compounds on columns with nonpolar stationary phases*

No.	Homologous series	$\delta I(CH_2)$/iu	
		Et/Me	Pr/Et
1	*N*-Alkylpyrroles	72	83
2	*N*-Alkylpyrazoles	66	84
3	*N*-Alkylimidazoles	61	78
4	4-Alkyl-2-methyloxazoles	90	90
5	4-Alkyl-2-methylthiazoles	87	90
6	2-Alkyl-4-methyloxazoles	88	83
7	2-Alkyl-4-methylthiazoles	83	83
8	2-Alkyl-5-ethyloxazoles	85	84
9	2-Alkyl-5-ethylthiazoles	80	85
10	Alkylpyrazines	93	92
11	2-Alkylpyridines	85	86
12	4-Alkylpyridines	99	89
13	Alkylbenzenes	96	91

* With OV-101, for series 1—10; with Apolan, for 11 and 12; and with SE-30, for 13.

injector and detector was 200 °C. Helium was used as the carrier gas. Splitting of the gas flow at the inlet of the column corresponded to a ratio of 1 : 30. For calculation of retention indices, the holdup time of the column was determined by methane, which was introduced simultaneously with a sample. The volume of a sample was 0.2—0.4 μL of 1—2% solutions of the analyzed substances in diethyl ether or benzene. The reproducibility of retention indices was ±2 iu.

In addition to the data presented in Table 1, the retention indices obtained previously on a column with OV-101/KF for oxazoles and thiazoles,[3,4] imidazoles,[5] and some other five- and six-membered aromatic compounds with one—three heteroatoms in the cycles[13] were used to calculate increments of specific fragments of the molecule (Tables 2—5).

Results and Discussion

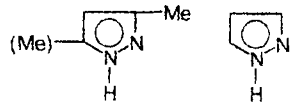
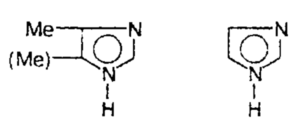
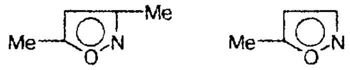
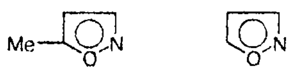
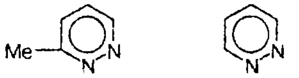
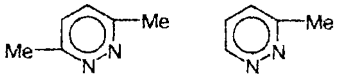
Molecules of five- and six-membered heterocyclic aromatic nitrogen-containing compounds are planar cycles in which the electron density distribution changes significantly when additional nitrogen atoms or alkyl substituents are introduced into the molecule.[14] This has a profound effect on the polarizability, dipole moments, boiling points, and other physicochemical properties[10,11] and, correspondingly, on the retention indices of azoles and azines (see Ref. 13 and Table 1).

The values of the homologous difference $\delta I(CH_2)$ calculated from the retention indices (see Table 1) for pairs of ethyl and methyl, and propyl and ethyl, members of the homologous series of *N*-*n*-alkylpyrazoles and *N*-*n*-alkylpyrazoles are presented in Table 2. The corresponding homologous differences for other nitrogen-containing heterocyclic compounds[3—5,9,15] and *n*-alkylbenzenes[16] are presented for comparison in Table 2. It is known that for the majority of homologous series of organic compounds, the lowest value of $\delta I(CH_2)$ for the pair of homologs is observed on going from the methyl to ethyl group, and then with an increase in the length of the *n*-alkyl chain the increment of $\delta I(CH_2)$ increases sharply followed by a slow increase to approach a value of 100 iu.[17] However, in the series of alicyclic amines (*N*-alkyl-substituted piperidines, morpholines, and thiomorpholines) and in the series of alkyl-*cyclo*-hexanes,[18] the pairs of Et/Me homologs show unexpectedly high $\delta I(CH_2)$ values. In these cases, the anomalous behavior is explained by the appearance of the sorption-active *gauche*-fragment in the ethyl homolog molecule that includes two C atoms of the alkyl chain and two atoms of the cycle.[19]

The data in Table 2 show that for some heteroaromatic compounds the $\delta I(CH_2)$ value for the pair of Et/Me homologs is equal or higher than the similar value for the Pr/Et pair. Since the same deviation is also observed for the series of alkylbenzenes, we may conclude that the high $\delta I(CH_2)$ values for the first pair of homologs are not related to the presence of heteroatoms in aromatic cycles. However, the presence of heteroatoms affects the difference in $\delta I(CH_2)$. For example, on approaching the alkyl substituent to the nitrogen atom, this difference decreases for 2- and 4-alkylpyridines. As shown by comparison of the series containing the oxazole or thiazole cycles, the replacement of the O heteroatom by S gives the same effect. If the alkyl chain is directly attached to the nitrogen atom of the heterocycle (series of *N*-alkyl-substituted pyrroles, pyrazoles, and imidazoles), the $\delta I(CH_2)$ value for the Et/Me pair decreases significantly and becomes lower than that for the pair of Pr/Et homologs. On going from *n*-alkyl-*cyclo*-hexanes to *N*-*n*-alkylpiperidines, a considerable decrease in the CH_2 increments calculated for the first pair of homologs is observed.[19] It can be assumed that the reason for the appearance of the anomalous difference in $\delta I(CH_2)$ values for aromatic *n*-alkyl-substituted cycles is related, as in the case of *n*-alkylalicycles,[19] to the appearance of a conformationally strained fragment in the ethyl homolog. For this fragment, a free rotation of the methyl group of the ethyl substituent is difficult because of the proximity of the aromatic ring.[20]

The increments of the methyl substituent (δI(Me)) in retention indices are presented in Table 3. The position of the methyl group in the cycle is indicated relative to the nearest N atom. As seen in Table 3, the lowest δI(Me) value is observed in the case of tautomeric 3(5)-methylpyrazole. Due to migration of the H atom between N atoms of the pyrazole cycle, the methyl group finds itself in the α-position alternatively to the pyrrole N(1) and pyridine N(2) atom. The increments δI(Me) in these positions are nonequivalent because of the different natures of the N atoms in the pyrazole ring, and a value of 66 iu depends on the ratio of tautomers. A much higher increment of the methyl group in tautomeric 4(5)-methylimidazole calculated from the previously obtained data[5] indicates that the ratios of the tautomeric forms for this compound and 3(5)-methyl-

Table 3. Increments of the methyl group (δI(Me)) in retention indices of methyl-substituted nitrogen-containing heterocyclic compounds

Compounds compared	Position of Me in cycle	δI(Me) /iu
	α_{N-2} (α_{N-1})	66
	β_{N-1}; β_{N-2}	100
	α_{N-3} (α_{N-1})	129
	α_N	99
	γ_N	112
	β_N	128
	α_N	67
	α_N	70
	β_N	130
	α_N	83
	β_N	120
	α_N	90

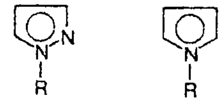
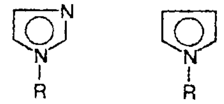
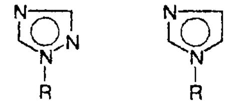
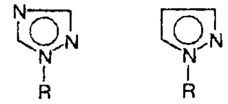
pyrazole are different. The removal of the methyl group in the pyrazole ring from the N atoms leads to an increase in its increment in the retention index from 66 to 100 iu. A similar trend of increasing the δI(Me) values with removal of the alkyl group from the N atom is also observed for substituted isoxazole and six-membered cyclic compounds, pyridazines and pyrimidines (see Table 3). The same situation was observed for pyridines, oxazoles, and thiazoles.[2–4] The high δI(Me) values, which attain 130 iu for heterocycles with several heteroatoms (see Table 3), have not been reported earlier for organic compounds.

A priori schemes for calculation of retention indices of polyalkyl-substituted pyridines and pyrazines,[2,9] and oxazoles and thiazoles,[3] have previously been developed. The δI(Me) values presented in Table 3 can also be used for calculation of retention indices of polymethyl-substituted pyrazoles, isoxazoles, pyrimidines, and pyridazines. It was of interest to verify the possibility of calculation of I values taking into account the increments of heteroatoms.

The change in the retention indices (δI(N)) which should accompany the introduction of the N atom in molecules of five- and six-membered heterocyclic compounds, *i.e.*, the replacement of the =CH— fragment of the heteroaromatic cycle by the pyridine N atom, is presented in Tables 4 and 5. The position of the inserted nitrogen atom is indicated with respect to the already present N heteroatom. The values of retention indices of pyridine and pyrazine and their methyl derivatives necessary for the calculation of δI(N) were taken from the previously published work,[2] which also was conducted on a column containing the methylsiloxane stationary phase. As can be seen in Tables 4 and 5, the highest δI(N) values are observed when the nitrogen atom is introduced in the β-position of the nonalkylated cycle. For example, if the nitrogen atom appears in the β-position of the pyrrole ring, the retention index increases by 295 iu, and when the N atom in the pyrazole molecule occupies the β-position to two already available N atoms, it increases by 252 iu. The appearance of the N atom in the α-position of the pyrrole cycle is accompanied by a much lower increase in I. When the pyrrole N atom has alkyl substituents, δI(N) attains much lower values than in the case of unsubstituted cycles. Again a familiar trend can be traced: a N atom in the α-position of the cycle brings about lower δI(N) values than one in the β-position.

On introducing the N atom to the six-membered nitrogen-containing cycle, *i.e.*, on going from pyridine to pyridazine, pyrimidine, or pyrazine (see Table 5), the highest δI(N) values were obtained for compounds where the introduced N atom occupies the α-position to the already available N atom (pyridazine and its methyl-substituted derivatives). Compared to other six- and five-membered heterocycles, pyridazine and imidazole exhibit an increased GC sorption activity and higher δI(N) values and also show unusual physicochemical properties, for example, high values of boiling tempera-

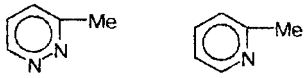
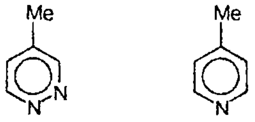
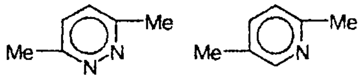
Table 4. Change in retention indices ($\delta I(\mathrm{N})$) upon insertion of the pyridine N atom into the five-membered heteroaromatic cycle

Compounds compared	Position of the inserted N atom	R	$\delta I(\mathrm{N})$ /iu
	α_{N-1}	H	117
		Me	12
		Et	6
		Pr	7
	β_{N-1}	H	295
		Me	198
		Et	188
		Pr	188
	α_{N-1}; β_{N-3}	H	74
		Et	−91
		Pr	−90
	β_{N-1}; β_{N-2}	H	252
		Et	91
		Pr	91

tures and dipole moments[10,11] and the ability of molecules for self-association.[13] The introduction of the N atom in the β- or γ-position of pyridine gives a much lower increment in the retention (see Table 5, pyrimidine—pyridine and pyrazine—pyridine pairs). When the N atom takes the β-position to two already available N atoms, the $\delta I(\mathrm{N})$ value becomes negative (*s*-triazine—pyrimidine pair). The presence of methyl groups in the β- or γ-positions with respect to the inserted N atom increases the $\delta I(\mathrm{N})$ values, whereas the α-arrangement of the methyl group substantially decreases $\delta I(\mathrm{N})$ (see Table 5).

Taking into account the regularities observed for the replacement of the =CH— fragment of the heteroaromatic rings by the N atom, we can calculate retention indices of some substances. For example, the *I* value for other *N*-alkyl-substituted compounds can be easily calculated from the data for pyrroles or diazoles. However, the use of these schemes for calculation of retention indices of compounds with several heteroatoms in the cycle is difficult because the GC data for the initial heterocycles with one heteroatom are scarce. By contrast, numerous data on the retention of aromatic hydrocarbons on columns with nonpolar stationary phases are published.[16,21] We attempted to use these data and

Table 5. Change in retention indices ($\delta I(\mathrm{N})$) upon insertion of the pyridine N atom into the six-membered heteroaromatic cycle

Compounds compared	Position of the inserted N atom	$\delta I(\mathrm{N})$ /iu
	α_{N-1}	177
	α_{N-1}	178
	α_{N-1}	193
	α_{N-1}	193
	α_{N-1}	130
	α_{N-1}	135
	β_{N-1}	6

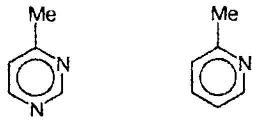
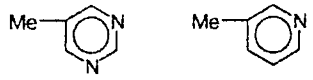
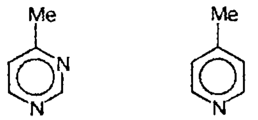
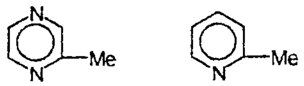
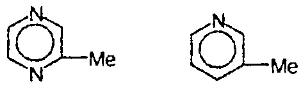
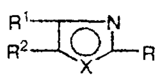
Compounds compared	Position of the inserted N atom	$\delta I(\mathrm{N})$ /iu
	β_{N-1}	23
	β_{N-1}	12
	β_{N-1}	−25
	γ_{N-1}	35
	γ_{N-1}	48
	γ_{N-1}	0
	β_{N-1}; β_{N-3}	−81

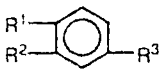
Table 6. Comparison of calculated (calc) by Eq. (1) and experimental (exp) *I* values of alkyloxazoles and alkylthiazoles

1				**2**			δ*I*(N,X)/iu	Azole				*I* of azole/iu	
X	R^1	R^2	R^3	R^1	R^2	R^3		X	R^1	R^2	R^3	calc	exp
O	H	Et	Me	H	Et	Me	−93	O	H	Me	Me	765	765
								O	H	Me	Et	855	851
								O	H	H	Me	665	662
S	H	Et	Me	H	Et	Me	62	S	H	Me	Me	920	922
								S	H	Me	Et	1010	1004
								S	H	H	Me	820	816
O	Me	H	Et	Me	H	Et	−130	O	Me	H	Me	728	730
								O	Et	H	Me	818	820
								O	Me	Et	Me	930	923
								O	Me	Me	Me	845	843
								O	Et	H	Et	907	903
S	Me	H	Et	Me	H	Et	22	S	Me	H	Me	880	887
								S	Et	H	Me	970	974
								S	Et	H	Et	1059	1053
								S	Me	Me	Me	997	997
								S	Me	Et	Me	1082	1072

calculate *I* of alkyl-substituted oxazoles and thiazoles



1



2

by the equation:

$$I(\mathbf{1}) = I(\mathbf{2}) + \delta I(\mathrm{N,X}), \qquad (1)$$

where $I(\mathbf{1})$ is the retention index of alkyl-substituted oxazole (X = O) or thiazole (X = S), $I(\mathbf{2})$ is the retention index of alkyl-substituted benzene with a similar structure, and δ*I*(N,X) is the value that characterizes the change in the retention index when the —CH=CH— and =CH— fragments of the benzene ring are replaced by the N and X atoms (O or S), respectively.

Oxazoles and thiazoles were chosen as model substances, because the *I* values for methyl and ethyl derivatives of these compounds have been determined experimentally on a nonpolar column with OV-101 at 110 °C.[3,4] In calculations of *I* by Eq. (1), oxazoles and thiazoles were divided into two groups by the degree of screening of the N atom by alkyl substituents. The first group contained compounds with R^1 = H, and the second group consisted of those with R^1 = Alk. In both groups, R^3 = Alk and R^2 = H or Alk. Division into groups based on the presence of alkyl substituents in the α- and α′-positions at the N atom was also used in other methods of calculation of retention indices of nitrogen-containing heterocycles.[4,8] The results of determination of *I* by Eq. (1) are presented in Table 6 along with the data of analysis of the compounds used for the calculation of δ*I*(N,X). The retention indices of aromatic hydrocarbons obtained on a column with SE-30 at 65 °C were taken from the reference book.[16]

As can be seen in Table 6, the calculated and experimental values of retention indices of azoles coincide, on the average, with an accuracy of ±4 iu, which is comparable with the results obtained previously by the use of results on the increments of alkyl groups and with the results expected from similarity in the GC behavior of O- and S-containing compounds.[3,4] Equation (1) can be applied for the calculation of *I* of oxazoles and thiazoles from the data for alkylbenzenes obtained with a hydrocarbon stationary phase (Squalan) at 80 and 100 °C.[16]

The advantage of Eq. (1) is the possibility to use published data on retention of aromatic hydrocarbons on nonpolar stationary phases. In this case, it is not necessary to calculate retention indices of hydrocarbons for a column on which *I* of nitrogen-containing heterocyclic compounds have been determined. Perhaps, equations similar to Eq. (1) are valid for the calculation of retention indices of other heterocyclic compounds.

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