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KOVÁTS RETENTION INDICES OF HIGH-MOLECULAR-WEIGHT MONOMETHYL-, CYCLOPENTYL-, CYCLOHEXYL- AND PHENYLALKANES

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SUMMARY

Kováts retention indices of monomethyl-, cyclopentyl-, cyclohexyl- and phenylalkanes containing 17–24 carbon atoms were measured on Apiezon L, OV-1, Dexsil 300, SE-52 and Carbowax 20M stationary phases. Good agreement between the calculated and measured retention indices was found. The calculations were made by summing the contributions of atoms, bonds and interactions to the retention index. The elution order of 3- and 2-methylalkanes was correlated only with the Van der Waals volumes of the molecules. It was found that cycloalkanes and phenylalkanes have a high $\Delta I/\Delta T$ coefficient. Therefore, to interpret the retention indices of these compounds we used $f_{i(T)}$ coefficients, which determine the influence of temperature on the contribution of interactions to the retention indices. A study of changes in the retention indices on individual stationary phases demonstrated that Dexsil 300 was the most suitable for the analysis of mixtures of isoalkanes, cycloalkanes and phenylalkanes.

INTRODUCTION

During chromatographic investigations of crude oil fractions¹, homologous 2-, 3-, 4- and 5-monomethylalkanes were identified by gas chromatography–mass spectrometry (GC–MS). However, certain methyl- and dimethylalkanes cannot be distinguished by means of mass spectra². With this in mind, we synthesized model homologous isoalkanes in order to confirm by retention index measurements the chemical structures that had been proposed earlier. The retention indices of methylalkanes for a number of liquid stationary phases will also permit a rapid and convenient identification of the types of compounds often found in natural products³.

Most data on the retention indices of high-molecular-weight branched alkanes were obtained on packed columns or short capillary columns and reported as equiv-

alents of chain length (ECL) indices^{4,5}. The accuracy of measurement was low, and 2- and 3-methyl isomers were not separated.

A number of mixtures of natural hydrocarbons, *e.g.*, cuticular lipids of insects, consist of mono-, di- and trimethylalkanes and it is extremely difficult to identify the components of such mixtures by GC-MS alone because, even if capillary columns are employed, the components are inseparable and their spectra do not indicate the presence of molecular ions. In such instances the measurement of retention indices would permit the easier identification of a compound with a known molecular weight (C_n).

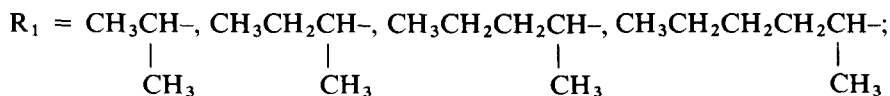
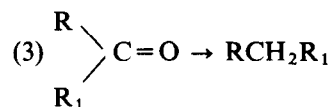
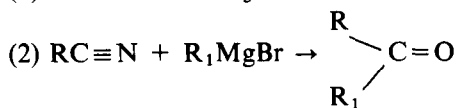
Kováts retention indices are commonly used to express the retentions of various substances^{6,7}. To characterize substances one may utilize not only the retention indices but also the temperature dependence of these indices ($\Delta I/\Delta T$)⁸⁻¹¹. Having found a relationship between retention index and structure, it is easy to identify compounds on the basis of their retention indices¹².

The retention indices of methylalkanes have been discussed in a number of publications^{13,14}; however, the values given in the literature pertain to low-molecular-weight compounds. Our investigations concerned the chromatographic properties of higher molecular weight compounds (up to 338 a.m.u.).

EXPERIMENTAL

Synthesis of the compounds investigated

Monomethyl-, cyclopentyl-, cyclohexyl- and phenylalkanes were prepared in a three-stage process:



Myristic, palmitic and stearic acids were used in the synthesis. The nitriles of the fatty acids were obtained by bubbling gaseous ammonia through molten fatty acids at 330°C for 6 h. The nitriles were purified by distillation at atmospheric pressure. The main fractions of the nitriles were purified by washing with a 50% solution of potassium hydroxide in ethanol and then with hot water, followed by crystallization from ethanol. Commercial isopropyl and cyclohexyl bromide and bromobenzene were used in the synthesis; other bromides were prepared by treatment of commer-

cially available 2-butanol, 2-pentanol, 2-hexanol and cyclopentanol with hydrobromic acid. Grignard reaction of the bromides with a mixture of three nitriles (prepared earlier) yielded long-chain ketones. Hydrocarbons were obtained by reduction of the ketones by means of the modified Clemensen method. Crude ketones dissolved in toluene were added to amalgamated zinc turnings in concentrated hydrochloric acid. The reduction was carried out by bubbling gaseous hydrogen chloride for 10 h. The mixture of crude hydrocarbons was purified on a column filled with silica gel-Kieselgel MN (Merck). The hydrocarbons eluted with *n*-hexane were chromatographically pure except that the phenylalkanes contained small amounts of phenylalkenes.

Gas chromatography

Kováts retention indices were measured on OV-1 (22 m × 0.3 mm I.D.), SE-52 (40 m × 0.3 mm I.D.), Dexsil 300 (40 m × 0.25 mm I.D.), Carbowax 20M (30 m × 0.25 mm I.D.) and Apiezon L (20 m × 0.3 mm I.D.) capillary columns. The columns were connected to a modified Varian Aerograph 1400 gas chromatograph. The samples were introduced into an injector consisting of a glass pre-column filled with Chromosorb and a splitter (1:20).

A 1- μ l volume of 0.1 mg/cm³ standard solution, which represented 5 ng of the sample substance, was introduced into the column.

The maximum sensitivity of the electrometer used for the measurements was 10⁻¹² A/mV. None of the chromatographic peaks exhibited any sign of overloading.

Helium was used as the carrier gas. The end of the capillary was connected to a detector in the make-up system and the flow velocity of the additional gas was 20 cm³/min. A Philips PM 8220 recorder was used to record the chromatograms; the paper speed was 2 cm/min. The absolute error, estimated by Student's method for a confidence level of 0.99, was 0.5 unit.

RESULTS AND DISCUSSION

Isoalkanes

Results of measurements of the retention indices of 2-, 3-, 4- and 5-methylalkanes are given in Table I. The measured retention indices on Dexsil 300 confirmed our earlier identification of the structure of crude oil fraction components¹ obtained by GC-MS.

Retention index measurements become even more important when it is possible to find a relationship between the retention indices and chemical structure. For this purpose we plotted the dependence of the difference in retention indices as a function of the number of carbon atoms in a homologous series. The differences in the retention indices were calculated by subtracting from the retention index of the sample compound the retention index of an earlier eluting *n*-alkane. Hence the differences in the retention indices may vary from 1 to 99. A straight-line dependence confirms an identical course of the dependence of the logarithm of retention volume on the number of carbon atoms in a homologous series. The dependences of homologues of 2-, 3-, 4- and 5-methylalkanes are shown in Fig. 1.

The observed constancy of the retention index differences results from a very small value of $\Delta I/\Delta T$ for monomethylalkanes. The published value of $\Delta I/\Delta T$ for isoalkanes of molecular weight of up to 142 a.m.u. on squalane¹⁵⁻¹⁷ is about 0.2 unit

TABLE I
COMPARISON OF RETENTION INDICES OF ISOALKANES WITH THOSE CALCULATED ACCORDING TO THE METHOD OF TAKÁCS *et al.*¹⁹

Hydrocarbon	Stationary phase									
	OV-1		Dexsil 300		SE-52		Apiezon L		Carbowax 20M ^{***}	
	<i>I</i> _{obs}	<i>I</i> _{calc}	<i>I</i> _{obs}	<i>I</i> _{calc}	<i>I</i> _{obs}	<i>I</i> _{calc}	<i>I</i> _{obs}	<i>I</i> _{calc}	<i>I</i> _{obs}	<i>I</i> _{calc}
2-Methylhexadecane*	1664.8	1664.6	1660.6	1661.5	1663.8	1663.9	1660.6	1661.5	1661.7	1662.3
2-Methyloctadecane	1864.4	1864.3	1860.3	1861.3	1863.2	1863.4	1860.4	1861.3	1861.7	1862.3
2-Methyleicosane	2064.3	2064.3	2060.3	2061.4	2063.1	2063.4	2060.5	2061.4	2061.7	2062.3
3-Methylheptadecane*	1772.2	1772.4	1770.8	1771.4	1771.2	1771.7	1768.4	1769.6	1769.2	1770.2
3-Methylnonadecane	1972.1	1972.2	1971.1	1971.6	1971.2	1971.7	1968.3	1969.5	1969.2	1970.2
3-Methylheneicosane	2172.0	2172.3	2172.2	2171.7	2171.3	2171.8	2168.5	2169.7	2169.3	2170.3
4-Methyloctadecane**	1858.9	1858.6	1856.3	1856.7	1857.9	1857.9	1654.7	1855.5	1857.0	1857.2
4-Methyleicosane	2058.5	2058.4	2056.6	2057.2	2057.4	2057.5	2054.6	2055.4	2057.0	2057.2
4-Methyldocosane	2258.3	2258.2	2256.2	2256.6	2257.2	2257.4	2254.3	2255.2	2256.9	2257.2
5-Methylnonadecane**	1952.2	1954.8	1949.2	1952.5	1951.6	1954.3	1946.6	1950.6	1948.7	1952.2
5-Methylheneicosane	2151.8	21454.5	2149.1	2152.5	2151.1	2154.0	2146.4	2150.5	2148.5	2152.0
5-Methyltricosane	2351.6	2354.3	2349.1	2352.5	2350.0	2353.1	2346.3	2350.4	2348.7	2352.2

* For 2- and 3-methylalkanes at 180°C.

** For 4- and 5-methylalkanes at 190°C.

*** For Carbowax 20M liquid phase at 150°C.

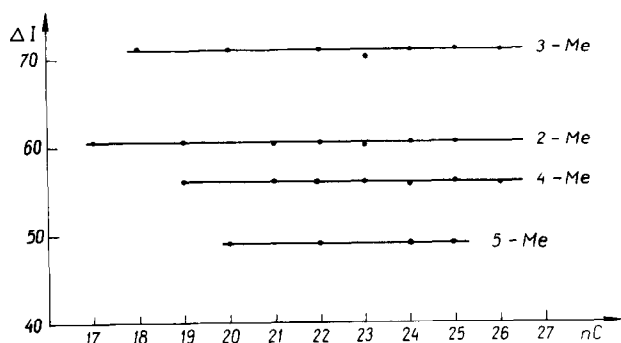


Fig. 1. Retention index differences (ΔI) vs. number of carbon atoms in methylalkanes.

per 10°C. Our measurement error exceeded the expected value of ΔI per 10°C, which made it impossible to determine the above quantity for the sample compounds.

The elution order of isoalkanes on all the stationary phases investigated (Table I) is 5-, 4-, 2- and 3-methylalkanes, which is consistent with the order of the respective low-molecular-weight isoalkanes¹⁸.

There are no significant differences between the retention indices measured on the various stationary phases. Even for polar Carbowax 20M the retention indices of isoalkanes are the same as on Dexsil 300.

The additivity rule of retention increments is the basis of the correlation of retention indices with chemical structure; much work on this subject has been carried out by Hungarian workers¹⁹⁻²³. Table I also lists the calculated retention indices of the isoalkanes studied.

Retention index calculations were carried out by the method of Takács *et al.*¹⁹, assuming that the retention index is the sum of the following quantities:

$$I = I_a + I_b + I_i$$

where I_a is the contribution related to atomic masses defined as one tenth of the mass, I_b is the contribution of particular kinds of bonds determined from experimental data and I_i is the contribution related to intermolecular interactions of the sample substance with the stationary phase. I_i for any stationary phase contains the factor of universal interactions, I_{ig} , and the factor of specific interactions, I_{ii} . Hence, for polar stationary phases

$$I_{ip} = I_{ig} + I_{ii}$$

and

$$I_{ii} = I_{ip} - I_{ig}$$

The I_{ig} interaction is defined as the contribution to the retention on a non-polar stationary phase according to

$$I_{ig} = I_{exp} \cdot 0.7455$$

TABLE II
RETENTION INDICES AND THEIR TEMPERATURE DEPENDENCE (I PER 10°C) FOR CYCLOPENTYL-, CYCLOHEXYL- AND PHENYLALKANES

Hydrocarbon	Stationary phase														
	OV-1			Dexsil 300			SE-52			Apiezon L			Carbowax 20M		
	I	ΔI per 10°C	I	ΔI per 10°C	I	ΔI per 10°C	I	ΔI per 10°C	I	ΔI per 10°C	I	ΔI per 10°C			
Cyclopentyltetradecane*	1961.7	3.1	1977.9	3.3	1966.8	2.7	1975.4	2.9	1986.7	4.7					
Cyclopentylhexadecane	2162.5	3.0	2178.7	3.3	2167.4	2.8	2175.9	2.9	2184.4	4.7					
Cyclopentyl-octadecane	2363.4	3.0	2379.5	3.2	2368.1	2.8	2376.4	2.8	2382.2	4.6					
Cyclohexyltetradecane**	2071.6	4.0	2093.5	4.6	2079.1	4.2	2084.7	5.2	2106.0	6.7					
Cyclohexylhexadecane	2272.5	3.9	2295.4	4.5	2280.1	4.3	2285.7	5.1	2304.2	6.7					
Cyclohexyl-octadecane	2473.4	4.0	2497.3	4.5	2481.1	4.3	2486.7	5.2	2502.4	6.6					
Phenyltetradecane***	2082.2	4.6	2117.9	4.5	2089.9	4.3	2104.3	3.2	2316.5	9.5					
Phenylhexadecane	2281.5	4.5	2320.0	4.6	2292.1	4.3	2306.7	3.1	2514.7	9.4					
Phenyl-octadecane	2482.8	4.5	2522.1	4.5	2494.3	4.4	2509.1	3.2	2712.9	9.6					

* For cyclopentylalkanes at 190°C (Carbowax 20M, 150°C).

** For cyclohexylalkanes at 210°C (Apiezon L, 190°C ; Carbowax 20M, 170°C).

*** For phenylalkanes at 210°C (Carbowax 20M, 190°C).

§ ΔI per 10°C was calculated from retention indices measured at temperatures 10°C above and 10°C below that of the sample hydrocarbon.

The values of the contributions needed for the calculations were taken from refs. 19–23. All values calculated within the retention index measurement error are in agreement with the experimental data. However, the application of this method to the interpretation of the retention indices of dimethylalkanes that we had identified in cuticular waxes of the Colorado beetle gave results differing by up to 10 units²⁴. This is probably due to the significant ΔI per 10°C values of these compounds.

The sum of the Reynolds numbers of the stationary phases is between 143 (Apiezon L) and 2308 (Carbowax 20M). The calculated values of I_{ii} are given in Table I. The isoalkanes studied should not have large I_{ii} contributions, which is in agreement with the experimental results (Table I).

As can be seen, the isoalkanes studied, just like low-molecular-weight isoalkanes^{18,25}, elute earlier than *n*-alkanes of the same molecular weight. To interpret the retention indices of isoalkanes a parameter is needed that would determine the branching of the compounds. Randic²⁶ suggested a branching or connectivity index,

χ , defined as the sum of factors for C–C bonds, $1/\sqrt{xy}$, where *x* and *y* are types of carbon atoms forming a bond. This approach was also used for alkanes²⁷. The following equation describes the retention index:

$$I = a\chi + b$$

Hence, higher χ values correspond to a higher retention index. Different isomers should have different values. In fact, for 2- and 3-methylalkanes the difference was found to be 0.03, which is consistent with the order of elution. However, for the higher isomers, 4-, 5-methyl etc., χ is constant, suggesting common elution, which is not true.

As above, the Van der Waals volume, V_m ²⁸, which is given by the equation

$$I = aV_m + b$$

does not distinguish the isomers, except 2- and 3-methylalkanes.

Cycloalkanes and phenylalkanes

Unlike isoalkanes, the $\Delta I/\Delta T$ values of cyclopentyl-, cyclohexyl- and phenylalkanes are measurable. The retention indices and ΔI per 10°C values are given in Table II. The dependences of the retention indices on the number of carbon atoms in a homologous series were obtained by plotting retention index differences against the number of carbon atoms (Figs. 2 and 3). It increases for Apiezon L, OV-1, SE-52 and Dexsil 300 (Fig. 2) and decreases for Carbowax 20M (Fig. 3). We also determined a direct dependence of the retention indices on the number of carbon atoms at a constant temperature by using the least-squares method to calculate the coefficients in the equation

$$I = a + bz$$

where *a* and *b* are constants and *z* is the number of carbon atoms in the molecule. The *a* and *b* values for individual compounds are given in Table III. The above equation makes it possible to calculate the retention index of any homologue for a given stationary phase at the same temperature.

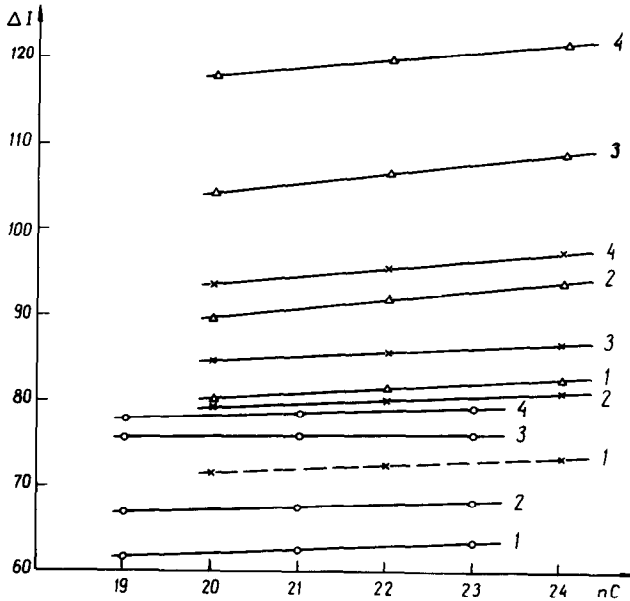


Fig. 2. Retention index differences (ΔI) vs. number of carbon atoms in cyclopentylalkanes (O), cyclohexylalkanes (x) and phenylalkanes (Δ) on OV-1 (1), SE-52 (2), Apiezon L (3) and Dexsil 300 (4).

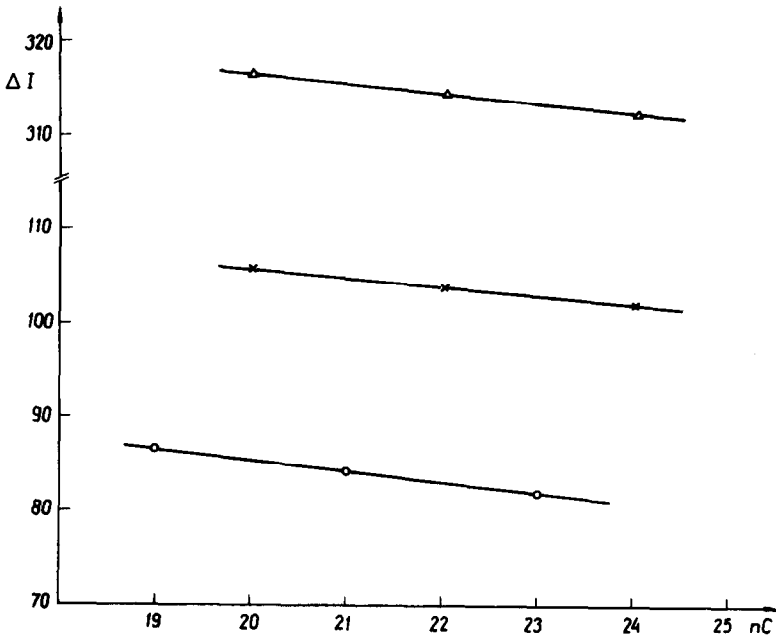


Fig. 3. Retention index differences (ΔI) vs. number of carbon atoms in cyclopentylalkanes (O), cyclohexylalkanes (x) and phenylalkanes (Δ) on Carbowax 20M.

TABLE III
VALUES OF a AND b IN THE EQUATION $I = a + bz$

Stationary phase	Cyclopentylalkanes			Cyclohexylalkanes			Phenylalkanes		
	$T(^{\circ}\text{C})$	a	b	$T(^{\circ}\text{C})$	a	b	$T(^{\circ}\text{C})$	a	b
OV-1	190	43.43	100.93	210	62.62	100.45	210	67.18	100.65
Dexsil 300	190	70.30	100.40	210	74.28	100.96	210	96.68	101.6
SE-52	190	60.51	100.33	210	69.1	100.50	210	67.9	101.1
Apiezon L	190	70.67	100.25	190	74.7	100.50	210	80.3	101.2
Carbowax 20M	150	107.84	98.88	170	124.0	99.1	190	334.5	99.1

As stated earlier, three groups of compounds studied have a significant $\Delta I/\Delta T$ value, which must be taken into account when trying to correlate retention indices with structure. According to Takács *et al.*²⁹, the influence of temperature on retention indices can be expressed by the equation

$$I(T) = \frac{I_m}{f_{ig}^{\text{st.ph.}}(T) + f_{ii}^{\text{st.ph.}}(T)}$$

where st.ph. = stationary phase, f_{ig} = universal interaction factor, f_{ii} = specific interaction factor and $I_m = I_a + I_b$. Hence the molecular contribution, I_m , to the retention index is independent of temperature. For a non-polar stationary phase such as Apiezon L this equation is reduced to

$$I(T) = \frac{I_m}{f_{ig}(T)} \quad \text{or} \quad I(T) = \frac{I_{ig}}{1 - f_{ig}(T)}$$

The results obtained for cycloalkanes and phenylalkanes showed that the above

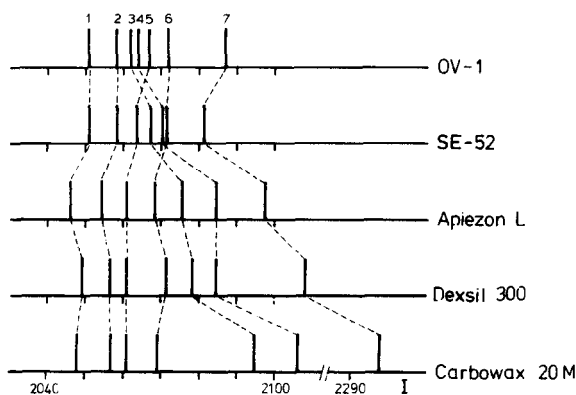


Fig. 4. Retention index shifts of branched, cyclo- and phenylalkanes. Temperature, 190°C (Carbowax 20M, 170°C). 1 = 5-Methyleicosane; 2 = 4-methyleicosane; 3 = cyclopentylpentadecane; 4 = cyclohexyltetradecane; 5 = 2-methyleicosane; 6 = 3-methyleicosane; 7 = phenyltetracosane.

method cannot be employed to correlate retention indices with the structures of high-molecular-weight cycloalkanes and phenylalkanes.

The influences of stationary phases on the retention indices of individual groups of compounds was determined by means of a shift diagram (Fig. 4), which leads to the conclusion that Dexsil 300 is the most suitable stationary phase for investigations of mixtures of isoalkanes, cycloalkanes and phenylalkanes.

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