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A METHOD FOR THE IDENTIFICATION OF BRANCHED-CHAIN PARAFFINS BY CALCULATION OF THEIR RETENTION INDEX VALUES ON THE BASIS OF MOLECULAR STRUCTURE AND PHYSICAL PROPERTIES*

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SUMMARY

The retention times of branched-chain paraffins were shown to depend on their physical properties, especially molecular volume, when non-polar phases were used for their separation.

As the molecular volume depends strongly on structure, the calculation of additive terms, which are characteristic of every structural group in a molecule, can permit the theoretical calculation of the retention indices of any branched isomer.

The additive terms can be calculated both by solving a system of equations and by comparison of the retention indices of selected compounds. The general application of the method to retention data taken from the literature is also possible.

INTRODUCTION

The complete gas chromatographic separation of complex mixtures of linear and branched-chain paraffins has been accomplished by using long packed or capillary columns¹⁻⁸. The identification of the separated compounds is a complex problem, and requires the use of many known standards or the combination of the gas chromatograph with a mass spectrometer⁹.

Some attempts to identify the components on the basis of their retention times have been made¹⁰, but the results were unsatisfactory. The parameter that can be used for this kind of calculation is the retention index, I (ref. 10), due to the fact that other retention values (retention time, volume, etc.) are strongly dependent on the conditions of the analysis. The retention index depends only on the type of stationary phase used, on the compound being analysed, and on temperature. The effect of temperature on I is generally low^{11,12} and a linear dependence was found for 250 compounds over the temperature range 27-86° with a non-polar squalane column¹¹.

By comparing the retention indices obtained from columns with different

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polarities, it seems possible to make some hypotheses on the structures of the analysed compounds.

KOVÁTS¹³ suggested the use of the difference in the I values obtained on a polar and a non-polar phase¹⁴. ROHRSCHEIDER¹⁵ used a series of stationary phases and many compounds with different structures in order to calculate constants that permit the calculation of retention indices, but this method was not widely applied to different compounds of the same series and seems to be useful for the characterization of stationary phases rather than for the identification of unknown compounds¹⁶.

SCHOMBURG¹⁷ tried to calculate the retention indices of dimethylheptanes and dimethyloctanes empirically by using some factors taken from the analysis of the methyloctanes and methylnonanes, respectively.

The agreement between the calculated and experimental values was good when the distance between the tertiary carbon atoms of the chain was large, but the presence of a quaternary carbon atom or of two tertiary atoms in the α -position results in large difference between the calculated and experimental values.

Recently, TAKÁCS *et al.*¹⁸ proposed an approximation method by means of which the retention index of a substance can be determined on the basis of its molecular structure. This method seems to be very promising, but many experimental values were necessary for the calculation of I on a squalane column, and the contributions to the index from different stationary phases are not yet available.

In this paper, we describe a method that permits the calculation of the retention indices of the branched-chain paraffins on non-polar stationary phases. The method, which is based on the use of structural factors taken from the analysis of a few selected compounds, gives satisfactory agreement between calculated and experimental values, even when quaternary atoms in α -positions are present in the molecules.

RELATIONSHIPS BETWEEN RETENTION INDICES AND PHYSICAL CONSTANTS

As the physical constants of many paraffins are well known^{19,20}, the possibility of calculating the retention time of any compound on the basis of these constants appears very attractive. Also, complex correlations could be used, as complex mathematical problems can be solved by using electronic computers. We shall discuss the correlations between the retention index and the boiling point at standard temperature and pressure, the number of carbon atoms, the heat (enthalpy) of vaporization and the molecular volume.

Boiling point

KOVÁTS¹³ found the following empirical relationship between the difference in the retention indices of two isomeric compounds on the same phase and the difference in their boiling points: $\delta I = 5\delta T_b$. The validity of this equation is not general, and some attempts to calculate the retention indices of paraffins were completely unsatisfactory. A linear relationship between I and T_b was also suggested, and we tried to verify its validity. Fig. 1 is a plot of I against T_b for various branched-chain paraffins. The values were taken from the literature^{2,4,5,7} and/or were experimentally determined.

The use of the boiling points to calculate I is therefore impossible on non-polar phases also, especially when a large T_b range has to be considered.

In fact, the dependence of T_b on the number of carbon atoms, n , is not linear for n -paraffins also (see Fig. 3). As, by definition^{10,13}, an increase in n of unity corresponds to 100 index units, then the dependence of I on T_b also cannot be linear. A prediction of the I value of a branched-chain isomer would be possible only if the curve of the plot of T_b against n were the same for n -paraffins and for all the series of isomers (2-methyl, 3-methyl, ... 2,2-dimethyl, etc.), but, from the data reported in the literature cited, it can be seen that the curves and the slopes of the plot are different for the various series.

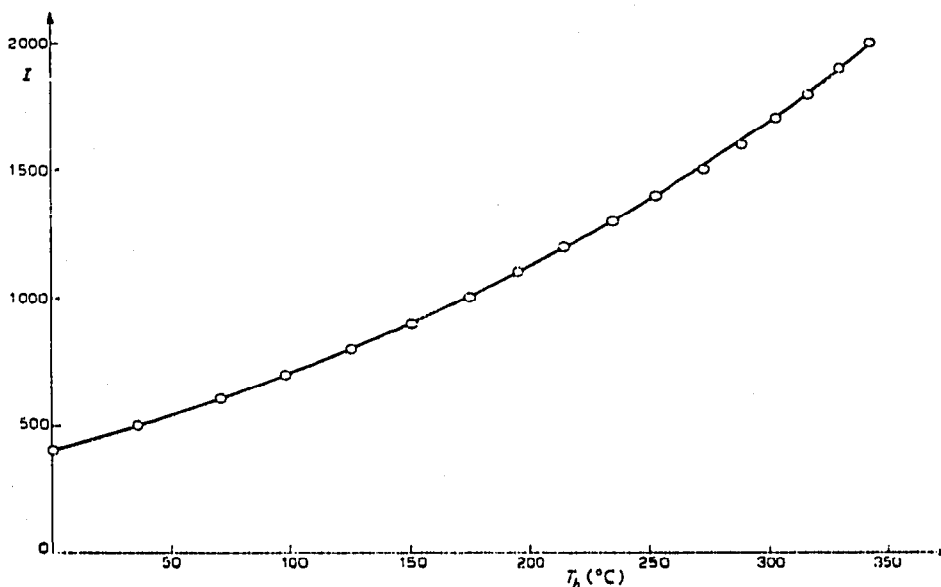


Fig. 3. Retention indices of linear paraffins as a function of the normal boiling point.

Number of carbon atoms

For every series of branched-chain paraffins (2-methyl, 3-methyl, etc.), the relationship between I and n is linear, and can be represented by an equation of the type:

$$I = E_0 + E_1 n \quad (1)$$

where E_0 and E_1 are constants. These constants were calculated by the least-squares method using the experimental data on several non-polar columns obtained from the literature (see Fig. 1) and from experimental determinations.

The values of the constants E_0 and E_1 are reported in Table I for the different series of homologues, and permit the calculation of the retention index of any branched-chain paraffin if the initial terms of the series can be analysed and their retention indices measured.

Heat of vaporization

The data for the heat of vaporization at saturation pressure at the normal boiling point were taken from the literature²⁰ and plotted against I .

TABLE I

VALUES OF THE CONSTANTS IN EQN. 1 FOR DIFFERENT SERIES OF PARAFFINS

Series of compounds	E_0	E_1
<i>n</i> -Paraffins	0	100
2-Methyl	-16.50	97.99
3-Methyl	6.28	96.20
3-Ethyl	25.17	94.25
2,2-Dimethyl	-76.76	100.10
2,3-Dimethyl	-13.80	97.37
2,4-Dimethyl	-29.77	95.00
3,3-Dimethyl	6.23	93.00
2,2,3-Trimethyl	6.37	92.90
2,2,4-Trimethyl	-72.33	95.70

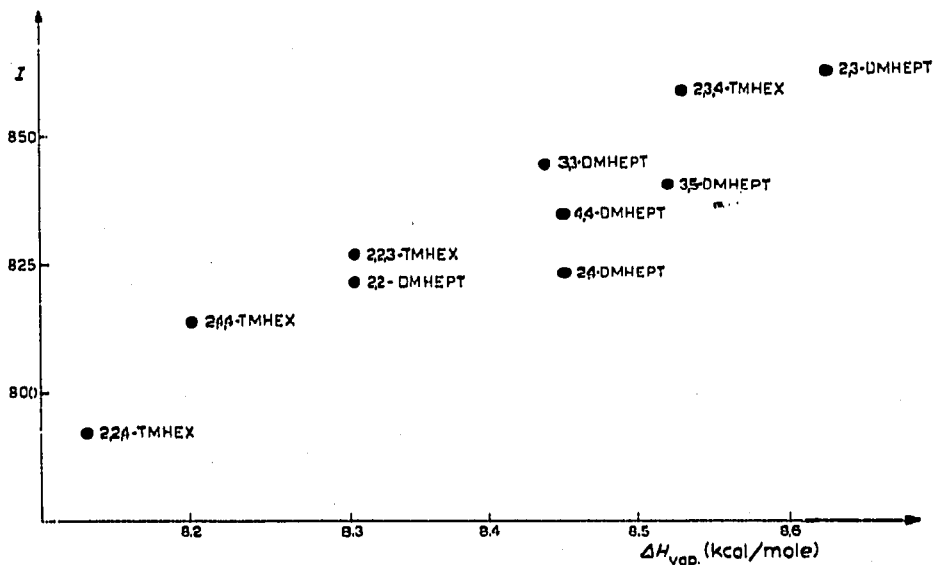


Fig. 4. Retention indices of some branched-chain nonanes as a function of their heats of vaporization at saturation pressure at the normal boiling point.

The isomers with the same value of n show irregular behaviour (see Fig. 4, where some data for nonanes are reported), while linear relationships were obtained for the different terms of every series with increasing n .

In Fig. 5 are reported the values for 2-methyl-, 3-methyl-, 2,2-dimethyl-, 2,3-dimethyl-, 2,4-dimethyl- and 3,3-dimethylparaffins with values of n between 6 and 9.

These results seem to be in agreement with the hypothesis that some specific structural groups of the molecule cause characteristic increments in the heat of vaporization, and these effects are additive when molecular interactions between these groups are absent²¹.

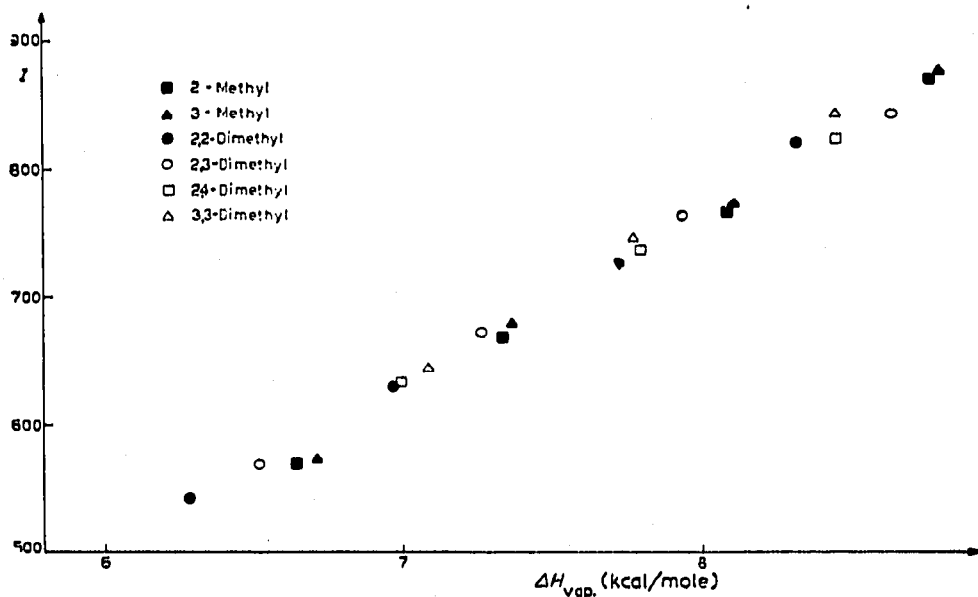


Fig. 5. Retention indices of some terms of different homologous series as a function of their heats of vaporization at saturation pressure at the normal boiling point.

Molecular volume

From the hypothesis reported above²¹ and taking into account the fact that a useful method of assigning a quantitative value to the structural groups of the molecule is the use of the molecular volume²², the relationship between this physical constant and *I* was examined.

The plot of the retention indices against the molecular volumes of the linear paraffins calculated by using literature values for the density²⁰ was linear, with a change of slope corresponding to hexadecane. This result may depend on the fact that hexadecane is the first linear paraffin that is solid at 25°, while the values of the density for higher paraffins are given at higher temperatures.

An approximate correction was therefore made, taking into account the coefficient of cubic expansion¹⁹.

The application of the formula $V_{25^\circ} = V_t (1 + 5.88 \Delta t)$ gives, for higher paraffins, values of the molecular volume shown as closed circles in Fig. 6, which show linear behaviour with a slope very close to that of the initial terms of the series. It seems probable that the correct knowledge of the molecular volumes of the higher paraffins at 25° would give one straight line only.

Also, the dependence of the *I* of the branched-chain paraffins on their molecular volumes was determined, and a series of parallel straight lines was obtained.

The linear relationships observed confirm the hypothesis that the increase in the length of the carbon chain (expressed by *n*) gives a constant contribution to the increase in *I*, while the differences between the various isomers are due to the different contributions of every structural group of the molecule.

The retention index can therefore be given by the sum of many terms, depending

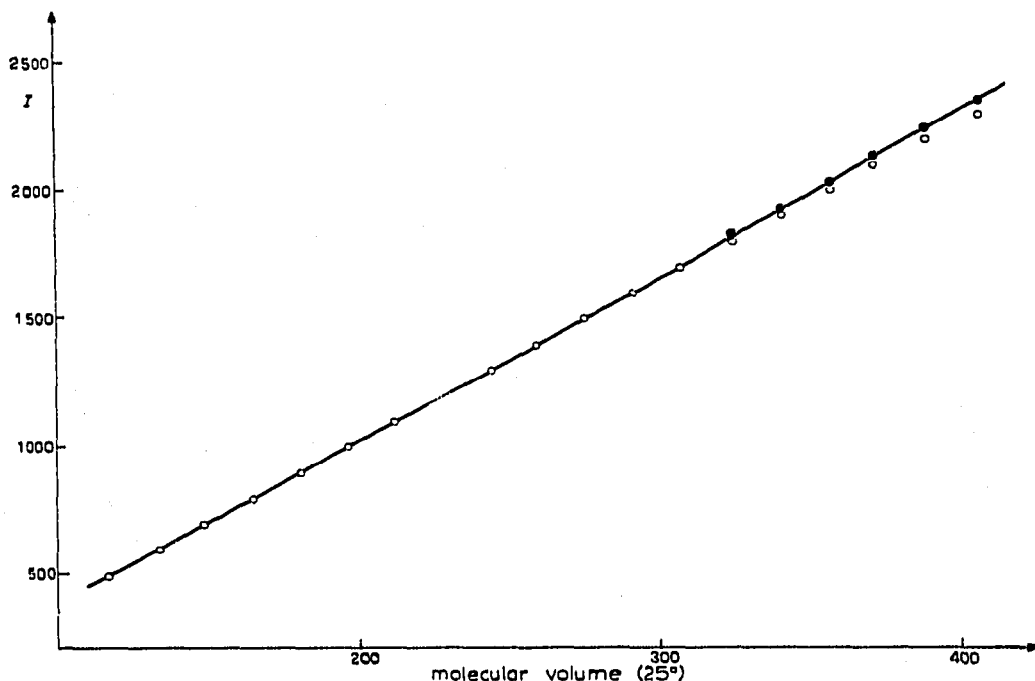


Fig. 6. Retention indices of *n*-paraffins as a function of their molecular volumes. Closed circles: values corrected by using the coefficient of cubic expansion (see text).

on the structural groups in the molecule. This hypothesis is in agreement with the well known relationship used in paper chromatography²³:

$$\log \left(\frac{I}{R_F} - 1 \right) = R_M \quad (2)$$

where the term R_M is additive in that it is composed of the partial R_M values for the individual functional groups or other groups of atoms in the molecule²⁴⁻²⁸.

DISCUSSION AND CONCLUSIONS

Some general conclusions on non-polar stationary phases can be made on the basis of the experimental data, as follows.

(1) The linear paraffin with *n* carbon atoms has the highest retention index of all the possible isomers with the same value of *n*. A decrease in the chain length and an increase in branching decrease the retention index. This decrease is indicated as δI , that is, the difference between the *I* values of the linear paraffin and of the branched-chain isomer¹⁹.

(2) When a quaternary carbon atom is in position 2 in the chain (external quaternary, EQ), δI is greater, while a lower δI is observed when the quaternary atom is in the middle of the chain (internal quaternary, IQ) or tertiary atoms are present (external or internal, ET or IT).

(3) The δI value decreases when the flexibility of the molecule decreases, owing to steric hindrance caused by more structural groups in the molecule. The δI value increases in the following order: two quaternary carbons in the α -position,

a quaternary and a tertiary carbon in the α -position, two tertiary carbons in the α -position, two tertiary or quaternary carbons in the β -position, divided by a secondary carbon atom.

(4) The presence of side-chains longer than methyl (ethyl, propyl, isopropyl, etc.) have an influence on I , as long as the length of the main carbon chain increases.

TABLE II

CHARACTERISTIC STRUCTURAL GROUPS WITH CORRESPONDING SYMBOLS

<i>Symbol</i>	<i>Group</i>
EQ	External quaternary C atom
IQ	Internal quaternary C atom
ET	External tertiary C atom
IT	Internal tertiary C atom
QQ	Two quaternary C atoms in α -position
TT	Two tertiary C atoms in α -position
TQ	Tertiary and quaternary C atoms in α -position
JO	Secondary C atom between two tertiary or quaternary atoms (joint)
EE	Terminal (external) ethyl group
DE	Two ethyls on the same C atom (dual ethyl)
SE	Ethyl group on a tertiary C atom (side ethyl)
EP	External propyl group

We tried to calculate the additive terms due to the different structural groups of the molecule by means of various systems of equations with eight to eleven variables, by using an electronic computer. The variables used were the additive terms of the structural groups that are reported, with the corresponding symbols, in Table II. The equations were constructed by taking into account the experimental retention indices of several compounds containing the structural groups (see Table V).

TABLE III

"SYSTEMS" METHOD FOR THE CALCULATION OF ADDITIVE VALUES, AND SUMMARY OF THE "COMPARISON" METHOD

<i>System</i>	<i>Compounds used for the system of equations</i>
1	2,2,3-TMB; 2,2-DMP; 3,3-DMP; 3-MHEx; 2,2,3,3-TMB; 2,3,4-TMP; 2,2-DMHEx; 2,3-DMHEx; 2,4-DMHEx; 3,3-DMHEx
2	2,2,3-TMB; 2,2-DMP; 2,3-DMP; 3,3-DMP; 2-MHEx; 2,2,3,3-TMB; 2,2,4-TMP; 2,4-DMHEx; 3,3-DMHEx; 2,3,4-TMP
3	2,2,3-TMB; 2,3-DMP; 3,3-DMP; 2-MHEx; 2,2,3,3-TMB; 2,2,4-TMP; 2,3,4-TMP; 2,2-DMHEx; 2,4-DMHEx; 3,3-DMHEx; 3,4-DMHEx
4	2,2,3-TMB; 2,2-DMP; 2,3-DMP; 3,3-DMP; 2-MHEx; 2,2,3,3-TMB; 2,2,4-TMP; 2,3,4-TMP; 2,2-DMHEx; 2,3-DMHEx; 3,3-DMHEx
5	As system 4, but replacing 2,3-DMHEx with 2,4-DMHEx
Comparison method	See Table V (SF-g6 at 80°)
Comparison method	See Table VI (squalane at 80°)

As an example, for 2,2,4-trimethylpentane, the equation is:

$$-\delta I(2,2,4\text{-TMP}) = EQ + ET + JO \quad (3)$$

and similar equations were written for selected compounds.

Depending on the group of compounds used for every system, the solution of the equation gave different values of the additive terms for every structural group. The calculated values are reported in Table III.

A negative sign indicates that the presence of the corresponding group decreases the retention time, while a positive sign indicates that the structural group causes an increase in the retention time.

Tertiary and quaternary carbon atoms, external and internal, therefore give a negative contribution, while the presence of two of these groups in the molecule (QQ, TT, TQ) gives positive terms, owing to steric hindrance to free rotation.

Some structural groups show very constant additive values, independent of the system (especially EQ, ET, TQ), but other groups have a strong fluctuation depending on the compounds used. In particular, the effect of the ethyl group (terms EE and DE) is very different for the various systems. This may be due to the fact that the effect of the ethyl group, as a terminal or side group, depends strongly on the length of the remaining chain and on other characteristic groups in the molecule.

The choice of the compounds to be used therefore has a strong influence on the results. For this reason, notwithstanding the fact that with the calculated terms (especially from systems 4 and 5) it is possible to calculate the retention indices of the compounds listed in Table III with satisfactory precision, the "systems" method is not convenient for general use owing to the great number of attempts that are necessary for the correct choice of the systems, and to the strong influence of incorrect experimental data, if used in the calculations.

To avoid the inconvenience of the "systems" method, a "comparison" method

additive terms for structural groups

	<i>IQ</i>	<i>ET</i>	<i>IT</i>	<i>QQ</i>	<i>TT</i>	<i>TQ</i>	<i>JO</i>	<i>EE</i>	<i>DE</i>	<i>SE</i>	<i>EP</i>
75	-60	-33	-27	+81	+19	+51	0	+2	+18	0	+5
71	-85	-33	-61	+73	+36	+47	0	+31	-15	0	+1
72	-87	-33	-63	+75	+37	+51	+1	+32	-15	0	+2
75	-72	-33	-27	+81	+19	+51	+4	+14	+6	0	+5
75	-94	-33	-71	+81	+41	+51	+4	+36	-16	0	+5
75	-69	-31	-39	+85	+29	+50	-7	+14	0	-24	+5
83	-87	-35	-53	—	+41	—	—	+22	—	—	+10

TABLE IV

"COMPARISON" METHOD FOR THE CALCULATION OF ADDITIVE VALUES
An example of application with the data of Table V.

Compound	Retention index	Structural groups	Value of any group
2-MP	569	ET	ET = - 31
2-MHEX	669	ET	
2-MHEPT	767	ET, EP	
2,2-DMB	542	EQ, EE	EQ = - 75
2,2-DMP	630	EQ, EP	EP = + 5
2,2-DMHEX	725	EQ	
2,3-DMB	568	ET, ET, TT	EE = + 14
2,3-DMP	673	ET, IT, TT, EE	
2,3-DMHEX	764	ET, IT, TT, EP	
3-MP	585	IT, EE, EE	IT = - 39
3-MHEX	680	IT, EE, EP	
3-MHEPT	775	IT, EE	
2,4-DMP	633	ET, ET, JO	
2,4-DMHEX	737	ET, IT, EE, JO	JO = - 6
3,3-DMP	662	IQ, EE, EE, DE	IQ = - 69
3,3-DMHEX	747	IQ, EE, EP	DE = + 3

TABLE V

COMPARISON BETWEEN EXPERIMENTAL AND CALCULATED RETENTION INDICES ON AN SF-96 COLUMN

	<i>I</i> (experimental) (80°)	<i>I</i> (calculated)	Difference (Exp. - calc.)
2,2-DMB	542	539	+ 3
2,3-DMB	568	567	+ 1
2-MP	569	574	- 5
3-MP	585	589	- 4
<i>n</i> -HEX	600	600	0
2,2-DMP	630	630	0
2,4-DMP	633	633	0
2,2,3-TMB	643	644	- 1
3,3-DMP	662	659	+ 3
2-MHEX	669	660	0
2,3-DMP	673	673	0
3-MHEX	680	680	0
3-ETP	685	678	+ 7
<i>n</i> -HEPT	700	700	0
2,2,4-TMP	696	687	+ 9
2,2-DMHEX	725	725	0
2,2,3,3-TMB	731	735	- 4
2,5-DMHEX	733	738	- 5
2,4-DMHEX	737	737	0
2,2,3-TMP	743 ^a	750	- 7
2,3,4-TMP	745	757	- 8
3,3-DMHEX	747	750	- 3

(Continued on p. 42)

TABLE V (continued)

	<i>I</i> (experimental) (80°)	<i>I</i> (calculated)	Difference (Exp. — calc.)
2,3,3-TMP	760	754	+ 6
2,3-DMHEX	764	764	0
2-MHEPT	766 ^a	769	- 3
4-MHEPT	768	771	- 3
3,4-DMHEX	775	779	- 4
3-MHEPT	774	775	- 1
2M-3ETP	—	773	—
3-ETHEX	775	770	+ 5
3M-3ETP	775	773	+ 2
<i>n</i> -OCT	800	800	0
2,2,4,4-TMP	785	743	+42
2,2,5-TMHEX	786	794	- 8
2,2,4-TMHEX	790	793	- 3
2,4,4-TMHEX	814	807	+ 7
2,3,5-TMHEX	816	821	- 5
2,2,3,4-TMP	816	824	- 8
2,2-DMHEPT	822	825	- 3
2,4-DMHEPT	825	828	- 3
2,2,3-TMHEX	826	831	- 5
2M-4ETHEX	830	827	+ 3
2,2M-3ETHEX	830	830	0
2,6-DMHEPT	835	838	- 3
4,4-DMHEPT	835 ^a	841	- 6
2,5-DMHEPT	841	844	- 3
3,5-DMHEPT	841	843	- 2
3,3-DMHEPT	845	845	0
2,4DM-3ETP	845	847	- 2
2,3,3-TMHEX	849	845	+ 4
2M-3ETHEX	854	854	0
2,2,3,3-TMP	858	855	+ 3
2,3,4-TMHEX	858 ^a	863	- 5
3,3,4-TMHEX	861	860	+ 1
3M-4ETHEX	862 ^a	869	- 7
2M-3ETHEX	862 ^a	864	- 2
4-ETHEPT	862 ^a	861	+ 1
2,3-DMOCT	862	859	+ 3
2,3,3,4-TMP	864 ^a	849	+15
3,4-DMHEPT	865	870	- 5
4-MOCT	871	866	+ 5
2-MOCT	872	869	+ 3
3-ETHEPT	875	865	+10
3-MOCT	877	875	+ 2
2,3DM-3ETP	878 ^a	868	+10
3,3-DETP	885 ^a	887	- 2
<i>n</i> -NON	900	900	0

^a Values taken from other sources.

was developed that permits the determination of the additive terms that are typical of the structural groups, by comparing different compounds with the same structural groups but with different numbers of carbon atoms.

As an example, from the retention indices of the following compounds:

2-MP = 569, 2-MHEN = 669 and 2-MHEPT = 767, one can calculate the value of the common group ET (external tertiary carbon atom), which is practically equal to -31.

The method is slightly complicated when many structural groups are simultaneously present in the same molecule.

In Table IV is reported an example of application of this method with the groups of compounds compared and the values of the additive terms calculated.

TABLE VI

COMPARISON OF THE EXPERIMENTAL AND CALCULATED VALUES OF SCHOMBURG¹⁷ WITH THE RESULTS OF THE "COMPARISON" METHOD ON SQUALANE AT 80°

Compound	Schomburg ^a		Difference (<i>g</i> - <i>b</i>)	This work	
	<i>g</i>	<i>b</i>		Calc.	Difference (<i>g</i> - calc.)
2-MOCT	865	---	---	865	0
3-MOCT	871	---	---	869	+2
4-MOCT	863	---	---	861	+2
3-ETHEPT	868	---	---	870	-2
4-ETHEPT	861	---	---	861	0
2,2-DMHEPT	817	830	-13	817	0
2,3-DMHEPT	857	836	+21	857	0
2,4-DMHEPT	822	828	-6	828	0
2,5-DMHEPT	834	835	-1	834	0
2,6-DMHEPT	827	830	-3	830	-3
3,3-DMHEPT	839	842	-3	839	0
3,4-DMHEPT(<i>E</i>) ^b	860	841	+19	866	-6
3,4-DMHEPT(<i>T</i>) ^b	860.5	841	+19.5	866	-6.5
3,5-DMHEPT(<i>m</i>) ^b	833.5	842	-8.5	837	-3.5
3,5-DMHEPT(<i>r</i>) ^b	835	842	-7	837	-2
4,4-DMHEPT	827	826	+1	833	-6
2-MNON	964	---	---	965	-1
3-MNON	970	---	---	969	+1
4-MNON	961	---	---	957	+4
5-MNON	958	---	---	955	+3
4-ETOCT	952	---	---	960	-8
2,2-DMOCT	916	928	-12	917	-1
2,3-DMOCT	953	934	+19	953	0
2,4-DMOCT	915.5	925	-9.5	909	+6.5
2,5-DMOCT	922	925	-3	916	+6
2,6-DMOCT	932	934	-2	934	-2
2,7-DMOCT	929	928	+1	930	-1
3,4-DMOCT(<i>E</i>) ^b	953	931	+22	961	-8
3,4-DMOCT(<i>T</i>) ^b	954	931	+23	961	-7
3,5-DMOCT(<i>E</i>) ^b	921	931	-10	926	-5
3,5-DMOCT(<i>T</i>) ^b	923	931	-9	926	-4
3,6-DMOCT	938	940	-2	938	0
4,4-DMOCT	921	922	-1	927	-6
4,5-DMOCT(<i>m</i>) ^b	944.5	922	+22.5	953	-8.5
4,5-DMOCT(<i>r</i>) ^b	946.5	922	+24.5	953	-6.5

^a *g* = experimental; *b* = calculated.

^b *E* = erythro form; *T* = threo form; *m* = meso form; *r* = racemic form.

All the additive terms are reported in the two last lines of Table III, and can be compared with the values obtained from the "systems" method. In many instances the values are very similar, but it must be pointed out that, when comparing different groups of compounds, the additive terms show a small variation (about one or two index units).

This reproducibility, and the easy application of the comparison method, which does not require the use of a computer for the handling of the complex systems, confirms that with this method the retention indices of the branched-chain paraffins can be easily calculated.

In Table V, the experimental and calculated values of I are shown. The agreement is satisfactory.

The calculation was also applied to nonane isomers, whose experimental retention indices were taken from the literature. The differences are greater, owing to dubious experimental values, but it is important to note that the elution order is the same for both the experimental and calculated values, and therefore the identification of the compounds in a complex mixture is still possible.

A great difference was only observed for 2,2,4,4-TMP, but this is due to the fact that this compound contains the first example of a secondary carbon atom between two quaternary groups, and the index value of this arrangement was not previously calculated.

The method can be applied to different types of stationary phases, and gives satisfactory results. The values of the additive terms change, depending on the type of column, but with the analysis of a few selected compounds the indices of all the isomers can be calculated.

As an example, we report in Table VI some experimental Kováts retention indices on squalane, the values and the differences calculated by SCHOMBURG¹⁷, the values calculated by us by the comparison method, and the differences between the experimental and our calculated values. The results of our method are particularly satisfactory for the compounds that contain quaternary or tertiary carbon atoms.

REFERENCES

- 1 L. R. DURRETT, L. M. TAYLOR, C. E. WANTLAND AND I. DVORETSKY, *Anal. Chem.*, 35 (1963) 637.
- 2 A. G. POLGAR, J. J. HOLST AND S. GROENINGS, *Anal. Chem.*, 34 (1962) 1227.
- 3 R. L. MARTIN AND J. C. WINTERS, *Anal. Chem.*, 35 (1963) 1930.
- 4 R. D. SCHWARTZ AND D. J. BROSSAUX, *Anal. Chem.*, 35 (1963) 1375.
- 5 E. S. JACOBS, *Anal. Chem.*, 38 (1966) 43.
- 6 R. D. SCHWARTZ, R. G. MATHEWS AND D. J. BROSSAUX, *J. Gas Chromatogr.*, 5 (1967) 251.
- 7 W. N. SANDERS AND J. B. MAYNARD, *Anal. Chem.*, 40 (1968) 527.
- 8 L. SOJAK AND A. BUCINSKA, *J. Chromatogr.*, 51 (1970) 75.
- 9 P. M. LLEWELLYN AND D. P. LITTLEJOHN, *Pittsburg Conference on Analytical Chemistry and Applied Spectroscopy*, 1966.
- 10 A. WEHRLI AND E. KOVÁTS, *Helv. Chim. Acta*, 42 (1959) 2709.
- 11 R. A. HIVELY AND R. E. HINTON, *J. Gas Chromatogr.*, 6 (1968) 203.
- 12 M. H. GUERMOUCHE AND J. M. VERGNAUD, *J. Chromatogr.*, 58 (1971) 169.
- 13 E. KOVÁTS, *Helv. Chim. Acta*, 41 (1958) 1915.
- 14 C. A. CRAMERS, J. A. RIJKS, V. PACAKOVA AND I. RIBEIRO DE ANDRADE, *J. Chromatogr.*, 51 (1970) 13.
- 15 L. ROHRSCHEIDER, *J. Chromatogr.*, 22 (1966) 6.
- 16 P. CHOVIN AND J. LEBBE, *J. Gas Chromatogr.*, 4 (1966) 37.
- 17 G. SCHOMBURG, *Anal. Chim. Acta*, 38 (1967) 45.
- 18 J. TAKÁCS, C. SZITA AND G. TARJÁN, *J. Chromatogr.*, 56 (1971) 1.
- 19 *Handbook of Chemistry and Physics*, 51st ed., Chemical Rubber Company, Cleveland, U.S.A., 1970-1971, p. C-75.

- 20 A.S.T.M. *Special Technical Publication No. 109 A*, A.S.T.M., Philadelphia, U.S.A., 1963.
- 21 M. DUNKEL, *J. Phys. Chem.*, **A138** (1928) 42.
- 22 E. HIRSCH, *Anal. Chem.*, **42** (1970) 1326.
- 23 E. C. BATE-SMITH AND R. G. WESTALL, *Biochim. Biophys. Acta*, **4** (1950) 427.
- 24 A. J. P. MARTIN, *Biochem. Soc. Symp.*, **3** (1948) 4.
- 25 E. R. REICHL, *Monatsch. Chem.*, **86** (1955) 69.
- 26 H. K. SCAVER AND R. Z. BURLISCH, *Naturforschung*, **106** (1955) 683.
- 27 S. MARCINKIEWICZ, J. GREEN AND D. McHALE, *J. Chromatogr.*, **10** (1963) 42.
- 28 O. MIKES, *Chromatographic Methods*, Van Nostrand-Reinhold, London, New York, Toronto and Melbourne, 1970, p. 34.