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STRUCTURE ASSIGNMENT BY RETENTION INDEX IN GAS-LIQUID RADIOCHROMATOGRAPHY OF SUBSTITUTED CYCLOHEXENES

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SUMMARY

Substituents and functional groups cause increase in retention indexes of six-membered ring compounds on apolar and polar columns. These retention index increments (δI) are characteristic and structure-dependent showing a structure-retention index relationship. The δI values obtained for methyl and halogen atom substitution, double bond conjugation and multiple substitution in substituted benzenes, cyclohexenes, and cyclohexanes are useful for predictive purpose in tritium labeling studies for tentative structure assignment of radioactive intermediates not amenable to analysis by conventional techniques.

INTRODUCTION

In our study of the mechanism of tritium labeling using "tritium charged" supported metal catalysts, a mixture of labeled products was formed¹. They were analyzed by gas-liquid radiochromatography (GLRC) and were shown to contain many radioactive peaks. With the exception of the radioactive peak corresponding to the original substrate, these products are of such high specific activity and negligible mass that even with preparative chromatography not enough material can be accumulated for identification by gas chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy. Identification by chromatography with authentic samples is limited by the lack of the latter; besides, of many intermediates little is known.

The Kováts retention index² (I), the difference between retention indexes on polar and apolar stationary phases (ΔI), and the increment of retention index due to substituents (δI) are unique and characteristic of chemical structure of individual compounds, and are useful for identification purposes. Cook and Rauschel³ observed the contribution of substituent groups to retention indexes of monosubstituted ben-

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zenes to be additive under isothermal conditions. They were able to obtain correct retention indexes for derivatives by summing the retention index of the benzene ring and that of the substituent, independent of the polarity of the stationary phase. High correlation was also observed for a few di- and tri-substituted compounds. West and Hall⁴ introduced an additional factor to account for the differences in retention indexes of positional isomers of toluene. Retention index was originally employed by Kováts for isothermal operation, but Van den Dool and Kratz⁵ found it equally valid for linear temperature programmed operations.

We have observed that this rule of additivity in the structure-retention index relationship (SRIR) for benzene and toluene derivatives may also apply to substituted cyclohexanes and cyclohexenes. This paper reports the SRIR observed in the study. With the use of Kováts retention index and SRIR it is possible to assign tentative structures to some radioactive side products formed in tritium labeling from their chromatographic behavior.

MATERIALS AND METHODS

All chemical compounds were obtained commercially* with the exception of 1-chloro-, 1-bromo-, and 1-iodocyclohex-1-ene, 1-bromo-4-methylcyclohex-1-ene, and 1,6-dibromocyclohex-1-ene from Bottini *et al.*⁶ and 4-bromocyclohex-1-ene from Fish and Broline⁷.

All runs were conducted on a Hewlett-Packard 5880A gas chromatograph equipped with a thermal conductivity detector and Level IV integration. Stainless-steel columns, 3.05 m × 3.175 mm I.D., containing 20% SE-30 on acid washed Chromosorb 100-200 mesh or 20% Carbowax 20M on Supelcoport 100-200 mesh, were used. Carrier gas was helium. The linear temperature program for Carbowax 20 M began at 40°C isothermally for 10 min after which temperature was increased to 210°C at a rate of 10°C per min and was maintained at 210°C for 30 min. The temperature program of SE-30 began at 40°C isothermally for 4 min with the final temperature maintained for 20 min (*vide infra*). The injection port was kept at 250°C. A standard mixture of *n*-paraffins (C₆-C₂₄) was injected simultaneously each time a sample of the analyte was injected. The equation reported by Van den Dool and Kratz⁵ was used to compute the retention index *I*. The equation is

$$I = 100 i \frac{X - M_{(n)}}{M_{(n+i)} - M_{(n)}} + 100 n \quad (1)$$

where *n* is the number of carbon atoms in *n*-paraffin marker; *X*, *M*_(*n*), and *M*_(*n*+*i*) are, respectively, the adjusted retention times (corrected for air peak) of the analyte, the *n*-paraffin marker with *n* carbon atoms eluting before, and that with (*n* + *i*) carbon atoms eluting after it. ΔI is the difference between retention indexes of the analyte on polar and apolar columns, *i.e.*, Carbowax 20M and SE-30; thus,

$$\Delta I = I^{\text{Carb.}} - I^{\text{SE-30}} \quad (2)$$

* From the following companies: Aldrich, Milwaukee, WI, U.S.A.; Chem. Service, Media, PA, U.S.A.; Eastman-Kodak; Rochester, NY, U.S.A.; Fluka, Buchs, Switzerland; Matheson, Coleman & Bell; Norwood, OH, U.S.A.; Pfaltz & Bauer; Stamford, CT, U.S.A.

The retention index increment δI represents the difference in retention index of the compound with the substituent ($I_{\text{subst.}}$) and the derivative without it (I_0) on the same stationary phase, *i.e.*,

$$\delta I = I_{\text{subst.}}^{\text{stat.ph.}} - I_0^{\text{stat.ph.}} \quad (3)$$

RESULTS AND DISCUSSION

Retention indexes obtained by Van den Dool and Kratz's method⁵ of computation may vary slightly when one of the two *n*-paraffin marker peaks appears in the isothermal region and the other in the temperature programmed region. Varying the initial isothermal region caused no fluctuation of the retention index on Carbowax 20M column. On SE-30 column, there was a trend for the retention index to decrease slightly as the duration of the isothermal region was increased. Shortening the isothermal region to 4-min duration to shift all the marker peaks to the temperature programmed region eliminates the variations observed. On Carbowax column a 10-min initial isothermal region was selected to ensure even spacing of the early emerging peaks and the constancy of the retention index on repeated runs. It should be pointed out that for the retention indexes to be reproducible the column helium flow-rate must be rigidly controlled.

The effect of substitution by methyl group, halogen atom, double bond, and conjugation on retention indexes of six-membered ring derivatives is given in the following.

TABLE I
CONTRIBUTION OF METHYL GROUP TO RETENTION INDEX

Compound	Stationary phase				
	SE-30		Carbowax 20M		
	<i>I</i>	δI	<i>I</i>	δI	ΔI
Cyclohexane	662	—	723	—	61
Methylcyclohexane	727	+65	759	+36	32
1,3-Dimethylcyclohexane	782	+60	814	+45	32
Cyclohexene	667	—	808	—	134
1-Methylcyclohex-1-ene	773	+106	909	+101	136
3-Methylcyclohex-1-ene	745	+78	847	+39	102
4-Methylcyclohex-1-ene	744	+77	849	+41	105
Benzene	654	—	965	—	311
Toluene	764	+110	1061	+96	297
Cyclohexylamine	862	—	1182	—	320
3-Methylcyclohexylamine	926	+64	1231	+49	305
1-Bromocyclohex-1-ene	975	—	1324	—	349
1-Bromo-4-methylcyclohex-1-ene	1036	+61	1361	+37	325
Cyclohexanone	881	—	1333	—	452
3-Methylcyclohexanone	945	+64	1376	+43	431
Cyclohexanol	885	—	1414	—	529
3-Methylcyclohexanol	947	+62	1463	+49	516

Methyl substitution

Table I lists the contribution of methyl groups to retention indexes (δI_M) of some cyclohexene derivatives. These derivatives contain bromo-, methyl-, -amine, -ene, -ol or -one functional groups in addition to methyl group in the molecule. The retention index increment δI for methyl group substitution in the benzene ring is in the range of 100–110 units; this high δI value is attributed to the proximity of the methyl group to double bond or to conjugated double bonds. According to Schomberg⁸, the methyl group linked to a double bond has a higher δI value than the methyl group not so linked. The presence of other functional groups as shown above exert little or no effect on the δI values from methyl group substitution.

When the methyl group is in alicyclic ring, the value of the retention index increment is in the range of 40–80 units; the lower value are from Carbowax column and the higher values from the SE-30 column. As methyl group is apolar, the δI has a slightly higher value on SE-30 column than on Carbowax 20 M column.

Table II shows the δI values of methyl substituents in aromatic and alicyclics on other stationary phases, from the literature. Methyl group increases the retention indexes by 99–107 units in the benzene ring and by 40–65 units in the alicyclic rings, with smaller values more prevalent in more polar stationary phases. Again, proximity to double bond increase the δI of methyl substitution, to a higher degree in aromatic than in alicyclic rings. The cyclohexene and cyclopentane derivatives are akin to benzene in molecular size, rigidity, and planarity and have intermediate δI values.

TABLE II

RETENTION INDEX INCREMENTS ON DIFFERENT STATIONARY PHASES DUE TO METHYL GROUP

Squalane, temp.: 80°C and 120°C (ref. 7). Apiezon L, on Chromosorb W; column: 1/4 in. O.D.; temp. extrapolated to 130°C (ref. 8). SE-30–20% on Chromosorb W; O.D. column: 6 ft. × 1/4 in. O.D.; temp.: 100°C, 130°C and 160°C (ref. 2). Emulphor O, temp.: 80°C and 120°C (ref. 7). Carbowax 6000 + Hyprose SP 80 (40:60), on Chromosorb W; column: 1/4 in. O.D.; temp. extrapolated to 130°C (ref. 8).

Compound	Squalane (apolar)	Apiezon L (apolar)	SE-30 (apolar)	Emulphor O (polar)	Carbowax 6000 + Hyprose SP 80 (polar)
Toluene	+ 107		+ 104	+ 99	
Methylcyclohexane	+ 65	+ 68		+ 51	+ 38
Methylcycloheptane	+ 65			+ 51	
Methylcyclooctane	+ 59			+ 50	
Methylcyclodecane	+ 55			+ 45	
3-Methylcyclohexanol*		+ 86			+ 37
3-Methylcyclohexanone		+ 73			+ 27
4-Methylcyclohexanone		+ 80			+ 36
4-Methylcyclohexanol*		+ 86			+ 38
1-Methylcyclopent-1-ene	+ 95			+ 89	
1-Methylcyclohex-1-ene	+ 88			+ 84	
1-Methylcyclohept-1-ene	+ 78			+ 69	
1-Methylcyclooct-1-ene	+ 74			+ 66	
1-Methylcyclodec-1-ene*	+ 74			+ 71	
1-Methylcyclododec-1-ene*	+ 76			+ 76	

* Average of *cis* and *trans* values.

TABLE III
CONTRIBUTION OF A HALOGEN ATOM TO RETENTION INDEX

Compound	Stationary phase					
	SE-30		Carbowax 20M			
	<i>I</i>	δI	<i>I</i>	δI	ΔI	$\delta \Delta I^*$
Benzene	654	—	965	—	311	—
Fluorobenzene	664	+10	992	+27	328	17
Chlorobenzene	839	+185	1241	+276	402	91
Bromobenzene	930	+276	1391	+426	461	150
Iodobenzene	1033	+379	1569	+604	536	225
Cyclohexene	667	—	808	—	141	—
1-Chlorocyclohex-1-ene	894	+227	1177	+369	283	142
1-Bromocyclohex-1-ene	975	+308	1324	+516	349	208
1-Iodocyclohex-1-ene	1079	+412	1484	+676	405	264
Cyclohexane	662	—	723	—	61	—
Fluorocyclohexane	738	+16	958	+235	220	159
Chlorocyclohexane	888	+226	1163	+440	275	214
Bromocyclohexane	969	+307	1303	+580	334	273
Iodocyclohexane	1070	+408	1449	+726	379	318

* $\delta \Delta I$ represents the change in polarity (ΔI) of the stationary phases from the halogenated derivatives to the base compounds benzene, cyclohexene and cyclohexane.

The increase in δI is, in general, dependent on ring aromaticity, molecular polarizability and immediate proximity to double bond. The presence of -ol and -one functional groups in the molecule has no effect. The values in Tables I and II are in good agreement.

Halogen atom substitution

The δI for halogen atom contribution (δI_x) in substituted benzenes, cyclohexenes, and cyclohexanes are given in Table III. The δI_x values are considerably higher in comparison with values from contribution of the methyl group and other substituents (*vide infra*). The high values may be attributed to the mass and polarizability of the halogen atoms which decrease in the order of $I > Br > Cl > F$. The highest δI and the largest increase in polarity (ΔI) for halogen atom substitution are found in the cyclohexane ring, the next highest in a cyclohexene ring and the least in a benzene ring. The polarity (ΔI) of the substituted derivatives increases with increase in conjugation within a ring system but the substituted benzenes have higher numerical values of ΔI than the corresponding members of the other ring systems.

Table IV shows the δI and ΔI values of some disubstituted derivatives of benzene. The δI in these derivatives is dependent upon the proximity of the two substituents, their mutual interaction, and their molecular polarizability towards the stationary phase. With identical or similar substituents the δI values for disubstitution may be approximated by values observed for the monosubstitution of the same substituent. With two dissimilar substituent groups where the probability of hydrogen bonding exists, the *ortho* effect becomes predominant. *Ortho*-isomers, such as

TABLE IV

CONTRIBUTION OF HALOGEN ATOMS TO THE RETENTION INDEX OF DISUBSTITUTED AROMATIC COMPOUNDS

Compound	Stationary phase				
	SE-30		Carbowax 20M		
	<i>I</i>	$\delta I/\text{atom}$	<i>I</i>	$\delta I/\text{atom}$	ΔI
Toluene	764	—	1061	—	297
<i>o</i> -Bromotoluene	1037	+273	1483	+422	446
<i>m</i> -Bromotoluene	1033	+269	1498	+437	465
<i>p</i> -Bromotoluene	1042	+278	1493	+432	451
Phenol	962	—	1967	—	1005
<i>o</i> -Bromophenol	1065	+103	1943	-24	878
<i>m</i> -Bromophenol	1262	+300	*		
<i>p</i> -Bromophenol	1256	+294	*		
Benzene	654	—	965	—	311
<i>o</i> -Dibromobenzene	1216	+281	1830	+433	614
<i>m</i> -Dibromobenzene	1190	+268	1756	+396	566
<i>o</i> -Iodotoluene	1150	+386	1668	+607	518
<i>m</i> -Iodotoluene	1148	+384	1674	+613	526
<i>o</i> -Iodophenol	1182	+220	*		
<i>m</i> -Iodophenol	1369	+407	*		
<i>p</i> -Iodophenol	1343	+381	*		
<i>o</i> -Diiodobenzene	1445	+395	2244	+640	799
<i>m</i> -Diiodobenzene	1431	+389	2161	+593	730
<i>p</i> -Diiodobenzene	1412	+379	2176	+606	764

* The retention times are too high for our reference compounds.

o-bromophenol and *o*-iodophenol, exhibit considerably lower retention indexes and δI values in comparison with *m*- or *p*-isomers.

Double bond conjugation

Table V shows the retention indexes and increment values when double bonds are present in the ring. Double bonds affect the electron density, configuration and polarizability of the molecule. The δI value on Carbowax column increases with the number of double bonds and ring conjugation (or aromaticity). The retention index values for the alicyclics increase more orderly with increase in ring size and conjugation on Carbowax column than on SE-30 column. The increase in δI and ΔI is the greatest in a six-membered ring and less so in a seven or eight-membered ring. On a SE-30 column, the retention index decreases with the introduction of the last double bond to achieve ring aromaticity, as in the transition of 1,4-cyclohexadiene to benzene, 1,3-cycloheptadiene to 1,3,5-cycloheptatriene or 1,5-cyclooctadiene to 1,3,5,7-cyclooctatetraene. The negative values observed for the δI of these compounds on the SE-30 column reflects their smaller values in retention index, as compared with those of the base compound cyclohexane, cycloheptane or cyclooctane. (Dreiding atom models show that 1,3,5-cycloheptatriene and 1,3,5,7-cyclooctatetraene are rigid but puckered molecules with alternate co-planar double bonds.)

Table VI lists the retention indexes *I* (δI , and ΔI) of the derivatives containing

TABLE V
CONTRIBUTION OF DOUBLE BONDS TO RETENTION INDEX

Compound	Stationary phase				
	SE-30		Carbowax 20M		
	<i>I</i>	δI	<i>I</i>	δI	ΔI
Cyclohexane	662	—	723	—	61
Cyclohexene	667	+5	808	+85	141
1,3-Cyclohexadiene	665	+3	861	+138	196
1,4-Cyclohexadiene	713	+51	939	+216	226
Benzene	652	-10	965	+242	313
Cycloheptane	794	—	892	—	98
Cycloheptene	783	-11	952	+60	169
1,3-Cycloheptadiene	811	+17	1060	+168	249
1,3,5-Cycloheptatriene	785	-9	1089	+197	304
Cyclooctane	932	—	1043	—	111
Cyclooctene	910	-22	1069	+26	159
1,5-Cyclooctadiene	949	+3	1188	+145	234
1,3,5,7-Cyclooctatetraene	880	-52	1226	+183	346

substituent groups that may or may not conjugate. When the substituent is not conjugated, an increase in the increment value is due, within the experimental error, to the contribution of the double bond.

Conjugation of a substituent with double bond results in an increase in the δI ; it depends on the combination of the electronic strength and the spatial configuration of the functional groups. Molecules with double bond in conjugation with electron-donating or electron-withdrawing substituents such as $-\text{OCH}_3$ or $-\text{NO}_2$ groups (e.g., Table VI, compounds 4, 6 and 18) have high values of δI on Carbowax 20M as compared to SE-30 columns. Conversion of a single bond to a double bond in halogenated cyclohexenes leads to an increase of δI , equal to about 1–2% of the retention index of the substituted derivatives (e.g., compounds 7–12). Comparison of the δI of halogenated derivatives with those similar derivatives not containing halogen atoms (compounds 2, 8, 10 and 12) shows that the introduction of a halogen atom substituent raises the δI to a similar magnitude as those given in Table IV.

The methyl group substituent, even though apolar in nature, can undergo hyperconjugation in series with a double bond to increase the δI values as in compounds 15, 16 and 22. If the methyl group is directly linked to a double bond, the δI value is even higher as in compound 14. The highest increase of δI is observed in compound 20, where the presence of a double bond brings forth also an extended system of conjugation. All these increases in δI values are distinctly recognizable on both SE-30 and Carbowax 20M columns.

Predicted retention indexes

The above tables (Tables I, III–VI) are the sources of SRIR relationship from which retention indexes of substituted cyclohexene and benzene molecules can be computed. The procedure involves the selection of a base compound and adding to it substituents to yield the desired compound; when translated into terms of retention

TABLE VI

CONTRIBUTION OF SUBSTITUENTS, CONJUGATED AND NON-CONJUGATED TO A DOUBLE BOND, TO RETENTION INDEX

Compound	Stationary phase				
	SE-30		Carbowax 20M		
	<i>I</i>	δI	<i>I</i>	δI	ΔI
1 Cyclohexane	662	—	723	—	61
2 Cyclohexene	667	+ 5	808	+ 85	141
3 1,3-Cyclohexadiene	665	+ 3	861	+ 138	196
4 1-Methoxy-1,3-cyclohexadiene	904	+ 239	1253	+ 392	349
5 1,4-Cyclohexadiene	713	+ 51	939	+ 216	226
6 1-Methoxy-1,4-cyclohexadiene	939	+ 226	1295	+ 356	356
7 Chlorocyclohexane	888	—	1163	—	275
8 1-Chlorocyclohex-1-ene	894	+ 6	1177	+ 14	283
9 Bromocyclohexane	969	—	1303	—	334
10 1-Bromocyclohex-1-ene	975	+ 6	1324	+ 21	349
11 Iodocyclohexane	1070	—	1449	—	379
12 1-Iodocyclohex-1-ene	1079	+ 9	1484	+ 35	405
13 Methylcyclohexane	727	—	759	—	32
14 1-Methylcyclohex-1-ene	773	+ 46	909	+ 150	136
15 3-Methylcyclohex-1-ene	745	+ 18	847	+ 88	102
16 4-Methylcyclohex-1-ene	744	+ 17	849	+ 90	105
17 Nitrocyclohexane	1079	—	1645	—	566
18 1-Nitrocyclohex-1-ene	1174	+ 95	1784	+ 139	610
19 3-Methylcyclohexanone	945	—	1376	—	431
20 3-Methyl-2-cyclohexen-1-one	1039	+ 94	1644	+ 268	605
21 3-Methylcyclohexanol	943	—	1463	—	520
22 3-Methyl-2-cyclohexen-1-ol	*	—	1552	+ 89	—
23 Cyclohexanol	885	—	1414	—	529
24 2-Cyclohexen-1-ol	887	+ 2	1481	+ 67	594
25 Cyclohexanone	881	—	1333	—	452
26 2-Cyclohexen-1-one	920	+ 39	1486	+ 153	566

* Very broad peak.

index of the base compound and the δI of the substituents, the final value constitutes the predicted or computed retention index of the desired compound.

Table VII lists the retention indexes of a few known compounds calculated on the basis of the above assumption using the rule of additivity of δI of the substituents for both SE-30 column and Carbowax 20M column. These compounds were chosen to represent a range of substituents which include double bond, methyl, bromo, iodo or hydroxyl group in mono- or di-substitution. When the predicted or calculated retention indexes of these compounds were compared with their observed values, agreement was good to within 1% in most cases. In only two cases, the error exceeds 2%. This essentially establishes the validity of the method for prediction of retention index based on SRIR relationship.

An example for computation of the retention index for 3-iodotoluene, shown in Table VII, is given as follows: The molecule is considered as composed of benzene with methyl- and iodo-substituents. The Kováts retention index (*I*) of benzene ob-

tained from Table I for SE-30 column is 654; δI for aromatic methyl substitution, obtained from the difference in I between toluene and benzene from Table I is 110; and the contribution from aromatic substituent, *i.e.*, the difference in I values between iodobenzene and benzene is 379 from Table III. All these values add up to a total of 1143 as the computed or predicted I value for 3-iodotoluene. An identical I value may also be obtained by using toluene as the base compound and adding to it the contribution of aromatic iodo substitution. The observed I value for 3-iodotoluene is 1148, indicating that the predicted value is accurate to within 0.48%. Similar calculation may be repeated for Carbowax 20M column. The retention indexes listed in both Table VII and Table VIII are similarly calculated.

In Table VIII we have listed the I values for intermediates of toluene, bromobenzene, iodobenzene, 2-iodotoluene, and 3-iodotoluene that may be formed in reaction with tritium atoms. These intermediates are not readily available or little known; not all of them are chromatographically separable, according to the predicted retention indexes but many of them can be differentiated by their retention indexes on apolar and polar stationary phases by the ΔI factor. The intermediates in Table VIII are arranged according to their increasing ΔI values. The basic fact of being able to predict their retention indexes from the SRIR relationship and also of knowing the limitation in their separation by GLRC, is essential to the study of mechanism of tritium labeling. We have shown here that the retention indexes of these intermediates can be predicted. Frequently, it is not the absolute value in I that one wishes to know but a rational basis for accepting or excluding alternatives in the interpretation of data.

Application

Fig. 1 shows the radiochromatograms of a mixture of ^3H -labeled products obtained from *m*-iodotoluene and tritium, run on SE-30 and Carbowax 20M columns. The radioactive peaks emerge at different times in accordance with the polarity of the stationary phase and the analyte. Peaks on two radiochromatograms from the same compound are designated by the same letter. Experimental details will be reported elsewhere¹⁰.

Table IX lists the observed retention index values of the radioactive peaks and the retention index values of reference standards and assigned compounds. Correspondence of the retention index values and the observed values are within 2%. Compounds A, C, D and E are identified, respectively, as cyclohexane, cyclohexene, methylcyclohexane, and toluene. Compound B is identified as benzene with its peak overlapping with cyclohexane on the SE-30 column. Compound F is predicted to represent methylcyclohexadiene or a mixture of its isomers. Compound G failed to appear on the Carbowax 20M column and as a result, no structure can be assigned to it. Compounds H and I, represented by a broad peak on SE-30 column, are assigned 1-iodo-3-methylcyclohexane and *m*-iodotoluene, respectively. The closeness of their retention index values on SE-30 column makes their chromatographic peaks overlap but they are well separated on Carbowax 20M column, as shown in both Fig. 1 and Table IX. This example illustrates that the retention indexes of the substituted cyclohexanes are predictable, and that these predicted values can be verified by the presence of radioactive peaks in radiochromatograms on apolar and polar columns. This technique of assigning structures to radioactive intermediates is useful

TABLE VII
COMPARISON BETWEEN CALCULATED AND OBSERVED RETENTION INDEXES

Compound	Stationary phase	Additive retention index increments	Retention indexes		
			Calculated	Observed	Difference (%)
3-Iodotoluene	SE-30	Benzene (T1*:654) + arom. methyl (T1:110) + arom. iodide (T3:379)	1143	1148	0.44
3-Bromotoluene	Carbowax 20M	(T1:965)	1665	1674	0.54
	SE-30	Benzene (T1:654) + arom. methyl (T1:110) + arom. bromide (T3:276)	1040	1033	0.68
1-Bromo-4-methylcyclohex-1-ene	Carbowax 20M	(T1:965)	1487	1498	0.73
	SE-30	1-Bromocyclohex-1-ene (T1:975) + aliph. methyl (T1:65) (T1:1324)	1040	1036	0.39
2-Cyclohexen-1-ol	Carbowax 20M	(T1:965)	1360	1361	0.07
	SE-30	Cyclohexanol (T1:885) + incr. cyclohexane to cyclohexene (T1:5)	890	880	1.14
3-Methylcyclohexanone	Carbowax 20M	(T1:1414)	1499	1481	1.22
	SE-30	Cyclohexanone (T1:881) + aliph. methyl (T1:65) (T1:1333)	946	945	0.11
3-Methylcyclohexylamine	Carbowax 20M	(T1:1333)	1369	1376	0.51
	SE-30	Cyclohexylamine (T1:862) + aliph. methyl (T1:65) (T1:1182)	927	926	0.11
	Carbowax 20M	(T1:1182)	1218	1231	1.06

1,3-Dimethylcyclohexane	SE-30	Cyclohexane (T1:662) + 2 × aliph. methyl (T1:130)	792	782	1.28
	Carbowax 20M	(T1:723)	795	814	2.33
1,6-Dibromocyclohex-1-ene	SE-30	1-Bromocyclohex-1-ene (T1:975) + aliph. bromide (T3:307)	1282	1288	0.47
	Carbowax 20M	(T1:1324)	1904	**	
3-Methyl-2-cyclohexen-1-ol	SE-30	2-Cyclohexen-1-ol (T6:887) + incr. cyclohexene to 1-methylcyclohex-1-ene (T1:106)	993	***	
	Carbowax 20M	(T6:1481)	1582	1552	1.93
3-Methylcyclohexanol	SE-30	Cyclohexanol (T1:885) + aliph. methyl (T1:65)	950	947	0.32
	Carbowax 20M	(T1:1414)	1450	1463	0.89
1,3-Cyclohexanediol	SE-30	Cyclohexanol (T1:885) + incr. cyclohexane to cyclohexanol (T1:223)	1108	1089	1.74
	Carbowax 20M	(T1:1414)	2105	2070	1.69
4-Bromocyclohex-1-ene	SE-30	Cyclohexene (T1:667) + aliph. bromide (T3:307)	974	969	0.52
	Carbowax 20M	(T1:808)	1388	1370	1.31
3-Methyl-2-cyclohexen-1-one	SE-30	2-Cyclohexen-1-one (T6:920) + incr. cyclohexene to 1-methylcyclohex-1-ene (T1:106)	1026	1039	1.25
	Carbowax 20M	(T6:1486)	1587	1644	3.59

* Data from Table I, III or VI are identified as T1, T3 or T6, respectively, with numerical values appended.

** Decomposed on the column.

*** A very broad peak.

TABLE VIII
COMPUTED RETENTION INDEXES FOR PARTIALLY SATURATED DERIVATIVES

	<i>Stationary phase</i>		<i>ΔI</i>
	<i>SE-30</i>	<i>Carbowax 20M</i>	
<i>Toluene</i>			
Methylcyclohexane*	727	759	32
6-Methyl-1,3-cyclohexadiene	730	897	167
6-Methyl-1,4-cyclohexadiene	778	975	197
1-Methyl-1,4-cyclohexadiene	791	999	208
1-Methyl-1,3-cyclohexadiene	791	997	206
3-Methyl-1,3-cyclohexadiene	791	997	206
Toluene*	764	1061	297
<i>Bromobenzene</i>			
Bromocyclohexane*	969	1303	334
3-Bromocyclohex-1-ene	974	1388	414
4-Bromocyclohex-1-ene	974	1388	414
1-Bromo-1,3-cyclohexadiene	980	1409	429
2-Bromo-1,3-cyclohexadiene	980	1409	429
1-Bromo-1,4-cyclohexadiene	1021	1455	434
Bromobenzene*	930	1391	461
6-Bromo-1,3-cyclohexadiene	972	1441	469
6-Bromo-1,4-cyclohexadiene	1020	1519	499
<i>Iodobenzene</i>			
Iodocyclohexane*	1070	1449	379
3-Iodocyclohex-1-ene	1075	1534	459
4-Iodocyclohex-1-ene	1075	1534	459
1-Iodo-1,3-cyclohexadiene	1077	1537	460
2-Iodo-1,3-cyclohexadiene	1077	1537	460
1-Iodo-1,4-cyclohexadiene	1125	1615	490
6-Iodo-1,3-cyclohexadiene	1073	1587	514
Iodobenzene*	1033	1565	532
6-Iodo-1,4-cyclohexadiene	1121	1665	544
<i>2-Iodotoluene</i>			
1-Iodo-2-methylcyclohexane	1135	1485	350
1-Iodo-6-methylcyclohex-1-ene	1157	1523	366
1-Iodo-2-methylcyclohex-1-ene	1185	1585	400
4-Iodo-3-methylcyclohex-1-ene	1153	1573	420
3-Iodo-4-methylcyclohex-1-ene	1152	1575	423
4-Iodo-5-methylcyclohex-1-ene	1152	1575	423
1-Iodo-6-methyl-1,4-cyclohexadiene	1203	1654	451
6-Iodo-1-methylcyclohex-1-ene	1181	1635	454
2-Iodo-1-methyl-1,3-cyclohexadiene	1183	1638	455
1-Iodo-2-methyl-1,3-cyclohexadiene	1183	1638	455
5-Iodo-6-methyl-1,3-cyclohexadiene	1151	1626	475
2-Iodo-1-methyl-1,4-cyclohexadiene	1231	1716	485
6-Iodo-1-methyl-1,3-cyclohexadiene	1179	1688	509
2-Iodotoluene*	1150	1668	518
6-Iodo-1-methyl-1,4-cyclohexadiene	1227	1766	539

TABLE VIII (continued)

	Stationary phase		ΔI
	SE-30	Carbowax 20M	
<i>3-Iodotoluene</i>			
1-Iodo-3-methylcyclohexane	1135	1485	350
1-Iodo-3-methylcyclohex-1-ene	1144	1520	376
1-Iodo-5-methylcyclohex-1-ene	1144	1520	376
4-Iodo-6-methylcyclohex-1-ene	1153	1573	420
1-Iodo-5-methyl-1,3-cyclohexadiene	1155	1576	421
2-Iodo-6-methyl-1,3-cyclohexadiene	1154	1578	424
1-Iodo-3-methyl-1,4-cyclohexadiene	1203	1654	451
3-Iodo-1-methylcyclohex-1-ene	1181	1635	454
5-Iodo-1-methylcyclohex-1-ene	1181	1635	454
1-Iodo-3-methyl-1,3-cyclohexadiene	1183	1638	455
2-Iodo-4-methyl-1,3-cyclohexadiene	1183	1638	455
1-Iodo-5-methyl-1,4-cyclohexadiene	1231	1716	485
3-Iodotoluene*	1148	1674	526
3-Iodo-1-methyl-1,4-cyclohexadiene	1227	1766	539

* Known compound included for comparative purpose.

when the mass of the analyte required for structural identification by conventional analytical techniques is either unavailable or cannot be accumulated or collected.

CONCLUSION

Contribution of substituent groups to retention indexes of compounds is characteristic, its effect is additive, and it can lead to prediction of retention indexes of derivatives not readily available or little known. The use of retention index increment to predict retention index has been applied to substituted benzenes, cyclic hydrocarbons and phenols^{3-5,8,9}. In published literature⁴, the retention index predictions are directed more to stationary phases of optimum polarity than to the same stationary phase employed for analyzing the reference compounds. The former approach favors inter-column comparison but requires polarity characteristics and related parameters of the stationary phases which are obtained by approximation or by averaging; whereas the latter approach elucidates the SRIR and proves to be conceptually simple, convenient and precise, as we have shown here.

By selecting a few basic reference compounds to focus on the influence of the substituent group, it is feasible to compile a list of δI values of substituent groups on apolar and polar columns for general use. The substituted cyclohexene derivatives that we have studied have δI values influenced by ring size, ring aromaticity, molecular polarizability, proximity of substituent to double bond, the *ortho* effect, hyperconjugation and hydrogen bonding. In general, the mass, polarizability and conjugation of the incoming groups and their influence on the ring can cause a large increase in δI . For example, in halogen atom substitution the introduction of a haloatom causes a substantial increase in I irrespective of the kind of base molecule it combines with; it appears that the mass, polarizability, and inductiveness of the sub-

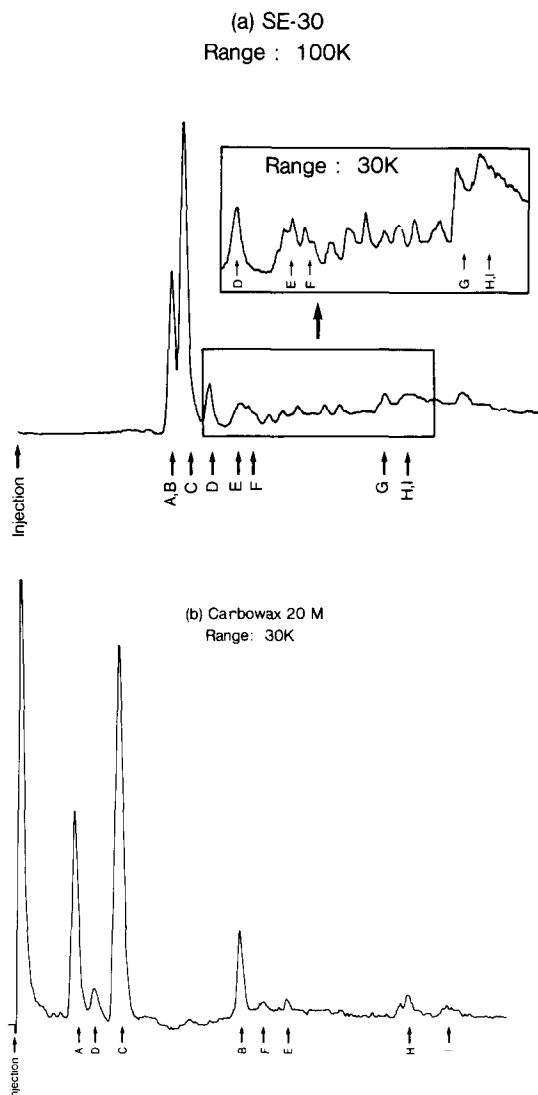



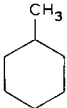
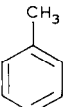
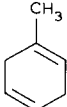
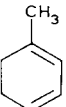
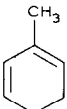
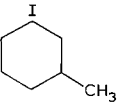
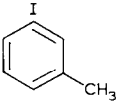


Fig. 1. Radiochromatograms of a mixture of ^3H -labeled products from *m*-iodotoluene and tritium, run on SE-30 (a) and Carbowax-20M (b) columns. Peaks are designated by letters; the same letter on these radiochromatograms indicates the same compound but with different retention index values due to polarity change of the stationary phase. See text.

stituent haloatom predetermines the δI . In monosubstituted derivatives the presence of the substituent may induce an electronic interaction with the ring, whereas in disubstituted derivatives, in addition to the electronic interaction of the two substituents with the ring, they may also interact in a different way with each other, especially in adjacent positions. Thus, a methyl group and adjacent double bond may hyperconjugate, and a halogen and adjacent hydroxyl group may hydrogen bond. The resulting interference of the electronic structure is reflected by the changes in

TABLE IX

COMPARISON OF RETENTION INDEX VALUES TAKEN FROM FIG. 1 WITH THOSE FROM REFERENCE COMPOUNDS AND PREDICTION

Compound designation	Observed I values		I Values from standards		Predicted I values		Assigned structure
	SE-30	Carb*	SE-30	Carb	SE-30	Carb	
A	660**	717	662	723	—	—	
B	660**	960	654	965	—	—	
C	675	804	667	808	—	—	
D	720	757	727	759	—	—	
E	770	1048	764	1061	—	—	
F	784	988	—	—	791	999	
			—	—	791	997	
			—	—	791	997	
G	1098	—	—	—	—	—	
H	1154***	1490	—	—	1135	1485	
I	1154***	1673	1148	1674	—	—	

* Carb = Carbowax 20M.

** Cyclohexane and benzene are not separable on SE-30 column.

*** A broad peak indicating that 1-iodo-3-methylcyclohexane and *m*-iodotoluene peaks overlap with each other.

δI . In the former, a methyl group adjacent to a double bond has invariably higher I values than one which is not. In the latter, the hydrogen bonding leads to smaller retention indexes in *o*-substituted derivatives as compared to *m*- and *p*-disubstituted derivatives or monosubstituted derivatives in the absence of *ortho* influence.

This method of using δI and ΔI to predict retention indexes for derivatives and to assign them chemical structures from these parameters is very simple in concept and will be very useful in the study of reaction mechanisms in tritium labeling. Our

results show that the δI values of the substituent groups are unique and relatively unaffected by the presence of other groups already in the molecule and that a high degree of accuracy and sophistication can be achieved by this approach.

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