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RETENTION PREDICTION OF ANALYTES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY BASED ON MOLECULAR STRUCTURE

III. MONOSUBSTITUTED ALIPHATIC COMPOUNDS

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SUMMARY

A system has been developed to predict retentions in reversed-phase high-performance liquid chromatography based on the molecular structure of the analyte. The contributions of aliphatic functional groups to the retention index of an analyte have been determined based on a series of substituted alkylbenzenes. These contributions are expressed as quadratic equations which relate the contribution to the proportion and type of organic modifier. The influence of phenyl groups in the proximity of the substituents has been examined.

INTRODUCTION

Attempts have been made in a number of laboratories to devise methods to aid the development of high-performance liquid chromatographic (HPLC) separations. These attempts have been primarily based on either the prediction of isocratic conditions from a gradient elution[1] or optimisation methods, which aim to satisfy a set of empirical separation and resolution criteria[2]. Both techniques combine experimental observation with calculations and require no knowledge of the structure of the analyte. However, in many cases the structure or partial structure of the analyte is known or the structural differences between related compounds, such as isomers or metabolites are known.

The aim of the present project has been to develop a retention prediction method for reversed-phase HPLC based on the molecular structure of the analyte[3,4]. A series of studies has therefore been carried out to determine the contributions of different substituents, both aliphatic and aromatic, to the retention of an analyte. The intention is to calculate the retention index (I) as the summation of a series of terms:

$$I = I_P + I_{S,R} + \Sigma I_{S,Al-X} + \Sigma I_{S,Ar-X} + \Sigma I_{I,Y-Z}$$

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These represent the retention index of a parent compound (I_P), a contribution for saturated alkyl chains ($I_{S,R}$), contributions for substituents on saturated aliphatic carbons ($I_{S,Al-X}$), contributions for aromatic substituents ($I_{S,Ar-X}$), and terms to account for any interactions between substituents ($I_{I,Y-Z}$) caused by electronic, hydrogen bonding and steric effects. Each of these terms will be sensitive to eluent composition and the organic modifier in the eluent and will be related to the percentage of modifier using a quadratic equation. Benzene was selected as the parent compound as its substituted derivatives can be readily detected. Retention indices based on the alkyl aryl ketones were used as the basis of the study as they are more robust than capacity factors and can be more readily transferred between systems[5,6]. The coefficients of the regression equations are held in a database which can be interrogated by an expert system program (CRIPES, Chromatographic Retention Index Prediction Expert System) for the calculation of predicted retention indices[7].

So far the coefficients of the equations for benzene and the substituent index coefficients for individual groups attached to an aromatic ring have been described in a range of methanol–pH 7 buffer and acetonitrile–pH 7 buffer eluents[3] and the methods used to ensure the reproducibility of the retention values throughout the study have been reported[8].

The present paper describes the determination of the contributions to the retention of substituents on an aliphatic side chain. By examining side chains of different lengths, it has been possible to examine the effects of interactions of the substituents with a phenyl ring. The magnitude of the contributions have been compared with the contribution of these groups to octanol–water partition constants. Despite considerable previous interest in the contribution of aromatic substituents to retention few studies have examined aliphatic substitution[9].

EXPERIMENTAL

Chemicals, equipment and procedures were as described previously[3].

Calculation of aliphatic substituent indices

The retention index increments for each aliphatic substituent were calculated as the difference between the retention index of the substituted model compound and the calculated retention index in the same eluent for the corresponding alkylbenzene based on the parent index value of benzene (I_P) and contributions for the alkyl side chain. The increments were fitted to a quadratic expression to relate the substituent indices to the proportion of organic modifier.

DISCUSSION

Rather than base a study of the effect on retention of substituents on a saturated carbon by examining substituted aliphatic compounds, which would be difficult to detect spectroscopically, three series of substituted alkylbenzenes have been examined. Because it is possible that the phenyl group could interact with the substituent, alkyl chains of different length were compared. Any effects should decrease with the separation between the groups and would not be expected to be significant for terminal substituents on a propyl side chain.

Retention behaviour of alkylbenzenes

The first step was to examine the retention of the alkylbenzene parent compounds. It was necessary to establish whether the contribution to the retention for a aliphatic methylene group (CH_2 = 100 units), which is defined in the alkyl aryl ketone retention index scale, was also valid to predict the retention of the homologous alkylbenzenes, particularly as it has been suggested that the methylene group increments for different homologous series may differ[10,11].

The capacity factors (Tables I and II) and retention indices (Tables III and IV) of toluene, ethylbenzene, *n*-propylbenzene and *n*-butylbenzene were measured across the eluent ranges methanol–pH 7 buffer (40:60) to (80:20) and acetonitrile–pH 7 buffer (30:70) to (80:20). Using the calculated parent index for benzene[3] as the baseline, the increments for the successive additions of a methylene group to the alkyl side chain can be calculated as $\delta I_{CH_2} = I_{Ph(CH_2)n+1H} - I_{Ph(CH_2)nH}$ (where Ph = phenyl) (Table V). With the exception of the effect of adding the second methylene group

TABLE I

CAPACITY FACTORS FOR COMPOUNDS WITH AN ALIPHATIC SUBSTITUENT ON THE ALKYL CHAIN IN ELUENTS CONTAINING METHANOL

Compound	*Capacity factor (k')*				
	Methanol (%)				
	40	*50*	*60*	*70*	*80*
Toluene	29.51	14.02	7.37	3.37	1.74
Benzyl alcohol	2.31	1.31	0.84	0.56	0.41
Benzyl bromide	31.54	13.40	6.52	2.85	1.56
Benzyl chloride	24.56	10.90	5.51	2.38	1.24
Benzyl cyanide	4.79	2.28	1.31	0.73	0.48
Methyl phenylacetate	10.39	4.68	2.53	1.25	0.74
Phenylacetaldehyde	5.35	2.01	1.13	0.70	0.56
Phenylacetamide	1.36	0.79	0.52	0.38	0.30
1-Phenyl-2-butanone	13.16	5.23	2.67	1.41	0.74
Ethylbenzene	65.28	27.38	13.73	5.02	2.35
Methyl phenylethyl ether	18.39	7.63	3.88	2.00	1.07
Methyl 3-phenylpropionate	24.04	9.58	4.64	1.99	1.06
4-Phenyl-2-butanone	12.60	5.24	2.42	1.35	0.75
2-Phenylethanol	4.11	2.42	1.28	0.74	0.50
2-Phenylethyl bromide	62.88	25.08	11.31	4.30	1.73
2-Phenylethyl chloride	45.99	18.81	8.30	3.49	1.66
3-Phenyl-1-propionamide	2.65	1.38	0.83	0.51	0.36
3-Phenyl-1-propionitrile	6.38	2.99	1.58	0.83	0.52
n-Propylbenzene	165.50	59.57	24.60	8.19	3.38
Methyl 4-phenylbutyrate	51.00	17.98	7.78	2.88	1.40
4-Phenyl-1-butyronitrile	15.22	6.13	2.94	1.32	0.72
3-Phenyl-1-propanol	8.55	3.84	2.06	1.03	0.62
3-Phenyl-1-propyl bromide	163.37	56.07	22.12	7.21	2.92
3-Phenyl-1-propyl chloride	121.95	42.36	15.69	5.86	2.45
n-Butylbenzene	420.37	130.97	46.25	13.55	4.94

TABLE II

CAPACITY FACTORS OF COMPOUNDS WITH ALIPHATIC SUBSTITUENTS ON AN ALKYL SIDE CHAIN IN ELUENTS CONTAINING ACETONITRILE

Compound	*Capacity factor (k')*					
	Acetonitrile (%)					
	30	*40*	*50*	*60*	*70*	*80*
Toluene	28.86	12.74	6.08	3.24	1.97	1.23
Benzyl alcohol	1.75	1.14	0.80	0.57	0.46	0.39
Benzyl bromide	34.10	13.07	5.69	2.84	1.66	1.10
Benzyl chloride	27.63	11.04	4.97	2.53	1.50	0.93
Benzyl cyanide	6.87	3.50	1.92	1.13	0.75	0.52
Methyl phenylacetate	10.63	4.99	2.59	1.47	0.95	0.65
Phenylacetaldehyde	5.30	2.56	1.81	1.17	0.77	0.47
Phenylacetamide	0.93	0.61	0.45	0.34	0.31	0.28
1-Phenyl-2-butanone	15.38	5.89	3.24	1.85	1.10	0.62
Ethylbenzene	59.53	22.38	9.46	4.66	2.67	1.58
Methyl phenylethyl ether	16.39	6.38	3.76	2.19	1.37	0.79
Methyl 3-phenylpropionate	20.39	8.26	3.85	2.04	1.26	0.81
4-Phenyl-2-butanone	13.05	5.05	3.16	1.66	1.01	0.58
2-Phenylethanol	2.71	1.56	1.00	0.68	0.53	0.43
2-Phenylethyl bromide	61.35	20.70	8.18	3.83	2.13	1.23
2-Phenylethyl chloride	47.24	16.74	6.85	3.28	1.86	1.09
3-Phenyl-1-propionamide	1.61	0.90	0.60	0.43	0.36	0.32
3-Phenyl-1-propionitrile	9.47	4.40	2.27	1.27	0.82	0.55
n-Propylbenzene	137.23	42.52	15.83	7.17	3.88	2.15
Methyl 4-phenylbutyrate	38.09	13.35	5.63	2.79	1.64	1.01
4-Phenyl-1-butyronitrile	18.40	7.38	3.41	1.78	1.08	0.69
3-Phenyl-1-propanol	4.90	2.39	1.38	0.87	0.64	0.50
3-Phenyl-1-propyl bromide	–	39.98	14.04	6.10	3.23	1.76
3-Phenyl-1-propyl chloride	106.66	31.94	11.60	5.14	2.76	1.53
n-Butylbenzene	308.86	79.97	26.33	11.02	5.63	2.94

(toluene to ethylbenzene) the increments ranged from 91 to 112 units. The variations from the anticipated values of 100 are generally within the expected experimental error range of ± 10 units for this study determined from the uncertainties in measurement and calculations[8], the only higher deviation being at 80% acetonitrile when measurements are the least reliable because of the short retention times. However, the change from toluene to ethylbenzene was consistently smaller and ranged from 87 to 92 (mean 88) units. A corresponding anomaly can also be seen in the octanol–water partition coefficient substituent constants (π) for the alkyl substituents on benzene (H, 0.0; methyl, 0.56; ethyl, 1.02; propyl, 1.55; butyl, 2.13)[12]. The step between methyl and ethyl of 0.46 is smaller than the other changes which range from 0.56 to 0.58.

Thus in the present study it will be valid to use the defined value of 100 units for the addition of a methylene group to an alkyl chain substituted on benzene but it will be necessary to apply an interaction index correction of −12 units ($I_{\mathrm{I,PhCH_2R}}$) if the addition is to a benzylic carbon.

TABLE III

RETENTION INDICES OF ALIPHATIC COMPOUNDS WITH METHANOL ELUENTS

Compound	*Retention index*				
	Methanol (%)				
	40	*50*	*60*	*70*	*80*
Toluene	983	1010	1038	1063	1090
Benzyl alcohol	689	691	698	684	675
Benzyl bromide	991	1004	1019	1030	1059
Benzyl chloride	962	976	992	992	994
Benzyl cyanide	773	766	763	738	722
Methyl phenylacetate	863	862	853	853	846
Phenylacetaldehyde	790	752	745	734	782
Phenylacetamide	627	623	591	598	582
1-Phenyl-2-butanone	884	885	883	877	867
Ethylbenzene	1075	1100	1126	1151	1177
Methyl phenylethyl ether	923	934	945	955	968
Methyl 3-phenylpropionate	960	959	954	953	948
4-Phenyl-2-butanone	888	883	879	871	863
2-Phenylethanol	756	774	759	741	729
2-Phenylethyl bromide	1072	1088	1107	1118	1090
2-Phenylethyl chloride	1033	1049	1066	1074	1077
3-Phenyl-1-propionamide	705	698	667	663	640
3-Phenyl-1-propionitrile	806	802	793	766	742
n-Propylbenzene	1182	1204	1227	1256	1282
Methyl 4-phenylbutyrate	1046	1043	1040	1033	1029
4-Phenylbutyronitrile	907	899	892	866	842
3-Phenyl-1-propanol	840	835	835	813	793
3-Phenyl-1-propyl bromide	1181	1196	1214	1229	1240
3-Phenyl-1-propyl chloride	1147	1159	1175	1184	1190
n-Butylbenzene	1290	1310	1331	1364	1392

Substituent indices of aliphatic substituents

The capacity factors (Tables I and II) of model substituted toluenes, ethylbenzenes and propylbenzenes, measured over the same eluent ranges as the alkylbenzenes, all showed systematic reductions with increased modifier in the eluent. At the pH of the buffer, carboxylic acid groups were ionised and aliphatic amino groups were protonated so these groups were not included in the study. The changes in the retention indices (Tables III and IV, Fig. 1a and b) were not as marked as for the capacity factors but all the compounds showed some dependence on the eluent composition. A number of the substituted toluenes had been examined previously as model substituted aromatic compounds[3] and are reported here for comparison with the longer chain compounds.

The effect of each of the substituents can be determined as a retention index increment (δI, Tables VI and VII). This value is the difference between the retention index and the predicted value of the corresponding unsubstituted alkylbenzene, which is calculated as the sum of the parent index for benzene, the methylene increments and as appropriate the interaction correction for methylene substitution on

TABLE IV

RETENTION INDICES OF ALIPHATIC COMPOUNDS IN ACETONITRILE ELUENTS

Compound	*Retention index*					
	Acetonitrile (%)					
	30	*40*	*50*	*60*	*70*	*80*
Toluene	1005	1021	1033	1042	1050	1056
Benzyl alcohol	654	640	630	624	636	645
Benzyl bromide	1026	1025	1020	1011	1002	986
Benzyl chloride	999	999	993	983	973	956
Benzyl cyanide	825	817	804	789	774	751
Methyl phenylacetate	879	873	864	853	843	829
Phenylacetaldehyde	781	784	781	790	790	782
Phenylacetamide	575	540	516	504	522	535
1-Phenyl-2-butanone	912	910	904	898	888	874
Ethylbenzene	1095	1110	1121	1130	1137	1144
Methyl phenylethyl ether	919	925	932	937	945	957
Methyl 3-phenylpropionate	961	952	942	932	922	907
4-Phenyl-2-butanone	896	889	880	869	864	855
2-Phenylethanol	708	689	675	667	676	682
2-Phenylethyl bromide	1099	1098	1092	1083	1073	1056
2-Phenylethyl chloride	1066	1064	1057	1045	1034	1013
3-Phenyl-1-propionamide	643	602	573	557	568	574
3-Phenyl-1-propionitrile	865	853	837	818	799	771
n-Propylbenzene	1200	1212	1223	1233	1244	1253
Methyl 4-phenylbutyrate	1039	1029	1018	1007	997	984
4-Phenylbutyronitrile	948	935	918	898	879	851
3-Phenyl-1-propanol	782	756	739	728	731	735
3-Phenyl-1-propyl bromide	–	1202	1200	1194	1191	1182
3-Phenyl-1-propyl chloride	1168	1167	1162	1153	1147	1132
n-Butylbenzene	1302	1313	1325	1337	1350	1365

the benzylic carbon ($\delta I_X = I_{Ph(CH_2)n-X} - [I_P + nI_{S,CH_2} + I_{I,PhCH_2R}$ (if $n>1$)]). A number of the substituents, CO_2CH_3, OCH_3, $COCH_2CH_3$ and $COCH_3$, also contained saturated alkyl groups not directly attached to the aromatic ring. The increments for the corresponding functional groups CO_2R, OR and COR have been calculated by subtracting 100 units for each aliphatic methylene group. The size of the increments for the substituents were markedly different from those for the corresponding groups directly attached to aromatic ring[3]. With the exception of the hydroxyl group, the retention index increments calculated from the ethyl- and propylbenzenes showed only small differences. This suggested that there was little interaction in these compounds between the substituent and the aromatic ring.

The coefficients for the quadratic equations (Table VIII) relating the increments to the eluent composition were calculated, for each substituent, based on the model compound with the longest available alkyl chain (indicated by '*a*' in Tables VI and VII). This was usually the substituted *n*-propylbenzene except for the amide and ketone groups which were based on ethylbenzene and the aldehyde group, which was

TABLE V

RETENTION INDEX INCREMENTS FOR THE ADDITION OF A METHYLENE GROUP TO AN ALKYLBENZENE

Compound	*Retention index increment*					
	Organic modifier proportion (%)					
	30	*40*	*50*	*60*	*70*	*80*
Methanol–buffer						
$I_{P.benzene}$[a]		885	913	938	961	982
Benzene–toluene	–	98	97	100	102	108
Toluene–ethylbenzene	–	92	90	88	88	87
Ethylbenzene–propylbenzene	–	107	104	101	105	105
Propylbenzene–butylbenzene	–	108	106	104	105	105
Acetonitrile–buffer						
$I_{P.benzene}$[a]	910	927	940	951	958	963
Benzene–toluene	95	94	93	91	92	95
Toluene–ethylbenzene	90	89	88	88	87	88
Ethylbenzene–propylbenzene	105	102	102	103	107	109
Propylbenzene–butylbenzene	102	101	103	107	106	112

[a] Calculated from ref. 3.

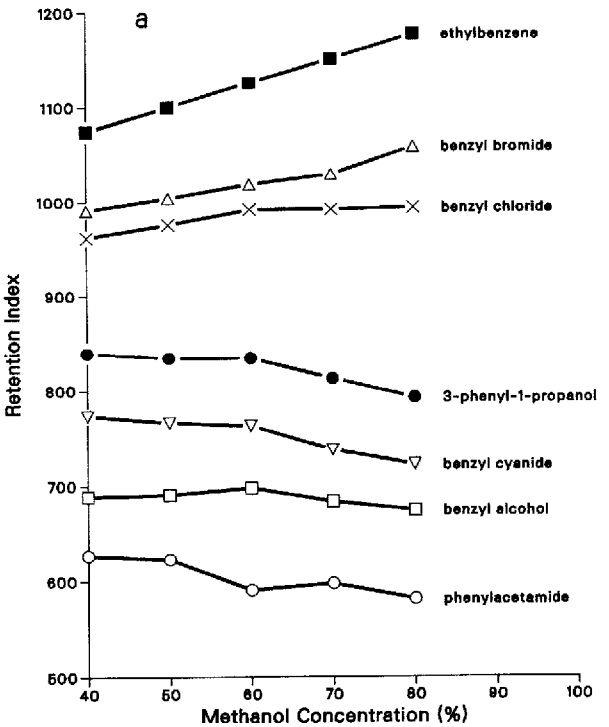

Fig. 1.

(Continued on p. 78)

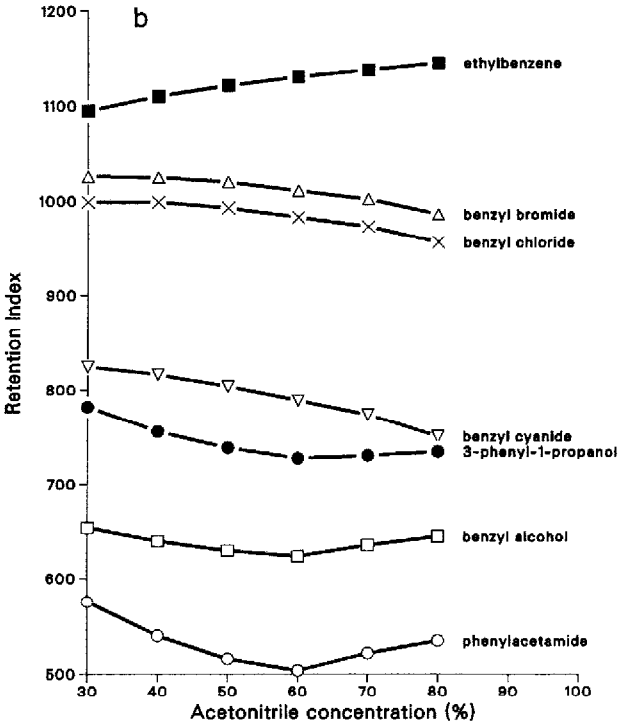

Fig. 1. Retention indices of selected substituted alkylbenzenes with proportion of (a) methanol and (b) acetonitrile.

based on toluene. These coefficients can be used to calculate the substituent indices and in each case there was a good correlation with the experimental values of the retention index increments (Fig. 2a and b) suggesting that within the composition ranges studied, the results would be a good prediction of retention changes.

Interactions of substituents with the phenyl group

The differences between the retention index increments derived from the different alkylbenzenes (Table VI and VII) are assumed to be caused by interactions between the substituents and the phenyl group. These interactions would be expected to depend on the distance of the substituent from the ring and to be strongest with substituents positioned on the benzylic carbon. The interaction increments (δI_I) for the substituted toluenes and ethylbenzenes have been examined by calculating the differences between the calculated substituent index (based on Table VIII) and the measured retention index increment ($\delta I_I = \delta I_X - I_{S,X}$) (Table IX).

The largest interaction increments (+55 to +88 units) were observed for the cyano and hydroxyl groups on the benzylic carbon (1-phenyl, 1–X interactions). These were much reduced for the substituted ethylbenzenes in methanolic eluents (1-phenyl, 2–X interactions) but were still significant for the hydroxyl group with acetonitrile–buffer eluents. Moderate interactions were observed for the amide and ester groups. The substitution of a ketonic carbonyl group on the benzylic carbon

TABLE VI

RETENTION INDEX INCREMENTS FOR ALIPHATIC SUBSTITUENTS IN METHANOL ELUENTS

	Fragmental constant[12]	*Retention index increment*				
		Methanol (%)				
		40	*50*	*60*	*70*	*80*
Substituents on toluene						
Calculated parent I ($I_P + I_{S,CH_2}$)		985	1013	1038	1061	1082
$CONH_2$		−358	−390	−447	−463	−500
OH		−296	−322	−340	−377	−407
CN		−212	−247	−275	−323	−360
CHO[a]	−1.10	−195	−261	−293	−327	−300
CO_2CH_3		−122	−151	−185	−208	−236
(CO_2R[b]		−222	−251	−285	−308	−336)
COC_2H_5		−101	−128	−155	−184	−215
(COR[c]		−301	−328	−355	−384	−415)
Cl		−23	−37	−46	−69	−88
Br		6	−9	−19	−31	−23
Substituents on ethylbenzene						
Calculated parent I ($I_P + 2I_{S,CH_2} + I_{I,PhCH_2R}$)		1073	1101	1126	1149	1170
$CONH_2$[a]	−2.18	−368	−403	−459	−486	−530
OH		−317	−327	−367	−408	−441
CN		−267	−299	−333	−383	−428
$COCH_3$	−1.13	−189	−216	−243	−272	−303
(COR[a,b]		−289	−316	−343	−372	−403)
OCH_3	−1.54	−150	−167	−181	−194	−202
(OR[a,b]		−250	−267	−281	−294	−302)
CO_2CH_3		−113	−142	−172	−196	−222
(CO_2R[b]		−213	−242	−272	−296	−322)
Cl		−40	−52	−60	−75	−93
Br		−1	−13	−19	−31	−80
Substituents on n-propylbenzene						
Calculated parent I ($I_P + 3I_{S,CH_2} + I_{I,PhCH_2R}$)		1173	1201	1226	1249	1270
OH[a]	−1.64	−333	−366	−391	−436	−477
CN[a]	−1.27	−266	−302	−334	−383	−428
CO_2CH_3	−0.72	−127	−158	−186	−216	−241
CO_2R[a,b]		−227	−258	−286	−316	−341)
Cl[a]	0.06	−26	−42	−51	−65	−80
Br[a]	0.20	8	−5	−12	−20	−70

[a] Values used for the calculation of substituent index equations.
[b] 100 subtracted for the methyl group contribution.
[c] 200 subtracted for the ethyl group contribution.

TABLE VII

RETENTION INDEX INCREMENTS FOR ALIPHATIC SUBSTITUENTS IN ACETONITRILE ELUENTS

	Retention index increment					
	Acetonitrile (%)					
	30	*40*	*50*	*60*	*70*	*80*
Substituents on toluene						
Calculated parent I ($I_P + I_{S,CH_2}$)	1001	1027	1040	1051	1058	1063
$CONH_2$	−435	−487	−524	−547	−536	−528
OH	−356	−387	−410	−427	−422	−418
CN	−185	−210	−236	−262	−284	−312
CHO[a]	−229	−243	−259	−261	−268	−281
CO_2CH_3	−131	−154	−176	−198	−215	−234
(CO_2R[b]	−231	−254	−276	−298	−315	−334)
COC_2H_5	−98	−117	−136	−153	−170	−189
(COR[c]	−298	−317	−336	−353	−370	−389)
Cl	−11	−28	−47	−68	−85	−107
Br	16	−2	−20	−40	−56	−77
Substituents on ethylbenzene						
Calculated parent I ($I_P + 2I_{S,CH_2} + I_{I,PhCH_2}$)	1098	1115	1128	1139	1146	1151
$CONH_2$[a]	−455	−513	−555	−582	−578	−577
OH	−390	−426	−453	−472	−470	−469
CN	−233	−262	−291	−321	−347	−380
$COCH_3$	−202	−226	−248	−270	−282	−296
(COR[a,b]	−302	−326	−348	−370	−382	−396)
OCH_3	−179	−190	−196	−202	−201	−194
(OR[a,b]	−279	−290	−296	−302	−301	−294)
CO_2CH_3	−137	−163	−186	−207	−224	−244
(CO_2R[b]	−237	−263	−286	−307	−324	−344)
Cl	−32	−51	−71	−94	−112	−138
Br	1	−17	−36	−56	−73	−95
Substituents on n-propylbenzene						
Calculated parent I ($I_P + 3I_{S,CH_2} + I_{I,PhCH_2R}$)	1198	1215	1228	1239	1246	1251
OH[a]	−416	−459	−489	−511	−515	−516
CN[a]	−250	−280	−310	−341	−367	−400
CO_2CH_3	−159	−186	−210	−232	−249	−267
(CO_2R[a,b]	−259	−286	−310	−332	−349	−367)
Cl[a]	−30	−48	−66	−86	−99	−119
Br[a]	−	−13	−28	−45	−55	−69

[a] Values used for the calculation of substituent index equations.
[b] 100 subtracted for the methyl group contribution.
[c] 200 subtracted for the ethyl group contribution.

TABLE VIII

SUBSTITUENT INDEX EQUATIONS FOR ALIPHATIC SUBSTITUENTS ON AN ALKYL SIDE CHAIN

$I_S = ax^2 + bx + c$; x = % organic modifier.

Substituent	*Coefficients*		
	a	*b*	*c*
Methanol–buffer			
$CONH_2$	0.0079	−5.013	−178
OH	−0.0257	−0.494	−273
CN	−0.0250	−1.050	−185
CHO	0.1314	−18.531	337
CO_2R	0.0071	−3.717	−90
COR	−0.0086	−1.851	−201
OR	0.0136	−2.939	−154
Cl	−0.0021	−1.053	19
Br	−0.0536	4.719	−99
CH_3	0.0	0.0	100
Acetonitrile–buffer			
$CONH_2$	0.0855	−11.786	−179
OH	0.0561	−8.139	−223
CN	0.0002	−2.997	−160
CHO	0.0073	−1.768	−184
CO_2R	0.0130	−3.580	−164
COR	0.0161	−3.654	−206
OR	0.0211	−2.644	−218
Cl	0.0018	−1.962	27
Br	0.0064	−2.161	63
CH_3	0.0	0.0	100

showed a negative interaction contribution in methanol as did the halides on both carbons.

The coefficients relating these interaction increments to the eluent composition were calculated but changes smaller than 10 units across the eluent range were regarded as within experimental error and a single mean value for the c term was used (Table X). Although general comments on the size of the interactions have been made, it was difficult to correlate these with any properties of the substituents.

Relationship between I_S and structural parameters

The substituent indices should be related to the contributions of the substituents to octanol–water partition coefficients and Hansch and Leo[12] have suggested that for aliphatic compounds fragmental constants F_r are a better guide than π constants. If a linear relationship between F_r and I_S exists, it could be useful as it could provide a method to estimate I_S for a substituent not included in the data set such as un-ionised aliphatic amino and carboxylic acid groups. The fragmental contributions (Table VI) have been compared with the substituent index values at different eluent compositions. However, there was only an approximately linear relationship with correlations decreasing from 0.925 to 0.869 in methanol and 0.924 to 0.860 in aceto-

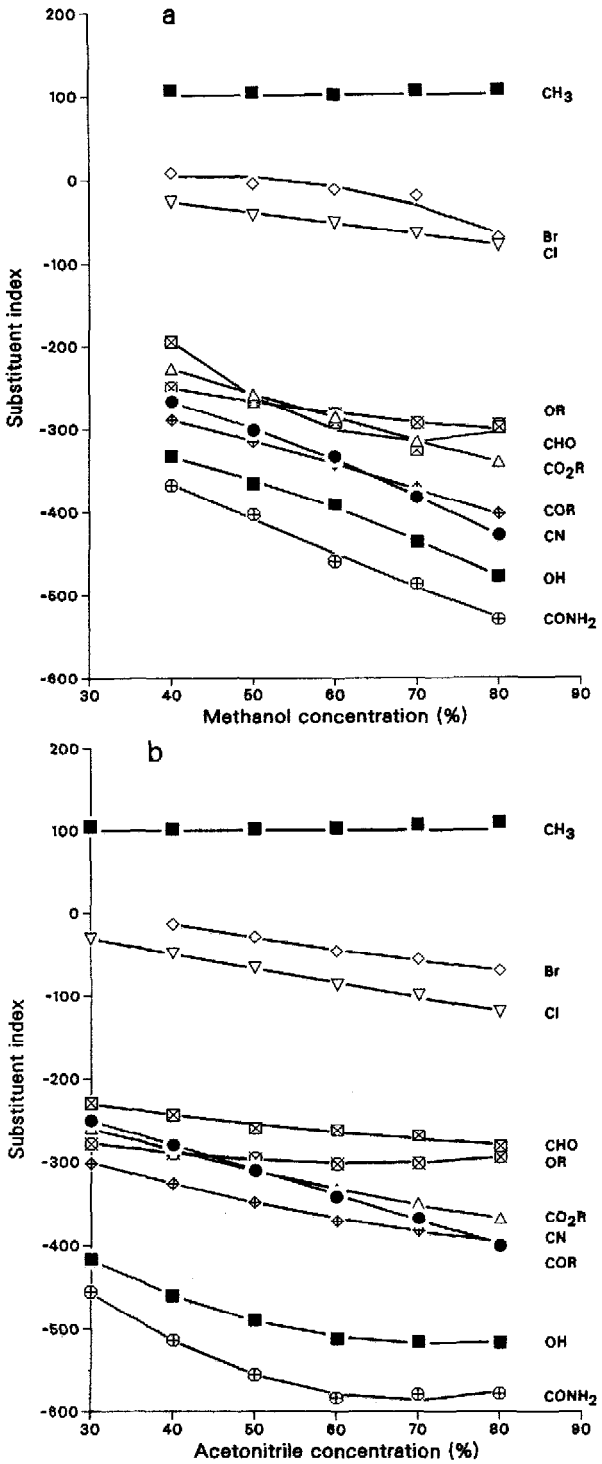

Fig. 2. Comparison of retention index increments for aliphatic substituents groups (points, from Tables VI and VII) with calculated substituents indices (solid line, calculated from Table VIII) in (a) methanol and (b) acetonitrile-containing eluents.

TABLE IX

INTERACTION INCREMENTS FOR INTERACTIONS OF ALIPHATIC SUBSTITUENTS WITH A PHENYL GROUP

Substituent	*Interaction increment*											
	Methanol (%)					*Acetonitrile (%)*						
	40	*50*	*60*	*70*	*80*	*30*	*40*	*50*	*60*	*70*	*80*	
1-phenyl, 1–X interactions ($PhCH_2$–X)												
$\delta I_I = \delta I_{PhCH_2-X} - I_S$												
$CONH_2$	11	19	3	27	28	19	27	30	31	49	47	
OH	38	40	55	67	70	61	72	80	82	96	97	
CN	55	53	53	58	69	65	70	73	77	85	86	
CO_2R	5	7	2	7	6	29	32	35	34	36	33	
COR	−12	−13	−12	−13	−12	3	9	12	14	13	6	
Cl	3	2	5	−4	−9	19	21	20	16	17	11	
Br	2	−11	−10	0	41	12	11	9	4	1	−8	
1-phenyl, 2–X interactions ($PhCH_2CH_2$–X)												
$\delta I_I = \delta I_{PhCH_2CH_2-X} - I_S$												
OH	17	35	28	26	36	27	33	37	37	48	46	
CN	0	−1	−5	2	1	17	18	18	18	22	18	
CO_2R	14	16	15	19	20	23	23	25	25	27	23	
Cl	−14	−13	−8	−10	−14	−2	−2	−4	−10	−10	−20	
Br	−5	−10	−10	0	−15	−3	−4	−5	−12	−16	−26	

TABLE X

COEFFICIENTS OF INTERACTION INDEX EQUATIONS FOR INTERACTIONS BETWEEN ALIPHATIC SUBSTITUENTS AND A PHENYL GROUP

$I_I = ax^2 + bx + c$; x = % organic modifier

Substituent	*Coefficients*					
	Methanol–buffer			*Acetonitrile–buffer*		
	a	*b*	*c*	*a*	*b*	*c*
1-phenyl, 1–X interactions ($I_{I,PhCH_2-X}$)						
$CONH_2$	0.0186	−1.809	56	0.0018	0.395	6
OH	−0.0007	0.996	−3	−0.0046	1.236	29
CN	0.0221	−2.327	113	0	0.440	52
CO_2R	0	0	0	0	0	33
COR	0	0	−12	0	0	10
Cl	−0.0143	1.414	−31	0	0	17
Br	0.0836	−9.139	235	−0.0079	0.479	5
1-phenyl, 2–X interactions ($I_{I,PhCH_2CH_2-X}$)						
OH	−0.0079	1.233	−16	−0.0021	0.636	10
CN	0	0	0	0	0	19
CO_2R	0	0	17	0	0	24
Cl	0	0	−12	−0.0075	0.482	−10
Br	−0.0071	0.757	−26	−0.0101	0.668	−14

nitrile as the proportion of the modifier increased. This trend agrees with previous reports which have suggested that the chromatographic system more closely resembles the octanol–water system when the organic content is lowest[13]. In both cases the correlations were poorer than the corresponding relationship for substituents on an aromatic ring[3].

CONCLUSION

The coefficients of the substituent index and interaction index determined for the aliphatic substituents generally reflect the effect on partitioning and the interaction of the phenyl groups and can be used in the prediction system to predict the retention indices of other substituents.

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