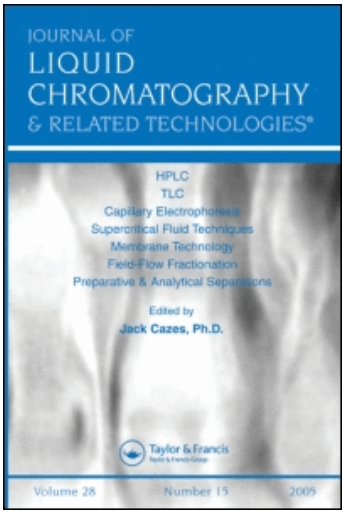

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NORMAL AND REVERSED PHASE THIN LAYER CHROMATOGRAPHY OF SELECTED 2,5-ANHYDROALDOHEXOSE ETHYLENE ACETAL DERIVATIVES

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NORMAL AND REVERSED PHASE THIN LAYER CHROMATOGRAPHY OF SELECTED 2,5-ANHYDROALDOHEXOSE ETHYLENE ACETAL DERIVATIVES

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ABSTRACT

The chromatographic behavior of 31 samples of variously substituted 2,5-anhydroaldohexose ethylene acetal derivatives has been studied on silica gel and C-18 modified silica gel layers with, respectively, binary non-aqueous and aqueous mobile phases. The slopes and intercepts of the linear relationships between the retention constant (R_M) and the logarithm of the volume fraction of the diluent in non-aqueous mobile phase, as well as of the volume fraction of organic component in aqueous-organic mobile phase, have been calculated and are discussed in relation to solute and mobile and stationary phase characteristics. The retention and relative retention of compounds depend largely on the retention behavior of their substituents.

INTRODUCTION

Variously substituted monosaccharide derivatives, frequently used as key intermediates in the synthesis of certain biomolecules,[1] are convenient for studying the relationship between the molecular structure of a compound and its chromatographic properties.

In a previous paper,[2] the normal phase chromatographic behavior of selected 1,2-O-isoprpylidene derivatives of aldohexoses and 1,2-O-cyclohexylidene derivatives of aldopentoses was studied on silica gel thin layers. Significant and distinct effects of the types of compounds and the type, number, and position of substituents in a molecule on retention were observed.

In this work, we have studied selected 2,5-anhydroaldohexose ethylene acetal derivatives of D-gluco, D-alo, D-gulo and L-ido series possessing a variety of substituents. The selected compounds have conveniently been used as key intermediates in the synthesis of C-nucleosides,[3] as well as of (+)-muscarine and its analogs.[4-6] The retention behavior of selected compounds has been studied by normal and reversed phase thin layer chromatography (TLC) using silica gel and C_{18} modified silica gel layers, and, respectively, non-aqueous and aqueous mobile phases.

EXPERIMENTAL

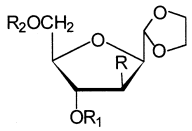
TLC was performed on 10 x 10 cm HPTLC plates pre-coated with silica gel 60 or C_{18} modified silica gel (Merck, Darmstadt, Germany). The samples were dissolved in chloroform (2 mg mL^{-1}) and 1-μL volumes of the solutions were applied to the chromatoplate with a micropipette.

The binary mobile phases in normal phase chromatography were cyclohexane (Cx) or toluene (Tl) mixed with ethyl acetate (EtAc), acetone (An), dioxane (Dx), or tetrahydrofuran (THF) in various proportions of mixture components.

In reversed phase chromatography, methanol (MeOH) or acetonitrile (ACN) were mixed in various proportions with water (H_2O). Spots were detected by spraying with a 50% aqueous solution of sulfuric acid, followed by heating at 120°C for 10-15 min.

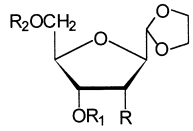
R_f values are averages from at least three chromatograms developed for each solute-mobile phase combination. R_M values were calculated by use of the formula $R_M = \log(1/R_f - 1)$. The structures of the compounds are given in Fig. 1.

D-*gluco*-series



1 R = OMs; R_1 = H; R_2 = Ms
2 R = OMs; R_1 = R_2 = Ms
3 R = OMs; R_1 = Bz; R_2 = Ms
4 R = OH; R_1 = Bn; R_2 = Bz
5 R = OMs; R_1 = Bn; R_2 = Bz
6 R = OH; R_1 = Bn; R_2 = H
7 R = OMs; R_1 = Bn; R_2 = Ms
8 R = OMs; R_1 = R_2 = Bz
9 R = N_3; R_1 = R_2 = H
10 R = N_3; R_1 = R_2 = Bz

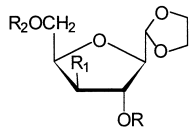
D-*allo*-series



11 R = OH; R_1 = R_2 = H
12 R = OBz R_1 = H; R_2 = Bz
13 R = OBz; R_1 = Ms; R_2 = Bz
14 R = OMs; R_1 = R_2 = Bz
15 R = OBz; R_1 = Tf; R_2 = Bz
16 R = N_3; R_1 = Bn; R_2 = Bz

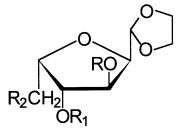
Bn, *benzyl*
Bz, *benzoyl*
Me, *methyl*
Ms, *mesyl (methanesulfonyl)*
N_3, *azide*
Ph, *phenyl*
Tf, *trifluoromethanesulfonyl*
Ts, *tosyl (p-toluenesulfonyl)*

D-*gulo*-series

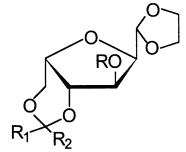


17 R = R_2 = H; R_1 = N_3
18 R = Bz; R_1 = N_3; R_2 = H
19 R = R_2 = Bz; R_1 = N_3
20 R = R_2 = Bz; R_1 = F

L-*ido*-series



21 R = Ms; R_1 = H; R_2 = OMs
22 R = R_1 = Ms; R_2 = OMs
23 R = Ms; R_1 = Ts; R_2 = OMs
24 R = Ms; R_1 = Tf; R_2 = OMs
25 R = Ts; R_1 = H; R_2 = OH
27 R = H; R_1 = Bz; R_2 = Br
28 R = Ms; R_1 = H; R_2 = I



26 R = Ts; R_1 = R_2 = Me
29 R = R_1 = H; R_2 = Ph
30 R = Ts; R_1 = H; R_2 = Ph
31 R = Ms; R_1 = H; R_2 = Ph

Figure 1. Structural formulas of the compounds examined.

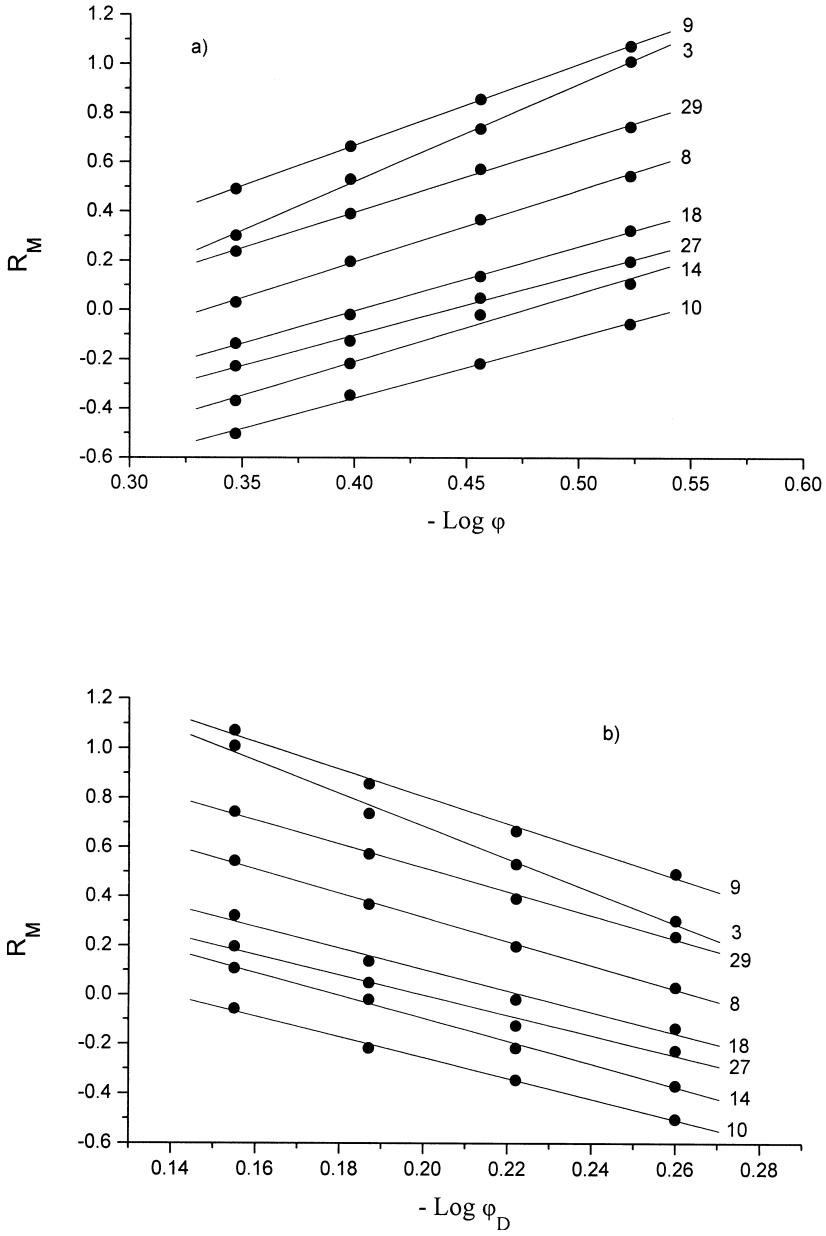


Figure 2. Plots of R_M *vs.* log φ (eqn. 1) (a) and R_M *vs.* log φ_D (eqn. 2) (b) for mobile phase Cx-THF. Designation of solutes is as in Fig. 1.

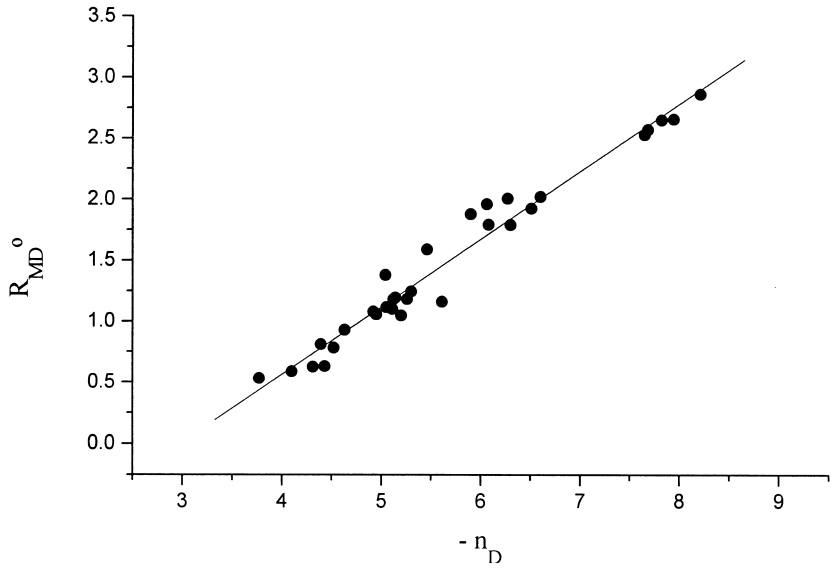


Figure 3. Plot of R_{MD}^{o} *vs.* n_D for mobile phase Cx-Dx.

RESULTS AND DISCUSSION

Normal Phase Chromatography (NPC)

The relationship between chemical structures of the compounds (Fig. 1) and their retention behavior in NPC has been studied using the well-known equation[7,8]

$$R_M = R_M^o - n \log \varphi \qquad (1)$$

where φ denotes the volume fraction of the polar constituent of a binary mobile phase, R_M^o is an extrapolated R_M value in pure polar solvent, and n is a constant. The numerical data of the constants n and R_M^o for each compound and mobile phase tested are presented in Table 1 for cyclohexane and in Table 2 for toluene as the diluent.

The data in Tables 1 and 2 show that slope n values of Eq. 1 mainly follow the polarities of the compounds, i.e., the n value decreases with the decrease of the compound retention.

Table 1

Constants n and R_M^0 of Eq. 1 for Eluents Containing Cyclohexane*

	Cx-EtAc φ = 0.35 - 0.55			Cx-An φ = 0.30 - 0.45			Cx-Dx φ = 0.30 - 0.45			Cx-THF φ = 0.30 - 0.45		
Cpd.	**n**	**R_M^0**	**r**	**n**	**R_M^0**	**r**	**n**	**R_M^0**	**r**	**n**	**R_M^0**	**r**
1	4.70	-0.538	.9963	3.93	-0.996	.9985	5.26	-1.248	.9968	4.81	-1.047	.9993
2	4.60	-0.511	.9972	3.67	-0.997	.9994	5.14	-1.245	.9993	4.60	-0.904	.9949
3	4.03	-0.715	.9983	3.18	-1.038	.9996	4.32	-1.295	.9974	4.01	-1.085	.9996
4	2.99	-0.899	.9991	2.34	-0.971	.9982	3.67	-.1417	.9910	3.00	-1.188	.9984
5	3.16	-1.028	.9989	2.46	-1.029	.9958	3.48	-1.413	.9962	2.85	-1.054	.9938
6	2.95	-0.672	.9947	2.86	-0.752	.9999	3.62	-1.107	.9982	3.29	-0.717	.9964
7	3.50	-0.816	.9986	3.10	-1.094	.9976	4.18	-1.326	.9983	3.68	-1.055	.9996
8	3.18	-0.925	.9987	2.49	-0.989	.9984	3.49	-1.366	.9941	2.95	-0.987	.9993
9	4.23	-0.645	.9975	3.17	-0.932	.9993	3.92	-1.039	.9987	3.34	-0.667	.9999
10	2.52	-1.122	.9946	1.96	-1.065	.9997	2.07	-1.126	.9990	2.52	-1.366	.9984
11	4.90	-0.532	.9932	4.07	-0.964	.9942	5.47	1.210	.9965	4.85	-0.891	.9943
12	2.99	-0.995	.9924	3.19	-1.340	.9976	3.70	-1.605	.9951	2.80	-1.187	.9990
13	3.24	-1.309	.9984	2.95	-1.246	.9960	2.72	-1.120	.9992	2.93	-1.292	.9999
14	3.13	-1.115	.9990	3.08	-1.276	.9976	3.39	-1.348	.9971	3.02	-1.169	.9993
15	3.02	-1.334	.9983	2.27	-1.111	.9991	3.01	-1.459	.9993	2.78	-1.323	.9928
16	2.47	-1.217	.9979	2.06	-1.169	.9991	3.24	-1.666	.9959	2.67	-1.549	.99785
17	4.22	-0.687	.9975	3.75	-1.049	.9925	4.22	-1.116	.9959	3.48	-0.795	.9949
18	2.73	-0.828	.9900	2.90	-1.167	.9935	3.42	-1.351	.9910	2.64	-1.064	.9985
19	2.48	-1.163	.9975	2.18	-1.161	.9736	3.18	-1.615	.9932	2.21	-1.312	.9874
20	2.52	-1.183	.9967	2.09	-1.084	.9685	2.70	-1.429	.9858	2.23	-1.299	.9906
21	4.76	-0.660	.9949	4.02	-1.068	.9999	5.36	-1.306	.9941	4.46	-0.985	.9980
22	4.68	-0.617	.9931	3.81	-1.095	.9998	5.10	-1.264	.9932	4.51	-0.950	.9960
23	3.83	-0.616	.9953	3.29	-0.798	.9992	4.40	-1.251	.9976	4.15	-1.110	.9964
24	3.77	-0.755	.9977	3.17	-0.876	.9992	4.07	-1.229	.9995	3.89	-1.096	.9979

25	4.44	-0.612	.9866	3.90	-0.991	.9987	4.04	-1.043	.9994	3.76	-0.774	.9971
26	2.66	-0.852	.9990	2.58	-1.196	.9999	2.94	-1.361	.9983	2.54	-1.112	.9966
27	2.53	-0.864	.9970	2.44	-1.056	.9943	3.08	-1.361	.9965	2.49	-1.100	.9964
28	2.94	-1.049	.9981	2.80	-1.203	.9866	3.39	-1.397	.9971	2.66	-1.115	.9983
29	3.88	-1.023	.9992	2.92	-0.961	.9998	3.36	-1.118	.9982	2.92	-0.775	.9991
30	2.82	-1.105	.9955	2.62	-1.206	.9995	3.46	-1.525	.9995	2.54	-1.115	.9974
31	3.02	-0.889	.9995	2.93	-1.158	.9971	3.29	-1.362	.9996	2.31	-1.044	.9959

* r = correlation coefficient.

Table 2

Constants n and R_M^0 of Eq. 1 for Eluents Containing Toluene*

	Tl-EtAc φ = 0.30 - 0.50			Tl-An φ = 0.25 - 0.45			Tl-Dx φ = 0.25 - 0.45			Tl-THF φ = 0.25 - 0.45		
Cpd.	**n**	**R_M^0**	**r**	**n**	**R_M^0**	**r**	**n**	**R_M^0**	**r**	**n**	**R_M^0**	**r**
1	2.67	-0.111	.9999	2.83	-0.879	.9983	3.05	-0.966	.9994	2.97	-0.963	.9972
2	2.27	-0.205	.9996	2.28	-0.811	.9957	2.20	-0.885	.9997	2.32	-0.843	.9999
3	2.24	-0.641	.9991	1.87	-1.096	.9996	1.69	-0.973	.9940	1.81	-0.904	.9973
4	1.91	-0.795	.9986	1.46	-1.091	.9954	1.58	-1.214	.9995	1.64	-1.130	.9916
5	1.60	-0.897	.9979	1.45	-1.205	.9995	1.49	-1.308	.9964	1.69	-1.260	.9929
6	2.49	-0.223	.9992	1.93	-0.689	.9942	2.40	-0.910	.9999	2.42	-0.854	.9998
7	1.94	-0.645	.9998	1.49	-1.016	.9989	1.51	-0.956	.9961	1.62	-0.920	.9997
8	1.70	-0.867	.9997	1.39	-1.235	.9947	1.49	-1.261	.9944	1.48	-1.098	.9995
9	2.60	-0.145	.9992	2.50	-0.698	.9979	2.80	-0.850	.9991	2.62	-0.750	.9978
10	1.22	-1.157	.9999	0.92	-1.315	.9998	1.20	-1.382	.9997	1.38	-1.581	.9950
11	2.91	-0.065	.9994	3.39	-0.447	.9938	4.00	-0.706	.9985	3.49	-0.589	.9989
12	2.01	-0.903	.9951	1.38	-1.053	.9952	1.61	-1.232	.9947	1.70	-1.190	.9918
13	1.62	-1.096	.9903	1.40	-1.374	.9999	1.53	-1.392	.9984	1.43	-1.393	.9998
14	1.52	-0.931	.9905	1.52	-1.334	.9982	1.60	-1.334	.9993	1.62	-1.297	.9999
15	1.72	-1.097	.9980	1.47	-1.346	.9957	1.38	-1.270	.9869	1.43	-1.240	.9993
16	1.10	-1.098	.9998	1.02	-1.218	.9973	1.34	-1.506	.9845	1.38	-1.581	.9950
17	2.65	-0.377	.9999	2.22	-0.689	.9994	2.53	-0.772	.9868	2.54	-0.723	.9999
18	1.70	-0.671	.9969	1.43	-0.999	.9957	1.80	-1.154	.9781	1.93	-1.303	.9999
19	1.11	-1.102	.9998	1.02	-1-187	.9951	1.22	-1.466	.9999	1.19	-1.392	.9474
20	1.11	-1.195	.9998	0.94	-1.210	.9951	1.28	-1.348	.9915	1.34	-1.568	.9918
21	2.77	-0.317	.9922	2.61	-0.832	.9984	2.72	-0.975	.9926	2.57	-0.982	.9976
22	2.52	-0.249	.9957	2.50	-1.070	.9939	2.29	-1.001	.9939	2.51	-0.979	.9940
23	2.08	-0.599	.9966	1.55	-0.869	.9980	2.09	-1.149	.9999	1.78	-0.996	.9921
24	2.08	-0.639	.9981	1.60	-0.985	.9980	1.73	-0.992	.9962	1.67	-0.959	.9897

25	2.55	-0.002	.9922	2.56	-1.023	.9992	2.40	-0.850	.9932	2.49	-0.881	.9998
26	1.51	-0.794	.9920	1.22	-1.177	.9957	1.66	-1.469	.9978	1.83	-1.293	.9969
27	1.47	-0.681	.9969	1.27	-0.978	.9983	1.52	-1.180	.9960	1.83	-1.298	.9999
28	1.83	-0.938	.9999	1.24	-0.963	.9941	1.66	-1.266	.9940	1.86	-1.378	.9999
29	2.32	-0.678	.9999	2.01	-1.062	.9957	1.86	-0.963	.9986	2.10	-1.087	.9999
30	1.55	-1.076	.9951	1.17	-1.133	.9903	1.57	-1.457	.9994	1.45	-1.359	.9968
31	1.87	-1.125	.9939	1.66	-1.183	.9997	1.67	-1.116	.9971	1.77	-1.144	.9933

* r = correlation coefficient.

Table 3

Constants n_D and R_{MD}^0 of Eq. 2 and Average R_{MD}^0 Values for Eluents Containing Cyclohexane*

Cpd.	Cx-EtAc			Cx-An			Cx-Dx			Cx-THF			Mean
	$-n_D$	R_{MD}^0	r	$-n_D$	R_{MD}^0	r	$-n_D$	R_{MD}^0	r	$-n_D$	R_{MD}^0	r	R_{MD}^0
1	5.83	2.657	.9979	8.09	2.335	.9978	7.82	2.649	.9823	7.93	2.659	.9931	2.575
2	5.70	2.613	.9936	7.53	2.112	.9965	7.68	2.569	.9907	7.68	2.660	.9998	2.544
3	4.90	1.998	.9859	6.66	1.690	.9982	6.51	1.925	.9970	6.64	2.012	.9959	1.906
4	3.66	1.119	.9948	4.92	1.040	.9996	5.11	1.101	.9975	4.99	1.135	.9994	1.099
5	3.88	1.110	.9970	5.19	1.090	.9999	5.26	1.184	.9987	4.76	1.157	.9986	1.135
6	4.90	2.013	.9883	5.98	1.700	.9955	5.46	1.589	.9981	5.49	1.833	.9993	1.783
7	4.35	1.565	.9985	6.53	1.573	.9998	6.30	1.789	.9966	6.10	1.786	.9978	1.678
8	3.90	1.227	.9943	5.24	1.155	.9995	5.30	1.245	.9993	4.89	1.293	.9986	1.230
9	5.25	2.232	.9825	6.58	1.768	.9920	5.90	1.880	.9955	5.52	1.908	.9962	1.947
10	3.13	0.593	.9896	4.09	0.616	.9973	3.77	0.534	.9996	4.18	0.581	.9983	0.581
11	6.08	2.800	.9982	8.45	2.502	.9957	8.21	2.861	.9994	8.08	2.865	.9835	2.757
12	3.70	1.035	.9951	6.50	1.355	.9860	5.61	1.162	.9998	4.63	0.974	.9938	1.132
13	3.98	0.980	.9940	6.08	1.260	.9948	4.95	1.058	.9995	4.84	0.967	.9957	1.066
14	3.86	1.026	.9917	6.36	1.341	.9983	5.12	1.180	.9986	4.98	1.158	.9925	1.176
15	3.71	0.723	.9918	4.70	0.821	.9955	4.52	0.783	.9938	4.64	0.831	.9973	0.790
16	3.06	0.459	.9967	4.33	0.603	.9989	4.43	0.632	.9999	4.41	0.510	.9947	0.551
17	5.23	2.179	.9903	7.67	2.126	.9839	6.27	2.007	.9819	5.71	1.884	.9824	2.049
18	3.39	1.029	.9965	6.02	1.304	.9995	5.14	1.195	.9995	4.35	0.973	.9902	1.116
19	3.08	0.525	.9965	4.59	0.709	.9932	4.31	0.626	.9853	3.63	0.390	.9736	0.563
20	3.15	0.534	.9887	4.41	0.710	.9904	4.10	0.589	.9922	3.66	0.418	.9789	0.563
21	5.90	2.573	.9966	8.22	2.334	.9919	7.94	2.657	.9762	7.39	2.460	.9945	2.506
22	5.80	2.561	.9932	7.90	2.148	.9931	7.65	2.530	.9989	7.52	2.542	.9912	2.445
23	4.71	1.975	.9932	6.91	2.021	.9936	6.60	2.021	.9911	6.82	2.086	.9860	2.026
24	4.64	1.798	.9960	6.60	1.830	.9842	6.08	1.793	.9912	6.48	1.916	.9881	1.834

25	5.50	2.401	.9985	7.97	2.308	.9906	6.06	1.961	.9922	6.21	2.124	.9912	2.199
26	3.26	0.945	.9952	5.39	1.013	.9950	4.39	0.812	.9885	4.20	0.849	.9900	0.897
27	3.11	0.850	.9949	5.06	1.020	.9992	4.63	0.931	.9939	4.11	0.819	.9916	0.905
28	3.61	0.941	.9957	5.83	1.185	.9974	5.05	1.115	.9874	4.39	0.934	.9940	1.044
29	4.81	1.613	.9927	5.89	1.503	.9966	5.04	1.380	.9927	4.85	1.485	.9979	1.495
30	3.51	0.816	.9960	5.51	1.048	.9983	5.20	1.049	.9952	4.20	0.845	.9934	0.940
31	3.74	1.161	.9909	5.97	1.315	.9866	4.92	1.080	.9906	3.83	0.742	.9938	1.075

* r = correlation coefficient.

Table 4

Constants n_D and R_{MD}^0 of Eq. 2 and Average R_{MD}^0 Values for Eluents Containing Toluene*

Cpd.	Tl-EtAc			Tl-An			Tl-Dx			Tl-THF			Mean
	$-n_D$	R_{MD}^0	r	$-n_D$	R_{MD}^0	r	$-n_D$	R_{MD}^0	r	$-n_D$	R_{MD}^0	r	R_{MD}^0
1	4.42	1.949	.9968	5.94	1.546	.9952	6.41	1.650	.9974	6.25	1.584	.9972	1.682
2	3.76	1.550	.9940	4.78	1.140	.9992	5.19	1.083	.9987	5.46	1.231	.9964	1.251
3	3.71	1.092	.9963	3.39	0.402	.9948	3.94	0.528	.9847	3.81	0.650	.9999	0.668
4	3.17	0.683	.9989	3.07	0.162	.9857	3.72	0.200	.9944	3.45	0.277	.9999	0.331
5	2.66	0.341	.9987	3.05	0.039	.9967	3.53	0.028	.9995	3.00	-0.013	.9985	0.099
6	4.18	1.714	.9975	4.02	0.961	.9910	5.05	1.150	.9958	5.70	1.309	.9985	1.284
7	3.20	0.850	.9942	3.09	0.275	.9989	3.55	0.394	.9999	3.35	0.458	.9906	0.494
8	2.79	0.445	.9983	2.92	-0.038	.9809	3.51	-0.070	.9998	3.11	0.174	.9922	0.128
9	4.35	1.871	.9992	5.25	1.446	.9881	5.74	1.526	.9909	6.13	1.583	.9901	1.607
10	2.06	-0.200	.9987	1.94	-0.525	.9941	3.43	-0.368	.9978	2.89	-0.401	.9999	-0.373
11	4.88	2.327	.9957	7.13	2.462	.9996	7.26	2.500	.9933	7.33	2.401	.9920	2.423
12	3.33	0.651	.9907	2.86	0.151	.9958	3.71	0.194	.9856	3.63	0.280	.9994	0.319
13	2.70	0.160	.9907	2.97	-0.164	.9965	3.56	-0.032	.9999	3.02	-0.163	.9931	-0.050
14	2.40	0.230	.9983	3.20	-0.024	.9884	2.91	-0.054	.9994	3.30	0.077	.9954	0.057
15	2.85	0.231	.9979	3.08	-0.089	.9830	2.86	-0.091	.9892	3.00	-0.016	.9908	0.009
16	1.82	-0.249	.9956	2.14	-0.374	.9997	2.47	-0.426	.9947	2.89	-0.401	.9999	-0.363
17	4.44	1.680	.9974	4.73	1.235	.9978	5.32	1.398	.9994	5.33	1.451	.9951	1.441
18	2.80	0.641	.9999	2.99	0.224	.9989	3.78	0.387	.9992	4.06	0.351	.9960	0.401
19	1.87	-0.237	.9985	2.15	-0.311	.9999	2.48	-0.434	.9940	2.51	-0.369	.9665	-0.338
20	1.87	-0.330	.9985	1.97	-0.407	.9999	2.70	-0.249	.9992	2.83	-0.415	.9985	-0.350
21	4.59	1.830	.9990	5.50	1.410	.9975	4.95	1.203	.9996	5.39	1.217	.9999	1.410
22	4.22	1.706	.9991	5.25	1.074	.9986	4.81	0.961	.9997	5.27	1.170	.9998	1.228
23	3.43	1.009	.9931	3.25	0.459	.9998	3.80	0.526	.9970	3.74	0.530	.9813	0.631
24	3.42	0.967	.9998	3.33	0.379	.9986	3.58	0.482	.9960	3.51	0.471	.9997	0.575

25	4.19	2.000	.9972	5.37	1.168	.9999	4.99	1.199	.9979	5.11	1.234	.9971	1.425
26	2.49	0.372	.9989	2.55	-0.135	.9996	3.37	-0.044	.9997	3.84	0.272	.9999	0.116
27	2.42	0.452	.9999	2.69	0.118	.9994	3.20	0.124	.9997	4.30	0.355	.9963	0.257
28	2.99	0.470	.9952	2.60	0.097	.9991	3.50	0.161	.9999	4.38	0.285	.9981	0.253
29	3.88	1.120	.9965	4.18	0.654	.9980	4.40	0.700	.9998	4.43	0.718	.9952	0.789
30	2.55	0.120	.9984	2.45	-0.133	.9978	3.20	-0.125	.9981	3.05	-0.115	.9882	-0.063
31	3.13	0.326	.9915	3.50	0.244	.9981	3.44	0.310	.9998	3.72	0.374	.9950	0.314

* r = correlation coefficient.

Table 5

Slopes and Intercepts of the Linear Relationship Between n_D and R_{MD}^0, and n_D and n in NPC, and Between m and R_{MW}^0 in RPC*

NPC	Slope	Intercept R_{MD}^0/n_D	r	Slope	Intercept n/n_D	r
Cx-EtAc	0.742	-1.702	0.9802	1.24	-0.021	0.9997
Cx-An	0.441	-1.251	0.9745	2.09	0.194	0.9986
Cx-Dx	0.551	-1.633	0.9829	1.43	0.276	0.9779
Cx-THF	0.534	-1.432	0.9703	1.67	-0.033	0.9998
Tl-EtAc	0.847	-1.927	0.9632	1.59	0.174	0.9759
Tl-An	0.530	-1.471	0.9759	2.10	-0.014	0.9971
Tl-Dx	0.587	-1.859	0.9790	1.80	0.556	0.9611
Tl-THF	0.558	-1.779	0.9672	2.19	-0.092	0.9852
		Average R_{MD}^0/n_D			**Average n/n_D**	
Cx	0.544	-1.437	0.9759	1.57	-0.092	0.9982
Tl	0.638	-1.813	0.9914	1.96	0.049	0.9952
RPC		**R_{MW}^0/n_D**				
MeOH-H_2O	0.977	-0.968	0.9977			
ACN-H_2O	1.145	-1.667	0.9912			

* r = correlation coefficient.

There is, however, no correlation between n and R_M^o values. For that reason, we have introduced into Eq. 1 the volume fraction of diluent, φ_D, instead of φ , so that Eq. 1 can be written as

$$R_M = R_{MD}^o - n_D \log \varphi_D \qquad (2)$$

where R_{MD}^o is an extrapolated R_M value at $\varphi_D = 1$, and n_D is a constant with negative sign. The numerical data for the constants n_D and R_{MD}^o are presented in Tables 3 and 4. It should be pointed out that Eq. 2 is valid for the narrow range of φ_D values, because there is no linear relation between log φ and log φ_D.

Table 6

Constants m and R_{MW}^0 of Eq. 3 for Eluents Containing Methanol and Acetonitrile

	MeOH-H_2O φ_{org} = 0.6-0.8		ACN-H_2O φ_{org} = 0.5-0.7			MeOH-H_2O φ_{org} = 0.6-0.8		ACN-H_2O φ_{org} = 0.5-0.7	
Cpd.	-m	R_{MW}^0	-m	R_{MW}^0	Cpd.	-m	R_{MW}^0	-m	R_{MW}^0
1	2.01	1.040			17	2.32	1.255		
2	2.13	1.190			18	3.13	2.285	2.23	0.907
3	3.01	1.937	2.38	1.002	19	6.51	5.329	3.53	2.512
4	5.13	4.094	2.92	1.609	20	5.51	4.797	3.30	2.230
5	5.92	4.730	3.43	2.189	21	1.97	0.990		
6	2.61	1.583	2.10	0.627	22	2.09	1.149		
7	3.39	2.335	2.62	1.308	23	3.63	2.484	2.33	1.087
8	5.56	4.568	3.30	2.059	24	3.85	2.707	2.21	0.979
9	2.20	1.099			25	2.49	1.371	2.15	0.707
10	6.65	5.457	3.52	2.288	26	4.94	3.752	3.03	1.687
11	1.91	0.803			27	4.43	3.254	2.51	1.218
12	5.33	4.135	2.72	1.460	28	4.95	3.950	2.66	1.413
13	6.12	4.870	3.32	2.066	29	3.27	2.165	2.20	0.832
14	5.94	4.736	3.28	2.057	30	5.19	4.190	3.06	1.942
15	7.22	6.086	3.70	2.501	31	3.03	2.093	2.25	1.003
16	6.45	5.460	3.56	2.532					

For φ values used and the same retention data, good correlation coefficients of the linear regression of experimental R_M values were obtained for both equations (Tables 1-4). Some correlation lines are given in Fig. 2.

Constants n_D and R_{MD}^o generally follow the retentivity of the compounds; therefore, there is good linear correlation between them (Fig. 3 and Table 5). Good linear relationship is also obtained between constants n and n_D (Table 5). As constants n_D and R_{MD}^o of a particular compound are similar for the same diluent and various eluting systems, their mean values (Tables 3 and 4) will be used for discussion of solutes retention behavior.

The retention of all studied compounds remarkably depends on the type of substituents and on the presence of other substituents in a molecule, but less on their positions or orientations. For example, compounds 1, 2, 21, and 22 co-migrated in mobile phases with cyclohexane, suggesting the same retentivity of hydroxy and mesyloxy functions. Likewise, compounds 4 and 5 were similarly retained, as were compounds 12, 13, and 14. Compounds 23 and 25, as well as compounds 29 and 31, were, however, clearly resolved. Compounds with hydroxy groups were more retained.

The effect of other substituents in a molecule on retention is evident. As expected, replacement of hydroxyl hydrogen or mesyl function with any other substituent resulted in reduced retention and, consequently, in decreased n and n_D values.

Substitution of cyclohexane with toluene as the diluent generally resulted in increased compound mobility, especially for those compounds with mesyloxy groups. This is evident from the R_{MD}^o data in Table 4. Exchange of hydroxy group for mesyloxy results in significantly higher ΔR_{MD}^o data for mobile phases containing toluene as diluent than those containing cyclohexane. Compound pairs 1-2, 21-22, and 4-5 were not resolved in systems with cyclohexane while, in systems with toluene, these pairs of compounds were completely separated with higher retention of those compounds possessing hydroxy groups. Increased compound mobility in systems with toluene also resulted in lower constants n, n_D, and R_{MD}^o in comparison with mobile phases containing cyclohexane.

The behavior of compounds bearing benzoyl or benzyl functions was generally as expected. The benzyl group is less polar than benzoyl; however, both groups caused low retentivity of the corresponding compounds. Compounds 6, 9, and 17 were of the similar retentivity, as were compounds 4 and 18, suggesting the similar effect of the benzyloxy or azide function on retention. A fluorine atom (compound 20) showed the same effect on retention as did an azide function (compound 19).

Reversed Phase Chromatography (RPC)

The retention data obtained in RPC were correlated with the volume fraction of organic component, φ_{org}, in a binary aqueous mobile phase according to the equation

$$R_M = R_{MW}^o + m\,\varphi_{org} \qquad (3)$$

where R_{MW}^o is an extrapolated R_M value in pure water, and m is a constant with negative sign. The numerical data for the constants m and R_{MW}^o are given in Table 6. Correlation coefficients of the linear regression of the experimental R_M values varied from 0.9971-0.9999. For some compounds, the retention constants were not measured, due to their great mobility in mobile phases containing acetonitrile.

There is good correlation between R_{MW}^o and absolute m values (Table 5). The slopes of the lines are about unity, indicating that the slope increments, Δm, are approximately equal to the retention increments, ΔR_{MW}^o (Table 7). According to Eq. 3, R_{MW}^o values for the particular compound and various

Table 7

Δm and ΔR_{MW}^0 Values for Conversion of Substituents in Compound Molecules

Converted Substituent	Compounds Compared	MeOH-H_2O Δm	MeOH-H_2O ΔR_{MW}^0	ACN-H_2O Δm	ACN-H_2O ΔR_{MW}^0	Converted Subsituent	Compounds Compared	MeOH-H_2O Δm	MeOH-H_2O ΔR_{MW}^0	ACN-H_2O Δm	ACN-H_2O ΔR_{MW}^0
3-OH→3-OMs	4 and 5	0.79	0.64	0.51	0.58	4-OH→4-Obn	1 and 7	1.38	1.30		
4-OH→4-OMs	1 and 2	0.12	0.15			3-OH→3-OTs	29 and 30	1.92	2.03	0.86	1.11
	12 and 13	0.79	0.74	0.60	0.61	4-OH→4-OTs	21 and 23	1.66	1.49		
	21 and 22	0.12	0.16			4-OH→4-OTf	12 and 15	1.89	1.95	0.98	1.04
3-OH→3-OBz	17 and 18	0.81	1.03				21 and 24	1.88	1.72		
4-OH→4-OBz	1 and 3	1.00	0.90			4-OH→4-N_3	12 and 19	1.18	1.19	0.81	1.05
6-OH→6-OBz	4 and 6	2.52	2.51	0.82	0.98	4-OBz→4-OBn	5 and 8	0.36	0.16	0.13	0.13
	18 and 19	3.38	3.04	1.30	1.61		3 and 7	0.38	0.40	0.24	0.31
3,6-OH→3,6-OBz	17 and 19	4.19	4.07			3-N_3→3-F	19 and 20	1.00	0.53	0.23	0.28
4,6-OH→4,6-OBz	9 and 10	4.45	4.36			6-OMs→6-J	21 and 28	2.98	2.96		

mobile phases should be equal at $\varphi_{org} = 0$. There is, however, significant difference between the R_{MW}^o values calculated for the mobile phases used, suggesting that the relationship between R_M and φ_{org} is not linear for the wide range of φ_{org}.[9-11]

The retention of the compounds depended on the hydrophobicity of substituents in a molecule. Compound 15, bearing three hydrophobic substituents, was most retained. Therefore, the effect of substituents on retention will be discussed using Δm and ΔR_{MW}^o values presented in Table 7. It is evident from these data that, for the same substitution and various solute pairs, both increments are reasonably constant and independent of the substituent position or orientation. The presence of other substituents in a molecule affects retention and, consequently, relative retention. For example, the mesyloxy function is slightly more hydrophobic than hydroxy; however, for conversion of hydroxy to mesyloxy function, the presence of hydrophobic benzyloxy or benzoyloxy functions in a molecule (compound pairs 4-5 and 12-13) increases both increments in comparison with the presence of other mesyloxy groups (compound pairs 1-2 and 21-22). This is also observed for replacement of hydroxy with a benzoyloxy function. For compound pairs 4-6 and 18-19, both Δm and ΔR_{MW}^o values are significantly higher than those for compound pairs 1-3 and 17-18. Hydrophobicities of benzyl, benzoyl, tosyl, or trifluoromethanesulfonyl functions are generally similar; azide is slightly more polar. Halogens, as electron-donating atoms, exhibit high hydrophobicity. Therefore, the retention difference between compounds 21 and 28 is about 3 (Table 7).

The retention sequence of compounds is generally opposite in RPC compared to that in NPC, especially when particular solute series are considered. However, an exception is observed, i.e., compound 15 was more retained than compound 16 in both NPC and RPC. Also, in both NPC and RPC, compounds 16 and 19, which are of similar functionality, co-migrated; therefore the trifluoromethanesulfonyl function is responsible for higher retention of compound 15. The trifluoromethyl moiety is hydrophobic and affects retention in RPC, while the sulfonyl moiety determines retention of compound 15 in NPC.

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