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Use of High-Pressure Liquid Chromatography for Quantitative Structure-Activity Relationship Studies of Sulfonamides and Barbiturates[1]

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Retention volumes for a group of sulfonamides obtained on three different HPLC columns were correlated with log P, pK_a, and biological activity. These data were compared with previously published R_M values obtained from five different TLC systems. In a similar manner, previously published chromatographic data of barbiturates from five different HPLC systems were correlated with log P, pK_a, and biological activity. Depending on the chromatographic system, good correlations can be obtained with log P or with biological activity, but not necessarily both, using the same chromatographic data.

The use of partition coefficients (log P) in an octanol–water system (the Hansch method) has become a standard method to carry out quantitative SAR studies.[2] The method requires either experimentally determining the partition coefficient or calculating log P using tables of π values for substituents.[3,4] The latter has its limitations, and there are innumerable compounds where log P between octanol–water must be determined.[5] This can be a tedious process complicated by instability in aqueous media, analytical procedures, and the tendency for the compound to dissociate.[2a]

Recently HPLC has been used to determine log P values.[6-8] This was a logical step as chromatography has been used to determine various physical constants. Paper impregnated with olive oil was used to determine R_M values for a series of phenols to measure hyperconjugation.[9,10] Other correlations of chromatographic behavior with chemical structure have been done using ethyl oleate coated cellulose.[11,12] Structure and biological data have been correlated in thin-layer chromatographic systems using silica gels impregnated with a nonpolar phase.[13-16] Finally, fairly linear correlations can be obtained when comparing the ion-pair partition chromatography retention volumes of a series of biogenic amines with their π values.[17] For our study, we selected sulfonamides due to the availability of biological data which have a reasonably well-defined end point and for comparison with previously published chromatographic studies in a variety of TLC systems.[14,18-21] There is also available an earlier traditional octanol–water system study.[22] For purposes of this study, the data from ref 20 were used as it had been utilized by Hansch in a previous study, and it appeared to be a well-controlled study. It was also decided to compare results obtained from three different HPLC columns: a bonded reverse phase pellicular packing, a pellicular packing coated with squalene, and a pellicular packing coated with octanol.

Experimental Section

Statistical correlations were performed [Oregon State University Statistical Interactive Programming System (SIPS)] using the log of the retention volume (log V_R). This is defined as

$$\log V_R = \log [(t_R - t_0)(\text{flow rate})]$$

where t_R = retention time of the compound and t_0 = retention time of the solvent front. It is not necessary to use the capacity factor (k') which is defined as

$$\log k' = \log [(t_R - t_0)/t_0] = \log (t_R - t_0) - \log t_0$$

The log t_0 term becomes a constant and drops out. Both V_R and k' can be compared with some other variable (log P, log $1/C$, or R_M) independent of the flow rates. Normally the log of the retention time (log R) requires a constant flow rate [log R = log ($t_R - t_0$)]. However, log R can be compared with other physical constants at programmed flow rates provided the change in flow rate is linear.[6]

Solvents were of analytical reagent quality and were used without further purification. Sulfonamides and barbiturates were obtained from commercial sources.

HPLC was performed on a Waters Model ALC/GPC 201 liquid chromatograph, equipped with two M-6000 pumps, a Model U-6 K injection valve, and a Model 660 programmer. A Varian Model 635 uv–visible spectrophotometer equipped with low dead volume flow cells was used as a detector. Sulfonamides were analyzed at 255 nm and barbiturates at 245 nm. Peaks were recorded on a Soltec dual pen recorder, and retention times were determined using a stopwatch.

The bonded reverse-phase packing material Bondapak C-18 Corasil, the pellicular silica Corasil II, and the porous silica Porasil A were all obtained from a commercial source (Waters Associates, Inc., Milford, Mass.; particle size 37–70 μ in each case). Approximately 1% loadings of octanol and squalene were prepared on Corasil II using published solvent–evaporation methods, with ether as a solvent.[26] Likewise, 15% loadings of squalene and octanol on Porasil A were prepared for packing precolumns.

Stainless steel columns, 60 cm × 2 mm i.d., were packed using published "Tap-fill" procedures.[26] Acetate buffers of pH 4.0 and 5.0 were prepared.[27] Sorensen phosphate buffer, pH 6.5, was also used.[28] The pH of the column eluent was monitored at periodic intervals.

An appropriate precolumn was placed between the pump and the injector when using the 1% squalene and 1% octanol on Corasil II columns. Also, the buffers were presaturated with the

Table I. Log V_R Values of Various Sulfonamides on C-18 Corasil, 1% Squalene on Corasil II, and 1% Octanol on Corasil II

			Log V_R[b]						Log P		Log $1/C$	
			C-18 Corasil		1% squalene		1% octanol					
	Drug	pK_a[a]	pH 4.0	pH 6.5	pH 4.0	pH 6.5	pH 4.0	pH 6.5	Obsd[c]	Calcd[d]	Obsd[e]	Calcd[f]
1	Sulfanilamide	10.08	−0.585	−0.677	−0.827	−0.842	−0.723	0.377	−0.83	−0.87	−2.11	−1.71
2	Sulfaguanidine	12.00	−0.702	−0.695	−0.899	−0.693	−0.796	−0.926	−1.22	−0.99	−1.81	−2.09
3	Sulfisoxazole	5.00	1.108	−0.777	0.187	−0.726	0.291	−0.753	1.15	0.78	−0.45	−0.12
4	Sulfamethazine	7.70	0.943	0.059	0.277	0.311	0.380	0.249	0.27	0.62	0.11	−0.01
5	Sulfacetamide	5.78	−0.111	−1.12	−0.686	−1.346	−0.583	−1.220	−0.01	−0.41	−0.52	−0.68
6	Sulfamerazine	6.98	0.652	−0.330	−0.027	−0.003	0.076	−0.117	0.13	0.33	0.06	0.03
7	Sulfadiazine	6.52	0.236	−0.638	−0.306	−0.519	−0.203	−0.475	−0.13	−0.07	−0.08	−0.10
8	Sulfathiazole	7.25	0.531	−0.357	−0.049	−0.125		−0.312	0.35	0.21	−0.18	−0.04
9	Sulfaethidole	5.65	1.326	−0.511	0.387	−0.148	0.494		1.10	1.00	−0.34	−0.36
10	Sulfamethoxazole	6.05	1.060	−0.747	0.017	−0.526	0.121		0.88	0.74	0.06	−0.08
11	Sulfamethoxy-pyridazine	7.20	1.056	−0.060	0.283	0.362	0.386		0.40	0.74	0.00	−0.08

[a] From ref 21. [b] Log V_R = log [($t_R - t_0$)(flow rate), ml/min]. [c] From ref 3. [d] Log P values calculated using eq 1, Table II (C-18 Corasil, pH 4.0). [e] C is in vitro minimum inhibitory concentration (μmol/l.) against *E. coli*, ref 20. [f] Log $1/C$ values calculated using eq 13, Table IV (C-18 Corasil, pH 4.0).

Table II. Correlation of Log P with Log V_R Values of Various Sulfonamides[a]

			Log $P = a$ log $V_R + b$				
Eq	HPLC column	pH	a	b	n	r	s	$F_{1,n-2}$
1	C-18 Corasil	4.0	0.98 (0.12)	− 3.03 (0.10)	10	0.937	0.27	63.53
2	C-18 Corasil	6.5			10	0.081		
3	1% squalene on Corasil II	4.0	1.37 (0.29)	+ 0.39 (0.14)	10	0.862	0.39	23.18
4	1% squalene on Corasil II	6.5			10	0.294		
5	1% octanol on Corasil II	4.0	1.38 (0.28)	+ 0.25 (0.13)	10	0.863	0.39	23.27
6	1% octanol on Corasil II	6.5			10	0.001		

[a] Values in parentheses are standard errors; r is the linear correlation coefficient; s is the standard deviation of the residual values for calculated log P; F is the standard variance ratio statistic.

stationary phase for 24 h prior to use, and the columns were allowed to equilibrate to a steady baseline before injection.

Samples for injection were dissolved in methanol and in 1% Na_2CO_3 solution at concentrations of 0.1–1.0 mg/ml. It was found that there was no difference in retention volumes between the samples dissolved in methanol and the samples dissolved in basic solution, indicating that the buffer capacity of the mobile phase was adequate to convert the sample to the same species in each case.

Injection volumes of the sulfonamides were 1.0 μl. Movement of the solvent front (t_0) for the sulfonamides was determined by injecting sulfanilic acid, while that of the barbiturates was determined with DMF. Repeat determinations for all of the compounds were made until the V_R values were constant to within ±0.05 ml. Retention times were calculated by subtracting t_0.

Flow rates were 0.4 ml/min for 1% squalene and 1% octanol on Corasil II columns. Constant flow rates of 1.0–3.0 ml/min were used whenever the Bondapak C-18 Corasil was used, depending on the compound. Linearity of the flow rate was checked, and variations in V_R on going from one flow rate to another were within experimental error for the C-18 Corasil column.

Results and Discussion

A summary of the experimental results is seen in Table I. Each log V_R value represents an average of two or three determinations. In general, the values of V_R did not vary by more than ±0.05 ml. In cases where the compound did not elute with sufficient resolution to allow measurement of V_R within these limits, the entry is missing.

It is shown in Table II that fairly good correlation can be obtained between log P of the sulfonamides and log V_R. As one might expect, the best correlations are obtained at pH 4.0. The highest correlation is obtained with C-18 Corasil (r = 0.937, $F_{1,8}$ = 63.53) though the 1% squalene on Corasil and 1% octanol on Corasil are also found to give correlations that are significant at better than a 99% level of confidence. In spite of these correlations, the individual variations between the experimental and the calculated log P values are fairly high. The fact that C-18 Corasil gave the highest correlation with log P may reflect a higher level of nonpolar stationary phase, hence, a greater degree of partitioning. It should be mentioned that little attempt was made to optimize the percent loading on the squalene and octanol columns. Calculated log P values for the sulfonamides, from eq 1, Table II, are seen in Table I.

Using the biological data for the in vitro minimum inhibitory concentration against *Escherichia coli*,[20] the equations in Table III were derived. Again, the highest correlations are obtained at pH 4.0. In going to a parabolic dependence of log $1/C$ on log V_R, even higher levels of correlation are obtained (Table IV). As before, retention data on C-18 Corasil give the closest fit to the observed potencies. A comparison between the calculated activity, using eq 13 of Table IV, and the observed activity is seen in Table I.

In dealing with sulfonamides, it was found by Fujita and Hansch that using values of log $1/C$ that were corrected for dissociation sometimes gave higher levels of correlation with π.[22] That this was not the case for log V_R at pH 4 is shown in Table V. Here, the correlations dropped considerably when using corrected log $1/C$ values. It was found, however, that significant increases in correlation could be obtained when log V_R values at pH 6.5 were corrected for ionization and then regressed with corrected log $1/C$ values. This is seen in Table VI. (It is possible, as was pointed out by one of the reviewers of this article,

Table III. Correlation of Log V_R Values from HPLC with Log $1/C$ for Sulfonamides

Eq	HPLC column	pH	Log $1/C = a \log V_R + b$ a	b	n	r	s	F
7	C-18 Corasil	4.0	0.87 (0.21)	−0.92 (0.18)	11	0.805	0.45	16.60
8	C-18 Corasil	6.5	0.91 (0.69)	−0.01 (0.43)	11	0.404	0.70	1.76
9	1% squalene on Corasil II	4.0	1.29 (0.34)	−0.29 (0.16)	11	0.785	0.47	14.48
10	1% squalene on Corasil II	6.5	0.79 (0.41)	−0.17 (0.26)	11	0.539	0.64	3.68
11	1% octanol on Corasil II	4.0	1.46 (0.33)	−0.36 (0.15)	10	0.843	0.45	19.81
12	1% octanol on Corasil II	6.5	−0.004 (0.63)	−0.62 (0.41)	8	−0.003	0.86	0.00

Table IV. Parabolic Dependence of Log $1/C$ on Log V_R for Sulfonamides from HPLC

Eq	HPLC column	pH	Log $1/C = a (\log V_R)^2 + b \log V_R + c$ a	b	c	n	r	s	$F_{2,n-2}$
13	C-18 Corasil	4.0	−1.09 (0.19)	+1.52 (0.16)	−0.48 (0.12)	11	0.963	0.22	50.69
14	C-18 Corasil	6.5	1.64 (0.86)	+2.70 (1.63)	+0.35 (0.57)	11	0.470	0.73	1.13
15	1% squalene on Corasil II	4.0	−2.47 (0.71)	−0.05 (.045)	+0.05 (0.14)	11	0.920	0.32	11.96
16	1% squalene on Corasil II	6.5	0.42 (0.78)	+1.14 (0.79)	−0.19 (0.28)	11	0.561	0.68	0.29
17	1% octanol on Corasil II	4.0	−2.67 (0.78)	+0.40 (0.35)	+0.01 (0.19)	10	0.924	0.31	20.58
18	1% octanol on Corasil II	6.5	−1.56 (1.27)	−1.24 (1.17)	−0.44 (0.42)	8	0.481	0.72	0.75

Table V. Correlation of Log V_R from HPLC at pH 4.0 with Corrected Log $1/C$ Values for Sulfonamides (Sauterne's Medium, pH 7.2)[a]

Eq	HPLC column	Log $1/C$ (cor) = log $1/C$ + log $[(K_a + 10^{-7.2})/10^{-7.2}] = a (\log V_R)^2 + b \log V_R + c$ a	b	c	n	r	s	F
19	C-18 Corasil	−0.95 (0.67)	+2.00 (0.52)	+0.06 (0.39)	11	0.840	0.73	2.03
20	1% squalene on Corasil II	−2.66 (1.93)	+0.50 (1.19)	+1.06 (0.41)	11	0.768	0.87	1.90
21	1% octanol on Corasil II	−3.83 (2.04)	+0.63 (0.91)	+1.29 (0.49)	8	0.810	0.79	3.53

[a] See ref 20.

Table VI. Correlation of Corrected Log $1/C$ Values with Corrected Log V_R Values (pH 6.5) of Sulfonamides

Log $1/C$ + log $[(K_a + 10^{-7.2})/10^{-7.2}] = a$ (log V_R + log $[(K_a + 10^{-6.5})/10^{-6.5}] + b$

Log $1/C$ (cor) = $a \log V_R$ (cor) + b

Eq	Column	a	b	n	r	s	F
22	C-18 Corasil	2.39 (0.58)	+0.59 (0.26)	11	0.825	0.76	17.07
23	1% squalene	1.11 (0.54)	+0.31 (0.37)	11	0.586	1.09	4.17
24	1% octanol	0.95 (1.03)	+0.18 (0.50)	8	0.354	1.26	0.86

Log $1/C$ (cor) = $a \log V_R$ (cor)^2 + $b \log V_R$ (cor) + c

Eq	Column	a	b	c	n	r	s	F
25	C-18 Corasil	−2.31 (1.06)	+2.32 (0.48)	+1.07 (0.30)	11	0.901	0.59	4.92
26	1% squalene	−1.75 (0.57)	+1.90 (0.46)	+1.07 (0.36)	11	0.847	0.72	9.32
27	1% octanol	−0.72 (1.94)	+0.84 (1.15)	+0.35 (0.71)	8	0.386	1.24	0.14

that the log V_R term at pH 4.0 may in fact contain a "hidden" σ term, which would cancel with the necessary correction in log V_R, and thus reduce the effects of this correction. This would result in lower correlations for the corrected data.)

As a further assessment of the relative importance of adsorption and partition processes, the TLC values in Table VII obtained from previously published papers[14,18] were correlated with pK_a, log P, and log $1/C$ values of the sulfonamides.

It is shown in Table VIII that the values of log V_R obtained from HPLC using the C-18 Corasil, 1% squalene on Corasil II, and 1% octanol on Corasil II columns do not, in general, have high levels of correlation with either the reverse phase partition or the adsorption TLC data. Again, this likely reflects the fact that the HPLC packing materials used were not completely coated with the nonpolar stationary phase. Thus the log V_R values reflect a combination of partitioning and adsorption effects. Presumably, correlations with reverse phase TLC data would have increased if the HPLC columns had been more heavily coated or vigorously silylated.[6,7]

Dependence of R_M values on pK_a is shown in Table IX. Clearly, the 20% silicone and 20% octanol reverse phase TLC systems (R_{M_1} and R_{M_2}) show a significant dependence on pK_a. This may be largely due to the fact that the log P and pK_a values for this sample of sulfonamides show a fairly high level of cross correlation (r = −0.905). The best correlation of pK_a vs. R_M for an adsorption TLC system was obtained with R_{M_4}, using petroleum ether–$CHCl_3$–butanol (1:1:1) (eq 34, Table X). These results for the adsorption TLC (R_{M_3}, R_{M_4}, R_{M_5}) could be rationalized by assuming that highly polar NH_4OH and MeOH, present in R_{M_3} and R_{M_5}, reduce the polar interactions of the

Table VII. TLC Retention Data for Sulfonamides

	Drug	R_M values[a]				
		R_{M_1}[b]	R_{M_2}[c]	R_{M_3}[d]	R_{M_4}[e]	R_{M_5}[f]
1	Sulfacetamide			0.105	−0.158	1.005
2	Sulfadiazine	1.24	0.74	0.140	−0.087	0.501
3	Sulfaethidole	2.11	1.18	−0.122	−0.550	0.689
4	Sulfaguanidine			−0.288	0.659	1.690
5	Sulfamerazine	0.90	0.41	0.052	−0.308	0.389
6	Sulfamethazine	0.74	0.43	−0.087	−0.327	0.347
7	Sulfamethoxazole	1.64	0.89	−0.070	−0.720	0.454
8	Sulfanilamide	−0.36	−0.68	−0.575	0.140	1.061
9	Sulfisoxazole	2.75	1.99	−0.105	−0.602	0.788
10	Sulfathiazole	0.79	0.22			
11	Sulfamethoxy-pyridazine	1.08	0.46			

[a] Corrected R_M values, $R_M = \log (1/R_f - 1) + \log [(K_a + 10^{-7.4})/10^{-7.4}]$. [b] 20% silicone DC 200 on silica gel, with aqueous buffer, pH 7.4; data from ref 14. [c] 20% octanol on silica gel, with aqueous buffer, pH 7.4, from ref 14. [d] Silica gel G, with ethyl acetate-methanol-25% NH_4OH (17:6:5), from ref 18. [e] Silica gel G, with petroleum ether-chloroform-butanol (1:1:1), from ref 18. [f] Silica gel G with chloroform-methanol (95:5), from ref 18.

Table VIII. Correlation Matrix for Chromatographic Retention Data of Sulfonamides[a]

	HPLC			TLC				
	C-18, pH 4.0	1% squalene, pH 4.0	1% octanol, pH 4.0	R_{M_1}[b]	R_{M_2}[b]	R_{M_3}[b]	R_{M_4}[b]	R_{M_5}[b]
C-18, pH 4.0	1.00	0.979	0.979	0.758	0.781	0.431	−0.915	−0.753
1% squalene, pH 4.0		1.00	0.998	0.662	0.673	0.381	−0.838	−0.760
1% octanol, pH 4.0			1.00	0.667	0.669	0.380	−0.838	−0.759
R_{M_1}				1.00	0.988	0.504	−0.824	−0.153
R_{M_2}					1.00	0.548	−0.792	−0.202
R_{M_3}						1.00	−0.418	0.782
R_{M_4}							1.00	−0.527
R_{M_5}								1.00

[a] Values are linear correlation coefficients representing the largest possible sample population in each case. [b] For explanation of these terms, see Table VII.

Table IX. Correlation of R_M Values from Reverse Phase (R_{M_1}, R_{M_2}) and Adsorption (R_{M_3}, R_{M_4}, R_{M_5}) Chromatography with pK_a Values of Sulfonamides

Eq		n	r	s	F
28	$R_{M_1} = -0.59\ (0.06)\ pK_a + 5.29\ (0.44)$	9	−0.964	0.23	91.41
29	$R_{M_2} = -0.47\ (0.06)\ pK_a + 3.92\ (0.41)$	9	−0.951	0.27	66.90
30	$R_{M_3} = -0.06\ (0.03)\ pK_a + 0.36\ (0.20)$	9	−0.676	1.62	5.88
31	$R_{M_4} = 0.16\ (0.03)\ pK_a + 1.41\ (0.24)$	9	0.887	1.02	25.96
32	$R_{M_5} = 0.12\ (0.05)\ pK_a - 0.16\ (0.39)$	9	0.681	1.61	6.06

Table X. Correlation of Log P with R_M Values from Adsorption TLC of Sulfonamides

Eq		n	r	s	F
33	$\log P = 1.60\ (1.27)\ R_{M_3} + 0.32\ (0.30)$	9	0.429	0.74	1.58
34	$\log P = -1.84\ (0.21)\ R_{M_4} + 0.25\ (0.09)$	9	−0.958	0.24	77.61
35	$\log P = -1.24\ (0.55)\ R_{M_5} + 1.10\ (0.48)$	9	−0.649	0.62	5.10

Table XI. Correlation of Reverse Phase TLC Retention Values of Sulfonamides with Log $1/C$ for in Vitro Inhibition of *E. coli*

Eq		n	r	s	F
36	$\log 1/C = 0.37\ (0.26)\ R_{M_1} - 0.77\ (0.38)$	9	0.472	0.61	2.01
37	$\log 1/C = 0.47\ (0.31)\ R_{M_2} - 0.62\ (0.29)$	9	0.494	0.61	2.26
38	$\log 1/C = -0.58\ (0.09)\ R_{M_1}^2 + 1.80\ (0.23)\ R_{M_1} - 1.25\ (0.16)$	9	0.954	0.21	45.67
39	$\log 1/C = -0.75\ (0.12)\ R_{M_2}^2 + 1.49\ (0.20)\ R_{M_2} - 0.61\ (0.11)$	9	0.949	0.22	39.96

sulfonamides with the silanol groups and, hence, reduce the dependence on pK_a. No significant correlations were found between log V_R values on the HPLC columns and the pK_a's of the sulfonamides tested.

Biagi found high levels of correlation between π and R_{M_1} and R_{M_2} mentioned above.[14] These correlations increased as the percent loading of stationary phase increased. Surprisingly, it is also possible to find a good negative correlation between log P and R_{M_4}, which is the R_M value on silica with petroleum ether–$CHCl_3$–butanol (1:1:1). It is likely that this reflects a "negative" partitioning between the relatively polar silanol groups of silica and the relatively

Table XII. Correlation of Reverse Phase TLC Retention Values with Corrected Log 1/C Values of Sulfonamides

Eq		n	r	s	F
40	Log 1/C (cor) = 1.22 (0.17) R_{M_1} − 0.92 (0.26)	9	0.936	0.48	49.32
41	Log 1/C (cor) = 1.50 (0.22) R_{M_2} − 0.38 (0.26)	9	0.934	0.49	47.82
42	Log 1/C (cor) = −0.38 (0.07) $R_{M_1}^2$ + 2.16 (0.18) R_{M_1} − 1.23 (0.12)	9	0.990	0.19	33.41
43	Log 1/C (cor) = −0.52 (0.08) $R_{M_2}^2$ + 2.20 (0.14) R_{M_2} − 0.37 (0.08)	9	0.992	0.17	41.41

Table XIII. Correlation of R_M Values of Sulfonamides from Adsorption TLC with Log 1/C

Eq		n	r	s	F
44	Log 1/C = 3.14 (0.87) R_{M_3} − 0.23 (0.18)	9	0.834	0.46	16.01
45	Log 1/C = −1.48 (0.48) R_{M_4} − 0.86 (0.22)	9	−0.760	0.54	9.58
46	Log 1/C = −1.65 (0.37) R_{M_5} + 0.70 (0.32)	9	−0.859	0.43	19.64
47	Log 1/C = −3.52 (3.23) $R_{M_3}^2$ + 1.66 (1.57) R_{M_3} − 0.20 (0.18)	9	0.863	0.42	1.11
48	Log 1/C = −0.66 (1.09) $R_{M_4}^2$ − 1.54 (0.51) R_{M_4} − 0.76 (0.31)	9	0.776	0.53	0.36
49	Log 1/C = 0.62 (0.89) $R_{M_5}^2$ − 2.86 (1.80) R_{M_5} + 1.17 (0.75)	9	0.870	0.41	0.48

Table XIV. Correlation of R_M Values from Adsorption TLC of Sulfonamides with Corrected Log 1/C

Eq		n	r	s	F
50	Log 1/C (cor) = 4.92 (1.62) R_{M_3} + 0.93 (0.38)	9	0.253	0.89	9.18
51	Log 1/C (cor) = −2.89 (0.65) R_{M_4} − 0.21 (0.30)	9	−0.858	0.70	19.68
52	Log 1/C (cor) = −2.10 (0.97) R_{M_5} + 2.03 (0.85)	9	−0.633	1.06	4.68
53	Log 1/C (cor) = −7.50 (6.63) $R_{M_3}^2$ + 1.75 (3.22) R_{M_3} + 1.00 (0.38)	9	0.802	0.82	1.25
54	Log 1/C (cor) = 0.04 (1.55) $R_{M_4}^2$ − 2.89 (0.72) R_{M_4} − 0.22 (0.43)	9	0.859	0.70	0.00
55	Log 1/C (cor) = −2.08 (2.28) $R_{M_5}^2$ + 1.99 (4.58) R_{M_5} + 0.46 (1.92)	9	0.689	0.99	0.84

Table XV. Correlation of Log P with Log 1/C and Log 1/C (cor) for Sulfonamides

Eq		n^a	r	s	F
56	Log 1/C = 0.73 (0.26) log P − 0.67 (0.21)	9	0.723	0.57	7.67
57	Log 1/C = −0.73 (0.24) $(\log P)^2$ + 0.77 (0.18) log P − 0.23 (0.20)	9	0.902	0.36	9.32
58	Log 1/C (cor) = 1.58 (0.28) log P + 1.81 (0.22)	9	0.902	0.62	30.21
59	Log 1/C (cor) = −0.52 (0.35) $(\log P)^2$ + 1.61 (0.26) log P + 0.50 (0.30)	9	0.929	0.53	2.13

[a] Using the same sulfonamides as in Tables XIII and XIV.

Table XVI. Log V_R Values of Barbiturates on C-18 Corasil, 1% Squalene on Corasil II, and 1% Octanol on Corasil II

		Log V_R (pH 5.0 buffer + 10% CH_3CN)			Log P	
	Drug	C-18	1% squalene	1% octanol	Obsd[a]	Calcd[b]
1	Barbital	0.161	0.193	0.170	0.65	0.76
2	Amobarbital	0.297	0.267	0.857	2.24	1.94
3	Pentobarbital	0.303	0.295	0.939	1.95	1.99
4	Secobarbital	0.344	0.241	1.132	2.15	2.36
5	Phenobarbital	0.228	0.217	0.857	1.42	1.35

[a] Data from ref 3. [b] Log P values calculated from eq 60, Table XVII.

Table XVII. Relationships between Log P and Log V_R Values of Barbiturates on C-18 Corasil, 1% Squalene on Corasil II, and 1% Octanol on Corasil II (pH 5.0 Buffer + 10% CH_3CN)

		Log P = a log V_R + b					
Eq	Column	a	b	n	r	s	F
60	C-18 Corasil	8.72 (1.54)	− 0.64 (0.42)	5	0.956	0.22	31.96
61	1% squalene on Corasil II	13.13 (5.67)	− 1.50 (1.39)	5	0.800	0.46	5.37
62	1% octanol on Corasil II	1.61 (0.46)	+ 0.41 (0.39)	5	0.894	0.34	11.96

nonpolar mobile phase. This is seen in eq 34, Table X.

Correlations of the retention data on reverse phase TLC with normal and corrected log 1/C values are seen in Tables XI and XII. In contrast to the results obtained with HPLC, it is seen that with R_{M_1} and R_{M_2}, the levels of correlation increase on going from normal to corrected log 1/C values. With the exception of R_{M_4}, which was shown to correlate with log P, the opposite is true of the

Table XVIII. Retention Data for Barbiturates on Ion-Exchange, Reverse Phase Partition, and Adsorption HPLC Columns

	Drug	pK_a	Log V_R SAX (acid)[a]	SAX (base)[b]	SCX[c] (acid)	Permaphase[d]	Corasil[e]	Log P Obsd[f]	Calcd[g]
1	Secobarbital	7.92	1.328	1.598	0.587	0.903	0.633	2.15	2.10
2	Amobarbital	7.78	1.057	1.305	0.373	0.671	0.778	1.95	1.85
3	Pentobarbital	8.02	1.125	1.382	0.481	0.699	0.614	1.95	1.88
4	Butabarbital	7.89	0.719	1.029	1.176	0.358		1.95	1.50
5	Butalbital	7.54	0.769	1.029	0.100	0.464	0.537	1.65	1.62
6	Hexobarbital	7.36	0.823	1.071	0.441	0.582		1.20	1.74
7	Butethal	7.76	0.732	1.009	0.025	0.324	0.886	1.65	1.47
8	Phenobarbital	7.26	0.981	1.290	−0.796	0.387	0.984	1.42	1.53
9	Aprobarbital	7.73	0.530	0.906	−0.102	0.207	0.841	1.15	1.33
10	Diallylbarbital	7.62	0.387	0.806	−0.553	−0.008		1.05	1.09
11	Barbital	7.75	−1.25	0.412	−2.00	−0.409	1.03	0.65	0.65

[a] SAX, Du Pont, 0.01 M citric acid solution, from ref 23. [b] SAX, Du Pont, 0.01 M sodium borate + 0.01 M sodium nitrate; data from ref 23. [c] SCX, Du Pont, 0.01 M citric acid solution, from ref 23. [d] ETH Permaphase, Du Pont, eluted with water, from ref 23. [e] Corasil II, Waters Associates, eluted with $CHCl_3$, from ref 25. [f] Data from ref 3. [g] Log P values calculated from eq 66, Table XX.

Table XIX. Correlation Matrix for Log V_R Values of Barbiturates on Various HPLC Columns

	SAX (acid)	SAX (base)	SCX (acid)	Permaphase	Corasil II
SAX (acid)	1.00	0.811	0.686	0.799	−0.602
SAX (base)		1.00	0.764	0.949	−0.564
SCX (acid)			1.00	0.896	−0.784
Permaphase				1.00	−0.725
Corasil II					1.00

absorption TLC data. That is, R_{M3} and R_{M5} behave more like the log V_R values obtained from HPLC with respect to normal and corrected biological activities. Again, this may reflect incomplete coating of the HPLC packing material, allowing a combination of adsorption and partitioning phenomena to occur. As a point of comparison, the corresponding correlations between normal and corrected log $1/C$ and log P values for the same sulfonamides used in Tables XIII and XIV are seen in Table XV.

The barbiturates are another group of compounds that have been studied by HPLC.[6,23] The log V_R values of five barbiturates on the C-18 Corasil, 1% squalene on Corasil II, and 1% octanol on Corasil II columns are given in Table XVI. Corresponding equations relating log P to log V_R are seen in Table XVII. Again, as with the sulfonamides, the highest correlation is seen with the C-18 Corasil column. As is seen in Table XVI, the individual variations between calculated and experimental log P values are not as great for the barbiturates as they were for the sulfonamides. This may be due to the fact that the aryl and alkyl substituents on the barbiturates more closely resemble the hydrocarbon coating of the C-18 packing material than do the heterocyclic substituents on the sulfonamides. Thus, higher correlations of log V_R with log P would be expected for the barbiturates than for the sulfonamides, since the latter show a more discriminating range of polar interactions with either octanol or the silanol groups of the Corasil.

Previously published retention data for several barbiturates on strong anion exchange (SAX), strong cation exchange (SCX), ether-bonded pellicular silica (Permaphase ETH), and pellicular silica (Corasil II) columns are given in Table XVIII.[23] These data are cross correlated by the matrix shown in Table XIX. The highest correlation (0.949), between SAX under basic conditions and Permaphase with water, is also reflected in Table XX. Here it is seen that similar levels of correlation exist when comparing log V_R values on the two columns with log P for barbiturates.

The SAX column under basic conditions also shows the highest level of correlation with pK_a (Table XXI). This is, of course, what one would expect for weak acids in basic media. For this set of compounds, the correlation of log P with pK_a is low (r = 0.462). Consequently, this is not a factor in the high level of correlation of log V_R on SAX with log P or with log V_R on the Permaphase column.

Hansch has correlated the inhibitory potencies of barbiturates on rat brain oxygen uptake and *Arbacia* egg cell division with log P and ΔpK_a values.[24] These same data, using the barbiturates available in this present study, are correlated with log V_R values in Tables XXII and XXIII. Hansch found log P to be of greatest significance

Table XX. Correlation of Log P Values of Barbiturates with Log V_R on Various HPLC Columns

Eq	Column	Log $P = a \log V_R + b$ a	b	n	r	s	F
63	SAX (acid)	0.31 (0.17) + 1.36 (0.15)		11	0.527	0.61	3.45
64	SAX (base)	1.25 (0.26) + 0.18 (0.29)		11	0.848	0.38	23.07
65	SCX (acid)	0.49 (0.13) + 1.58 (0.09)		11	0.788	0.45	14.72
66	Permaphase	1.11 (0.22) + 1.10 (0.12)		11	0.852	0.39	23.90
67	Corasil II	−1.97 (0.77) + 3.12 (0.62)		8	−0.722	0.50	6.54

Table XXI. Correlation of Log V_R Values of Barbiturates Obtained on Various HPLC Columns with pK_a Values

Eq	Column	Log $V_R = a$ p$K_a + b$ a	b	n	r	s	F
68	SAX (acid)	0.05 (0.03) + 0.495 (0.24)		11	0.457	0.55	2.38
69	SAX (base)	0.35 (0.03) − 1.56 (0.19)		11	0.974	0.14	166.93
70	SCX (acid)	0.06 (0.05) − 0.39 (0.38)		11	0.367	0.58	1.40
71	Permaphase	0.05 (0.03) + 0.05 (0.22)		11	0.504	0.54	3.07
72	Corasil II	0.023 (0.03) + 0.63 (0.20)		8	0.320	0.59	0.69

Table XXII. Relationships between Log V_R Values of Barbiturates on Various HPLC Columns and Inhibition of Rat Brain Oxygen Consumption

Eq	Column	Log $1/C = a \log V_R + b$ a	b	n	r	s	F
73	SAX (acid)	0.70 (0.11) + 2.16 (0.11)		8	0.931	0.24	39.10
74	SAX (base)	1.47 (0.33) + 0.98 (0.39)		8	0.874	0.33	19.36
75	SCX (acid)	0.70 (0.06) + 2.75 (0.05)		8	0.978	0.14	131.34
76	Permaphase	1.48 (0.18) + 2.03 (0.10)		8	0.960	0.19	70.56
77	Corasil II	−2.54 (0.93) + 4.63 (0.75)		8	−0.744	0.46	7.43

Eq	Column	Log $1/C = a (\log V_R)^2 + b \log V_R + c$ a	b	c	n	r	s	F
78	SAX (acid)	0.09 (0.19) + 0.71 (0.12) + 2.08 (0.24)			8	0.934	0.24	0.21
79	SAX (base)	−1.12 (0.76) + 3.70 (1.55) + 0.019 (0.75)			8	0.913	0.27	2.14
80	SCX (acid)	0.003 (0.09) + 0.70 (0.14) + 2.75 (0.07)			8	0.978	0.14	0.0008
81	Permaphase	−0.46 (0.37) + 1.69 (0.24) + 2.09 (0.10)			8	0.970	0.16	1.55
82	Corasil II	−0.13 (0.46) + 1.87 (0.74) − 3.29 (0.28)			8	0.912	0.28	8.30

Table XXIII. Relationships between Log V_R Values of Barbiturates on Various HPLC Columns and Inhibition of *Arbacia* Egg Cell Division at pH 8

Eq	Column	Log $1/C = a (\log V_R) + b$ a	b	n	r	s	F
83	SAX (acid)	0.65 (0.19) + 1.99 (0.18)		11	0.737	0.45	10.71
84	SAX (base)	1.60 (0.34) + 0.69 (0.38)		11	0.843	0.34	22.12
85	SCX (acid)	0.63 (0.16) + 2.49 (0.12)		11	0.796	0.38	15.56
86	Permaphase	1.45 (0.28) + 1.87 (0.14)		11	0.868	0.31	27.63
87	Corasil II	−2.65 (1.02) + 4.55 (0.83)		8	−0.726	0.43	6.68

Eq	Column	Log $1/C = a (\log V_R)^2 + b \log V_R + c$ a	b	c	n	r	s	F
88	SAX (acid)	0.53 (0.20) + 0.68 (0.16) + 1.52 (0.23)			11	0.867	0.32	6.72
89	SAX (base)	0.64 (0.83) + 0.30 (1.71) + 1.29 (0.87)			11	0.855	0.33	0.59
90	SCX (acid)	0.32 (0.18) + 1.06 (0.28) + 2.36 (0.13)			11	0.861	0.32	3.30
91	Permaphase	0.88 (0.58) + 1.01 (0.39) + 1.80 (0.14)			11	0.899	0.28	2.28
92	Corasil II	−0.11 (0.70) + 0.14 (0.11) + 1.63 (4.17)			8	0.821	0.36	2.27

in his correlations. This is also reflected here by the relatively high levels of correlation with log V_R on the Permaphase column. Surprisingly, though, the SCX column provides the best relationship of log V_R to log $1/C$ for the inhibition of oxygen uptake (Table XXII). We have no explanation for this result; in fact, it may be an artifact of the sample of barbiturates selected.

Finally, Table XXIV gives the observed and calculated log $1/C$ values for the biological activities of the barbiturates. It should be mentioned that comparisons of corrected log $1/C$ values for inhibition of *Arbacia* egg cell division and log V_R values gave much poorer correlations, both for linear and parabolic relationships, than normal uncorrected log $1/C$ values. This is in spite of the fact that the biological activities were determined at pH 8, which is higher than the pK_a values of most of the barbiturates studied, resulting in ionization of the compounds in solution.

It can be concluded that HPLC can be a useful method to carry out quantitative SAR studies. However, the user must be careful of what conclusion can be drawn with the results from a statistical point of view. Further, the user should not hesitate to try a variety of columns, should the initial results not be acceptable. There is still much theoretical work that must be done to understand the processes occurring on the surface of the column before one can predict which column or chromatographic technique would give useful information.

There have been two approaches in the use of HPLC in SAR studies. One uses the existing packing column materials without further modification. The other involves exhaustive silylation which is supposed to tie up active sites on the silica surface. In the course of another study, independent of any SAR studies, we silylated C-18 Corasil with dimethylchlorosilane and *N,O*-bis(trimethylsilyl)-acetamide. We repeated the injections described in this paper using 11 sulfonamides and 5 barbiturates.

Table XXIV. Observed and Calculated Activities of Barbiturates as Inhibitors of Rat Brain Oxygen Uptake and *Arbacia* Egg Cell Division

	Drug	Inhibn of O_2 uptake: Log $1/C^a$ obsd	Log $1/C^b$ calcd	Inhibn of *Arbacia* egg cells: Log $1/C^c$ obsd	Log $1/C^d$ calcd
1	Barbital	1.32	1.35	1.49	1.27
2	Butethal	2.80	2.77	2.40	2.33
3	Amytal	3.12	3.01	2.82	2.84
4	Phenobarbital	2.36	2.19	2.02	2.43
5	Secobarbital	3.19	3.16	3.62	3.17
6	Pentobarbital	3.07	3.09	2.92	2.88
7	Aprobarbital	2.41	2.67	2.01	2.17
8	Butalbital	2.80	2.82	2.41	2.54
9	Butabarbital			2.85	2.38
10	Hexobarbital			2.24	2.70
11	Diallylbarbituric acid			1.79	1.85

[a] Data from ref 24. [b] Calculated from eq 75, Table XXII. [c] Data from ref 24. [d] Calculated from eq 91, Table XXIII.

The equation obtained for the sulfonamides at pH 4.0 was

$$\log P = 1.23\ (0.13) \log V_R - 0.544\ (0.099) \quad (93)$$
$$n = 11;\ r = 0.951;\ s = 0.21$$

and the equation for the barbiturates was

$$\log P = 1.076\ (0.08) \log V_R + 0.311\ (0.12) \quad (94)$$
$$n = 5;\ r = 0.992;\ s = 0.05$$

The intercepts differed significantly ($p < 0.005$) but the slopes differ only at the 50% level. Our results using silylated columns more closely resemble those of Haggerty[6] than those of McCall.[7]

Further there is good correlation when comparing V_R (silylated) with V_R (nonsilylated) at pH 4.0 for sulfonamides

$$\log V_R \text{ (silylated)} = 0.975\ (0.05) \log V_R \text{ (nonsilylated)} - 0.44\ (0.04) \quad (95)$$
$$n = 11;\ r = 0.994;\ s = 0.09$$

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Adrenergic Sulfonanilides. 4. Centrally Active β-Adrenergic Agonists

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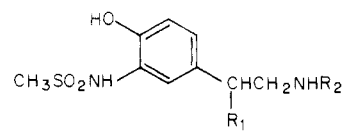
The central nervous system (CNS) activities of a number of soterenol analogs have been investigated, and several of these compounds possessed potent morphine antagonistic and anorexiant properties. The CNS activity of these compounds was enhanced by certain lipophilic [e.g., 1,1-dimethyl-2-phenethyl (**43**) or cyclopropyl (**40** and **44**)] nitrogen substituents; however, minor structural changes on either the aromatic or side-chain moieties drastically reduced central activity. Toxicity in this series was related to the inherent α-adrenergic stimulating component (direct or indirect).

While screening for potential narcotic antagonists, we observed that phenethylamine **40**, a weak β-adrenergic agonist, possessed potent morphine antagonism properties (ED_{50} = 0.21 mg/kg). Compound **40** as well as other appropriately substituted analogs of soterenol[1] was observed to have a multiplicity of CNS activities. The presence or absence of central activity seems to depend upon the nature of the nitrogen substituent; certain nitrogen substituents, especially cyclopropyl, allow pseudo-catechol derivatives which are normally peripheral agents devoid of central properties to elicit a variety of central activities.



soterenol, R_1 = OH; R_2 = $CH(CH_3)_2$
40, R_1 = H; R_2 = c-C_3H_5
43, R_1 = OH; R_2 = $C(CH_3)_2CH_2C_6H_5$
44, R_1 = OH; R_2 = c-C_3H_5

Compounds **40**, **43**, and **44** (all β agonists) are potent anorexiants ("free-feeding" method,[2] 5.5, 11, and 22 times *d*-amphetamine, respectively), and phenylethanolamines **43** and **44** are also active as narcotic antagonists (ED_{50} = 18.8 and 0.275 mg/kg, respectively), thus indicating CNS involvement. The concept of central β-adrenergic involvement is suggested by the work of Leibowitz[3] who has shown that β-"satiety" receptors may exist in the hypothalamus and play a role in regulating food intake. Furthermore, the existence of β-adrenergic receptors has been demonstrated in the rat hypothalamus[4] and cerebral cortex.[5] These receptors were sensitive to both norepinephrine and isoproterenol, causing significant increases in cyclic adenosine 3',5'-monophosphate (cAMP). As **43** is not a CNS stimulant, but is a potent anorexiant, and as **40** and **44** do not conform to normal amphetamine structure–activity relationships (SAR),[6] these compounds are not amphetamine-like. Instead, their anorexiant activity may be mediated via β-adrenergic receptors.

These observations prompted us to study a variety of related compounds in order to determine what structural parameters are necessary for central nervous system (CNS)