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R_m Values of Phenols. Their Relationship with Log P Values and Activity

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The experimental R_m values for a series of phenols were obtained by a reversed-phase TLC system. The extrapolation from a range of linear relationship between experimental R_m values and acetone concentration provided a set of extrapolated R_m values. This were used for studying the relationship between structure and activity in vitro and in vivo. The possibility to obtain by means of the extrapolation technique the R_m values in a standard system for several series of chemotherapeutic agents is pointed out.

The usefulness of the R_m values in studying the correlation of chemical structure with biological activity was shown with bis(dichloroacetamides) and vitamin K analogs,[1] penicillins and cephalosporins,[2] testosterone esters,[3] and sulfonamides.[4] The chromatographic R_m values, as an expression of the lipophilic character of molecules, were shown to be correlated with the Hansch π values in series of testosterone esters[3] and sulfonamides.[4]

In extending the use of a chromatographic technique for the determination of R_m values, we turned our attention in this paper to a series of phenols. In particular the purpose of the present work was to show that the present chromatographic technique can provide the R_m values of phenols in a standard system, where they can be compared with other series of chemotherapeutic agents. Another aspect of this paper was to further point out the possibility of relationships between partition data in different systems. Finally some experiments were carried out in order to give an additional contribution to the study of structure–activity relationships of phenols.

Experimental Section

Materials and Methods. The phenols reported in Table I were obtained from commercial sources. The experimental data of the present work were the result of at least 4–8 determinations.

R_m Values Determination. The chromatographic technique for the determination of the R_m values as an expression of the lipophilic character of molecules has previously been described.[5,6] The polar mobile phase was represented by veronal acetate buffer at pH 7.4 in various mixtures (v/v) with Me_2CO. The stationary nonpolar phase consisted of a silica gel G layer impregnated with silicone DC 200 (350 cSt) from Applied Sciences Laboratories. The concentration of Me_2CO in the mobile phase ranged from 5 to 55%. The developed plates were dried and sprayed with tetrazotized benzidine.[7] After a few minutes at 105° yellowish spots appeared on a white background. The R_m values were calculated by means of the formula

$$R_m = \log (1/R_f - 1)$$

Antibacterial Activity Determination. The phenolic compounds were assayed against *Staphylococcus aureus* 16 R by means of the turbidimetric method. Test tubes containing 4 ml of brain heart (Difco) liquid medium were added with 0.1 ml of EtOH solutions of phenols in order to obtain various serial dilutions. Finally 0.1 ml of an overnight culture of *Staph. aureus* was added to each tube. Minimal inhibitory concentrations (MIC) were determined after an 18-hr incubation at 37°. In Table IV the MIC's are expressed as log $1/C$ values where C is the average molar concentration which prevents the growth of microorganisms.

Hemolytic Activity Determination. The details of the procedure have already been described.[3] A volume of 3.8 ml of phosphate-buffered saline was added to 0.2 ml of a rat erythrocytes suspension. This was obtained by suspending the erythrocytes, separated from 0.8 ml of rat blood, in phosphate-buffered saline to a volume of 8 ml. EtOH solutions of the test compounds were finally added to the system in 1- to 10-μl amounts. Control and test suspensions were incubated for 3 hr at 37°. After incubation all suspensions were centrifuged and the optical densities of supernatants measured at 540 mμ in the Bausch and Lomb colorimeter. The results were expressed as percent of total hemolysis provoked by distilled H_2O by means of the formula

$$\frac{(\text{OD of sample} - \text{OD of EtOH control}) \times 100}{\text{OD of distilled } H_2O \text{ control}}$$

The linear relationship between the concentration of phenolic compounds and the percent of total hemolysis allowed calculation of the molar concentration of each compound provoking a 50% hemolysis. This is expressed in Table IV as log $1/C$. A "t" test showed the statistical significance of the above linear relationship for each compound.

LD_{50} Determination in Mice. Albino mice, weighing approximately 20–25 g each, were used. Five animals were injected at each dose level. Dimethyl sulfoxide (Me_2SO) solutions of test compounds were administered intraperitoneally. All mice were observed over a period of 24 hr for their death rate. The LD_{50} values were calculated by means of the Spearman and Kärber method.[8] In Table IV are reported the log $1/C$ values where C is the LD_{50} expressed as M/kg. Some preliminary experiments were carried out in order to rule out the possibility of toxic effects of Me_2SO.

Results

R_m and Log P Values. The early results of the chromatographic work had shown that the test compounds did not move from the starting line when the mobile phase was represented only by veronal acetate buffer. The addition of acetone was necessary in order to obtain longer migrations and therefore more reliable R_m values. The plots of the R_m values vs. the composition of the mobile phase showed that for each compound there was a range of linear relationship between R_m values and acetone concentration. The straight lines of Figure 1, where the data of only a few compounds

Table I. R_m and Log P Values of Phenols[a]

No.	Substituent	pK_a	Log $K_{a^*}[H^+]/[H^+]$	Extrapolated R_m values	R_m values at various acetone concentrations									Log P
					5	10	15	20	25	30	35	40	45	
1	H	9.95	0.001	0.230	−0.03	−0.06	−0.10	−0.09	−0.15	−0.23	−0.35	−0.48	−0.51	1.46
2	4-Cl	8.48	0.035	1.045	0.63	0.55	0.60	0.49	0.38	0.24	0.03	−0.12	−0.22	2.39
3	2-Cl	7.85	0.132	1.194	0.87	0.88	0.74	0.56	0.48	0.40	−0.01	−0.15		2.15
4	2,4-Cl_2	7.75	0.151	1.522	1.03	0.98	0.96	0.89	0.75	0.60	0.33	0.17	0.02	3.08
5	2-Br	8.42	0.039	1.086	0.53	0.54	0.50	0.50	0.32	0.20	−0.10	−0.20	−0.24	2.35
6	2-C_2H_5	10.20	0.000	1.077	0.74	0.66	0.64	0.52	0.38	0.28	0.10	−0.07	−0.19	2.46
7	4-C_2H_5	10.00	0.001	1.062	0.86	0.68	0.61	0.48	0.35	0.18	0.13	−0.17	−0.24	2.46
8	4-NO_2	7.14	0.450	0.235	0.13	0.07	0.01	0.02	−0.05	−0.12	−0.11	−0.17	−0.24	1.96
9	4-I	9.31	0.005	1.503	0.91	0.90	0.88	0.76	0.63	0.42	0.05	−0.07	−0.16	2.91
10	2,4-Me_2	10.60	0.000	0.995	0.73	0.67	0.57	0.44	0.32	0.10	0.12	−0.20	−0.26	2.44
11	3,5-Me_2	10.19	0.000	0.988	0.65	0.56	0.53	0.32	0.33	0.02	0.12	−0.29	−0.26	2.58
12	4-Ph	9.51	0.003	1.768	1.38	1.40	1.16	0.93	0.80	0.47	0.45	−0.04	−0.02	3.46
13	2-F	8.81	0.016	0.316	−0.07	−0.06	−0.13	−0.11	−0.12	−0.26	−0.26	−0.42	−0.49	1.71
14	4-F	9.34	0.005	0.483	0.20	0.15	0.15	0.08	0.08	−0.07	−0.13	−0.34	−0.42	1.77
15	2-Me	10.28	0.000	0.680	0.37	0.27	0.29	0.20	0.20	0.02	−0.12	−0.28	−0.38	1.96
16	2-Br, 4-Me			0.907	0.70	0.50	0.56	0.41	0.27	0.13	0.07	−0.10	−0.24	2.83
17	4-*n*-Pr			1.564	1.14	1.01	1.00	0.84	0.70	0.50	0.34	0.04	−0.03	2.96
18	4-*t*-Bu			1.721	1.34	1.27	1.14	0.96	0.88	0.64	0.46	0.15	0.05	3.14
19	2-*t*-Bu			2.000	1.41	1.37	1.36	1.27	1.09	0.95	0.69	0.39	0.27	3.14
20	2-*s*-Bu			1.932	1.44	1.39	1.29	1.18	0.99	0.84	0.58	0.28	0.18	3.26
21	4-*s*-Bu			2.035	1.35	1.38	1.36	1.19	1.00	0.82	0.49	0.23	0.13	3.28
22	2-*t*-Bu, 4-Me			2.111	1.48	1.35	1.45	1.35	1.28	1.04	0.77	0.55	0.36	3.62
23	4-*t*-Bu, 2-Me			2.181	1.48	1.38	1.41	1.34	1.19	0.97	0.55	0.32	0.25	3.64
24	4-Br	9.34	0.005	1.215	0.69	0.74	0.67	0.60	0.45	0.28	0.12	−0.12	−0.25	2.54
25	2-Cl, 4-NO_2	5.45	1.954	1.620	1.51	1.49	1.44	1.38	1.24	1.20	1.01	0.93	0.94	2.65
26	3-OH	9.44	0.004	−0.484	−0.47	−0.58	−0.61	−0.74	−0.66	−1.00	−0.69	−1.30	−0.86	0.80
27	4-OCH_3	10.20	0.000	0.065	0.02	−0.15	−0.15	−0.33	−0.25	−0.59	−0.37	−0.89	−0.66	1.34
28	2-NO_2	7.23	0.394	0.334	0.13	−0.10	0.05	0.04	−0.03	−0.06	−0.08	−0.12	−0.24	1.79

[a]Both extrapolated and experimental R_m values were corrected for ionization.

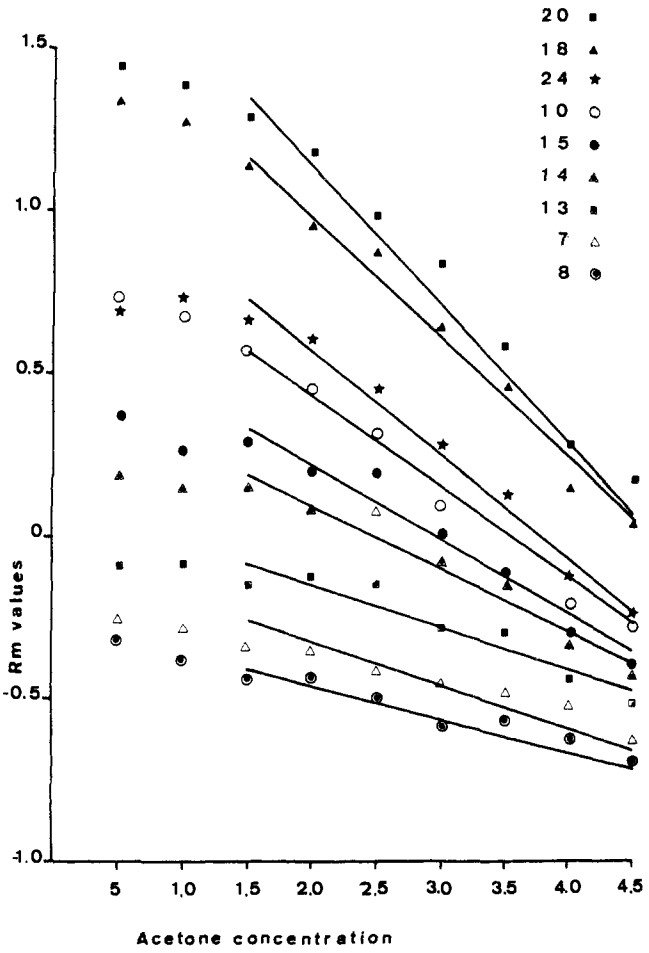

Figure 1. Linear relationship between uncorrected R_m values of phenols and acetone concentration in the mobile phase. The extrapolated R_m values of Table I were first calculated from the equations obtained with the uncorrected data in the range between 15 and 45% and then corrected for ionization.

are reported, were calculated by means of regression equations with the R_m values in the linearity range between 15 and 45% acetone concentration. A similar linear relationship had been previously obtained for penicillins and cephalosporins[5,6] and testosterone esters.[3] In particular, in the case of penicillins and cephalosporins, since not all the compounds could migrate without the addition of acetone to the mobile phase, the equations of the straight lines were used in order to calculate a theoretical R_m value corresponding to a 0% acetone concentration in the mobile phase. As already pointed out, the phenols did not migrate with only the veronal acetate buffer. Therefore, the same procedure described for penicillins and cephalosporins was adopted with the present data. The R_m values extrapolated to the system silicone oil–veronal acetate buffer are reported in Table I. On the other hand, in a previous work it was shown that the chromatography system silicone oil–veronal acetate buffer, i.e., without any addition of acetone to the mobile phase, could provide the R_m values of a series of sulfonamides.[4] In conclusion, either directly or by extrapolation the R_m values of penicillins, cephalosporins, sulfonamides, and phenols have been so far determined in the same chromatographic system and therefore they could be compared.

With acids, such as sulfonamides and phenols, the R_m values, in order to be an expression of the true partition coefficient, have to be corrected according to their degree of ionization at the pH 7.4 of the mobile phase. In Table I are reported the data necessary for the calculation of the $[R_m + \log (K_a + H^+)/(H^+)]$ values. In the case of compounds 16–23 the pK_a values were not available in the literature and therefore it was not possible to obtain the corrected R_m values. Their pK_a values were assumed to be close to those of the phenolic compounds with alkyl groups listed in Table I, i.e., high enough to make the log $(K_a + (H^+))/(H^+)$ term very small. All this could be supported also by the low σ values of compounds 16–23.[9] However, for compound 16 a pK_a similar to that for compound 5 would be expected. In this case it would require a significant correction as the log $(K_a + (H^+))/(H^+)$ term for compound 5 is 0.039. In the regression analysis of the present data the uncorrected R_m values of compounds 16–23 were used. The log P values in octanol–water were taken or calculated from the data in the literature.[9-11]

It is interesting to note that the range of the extrapolated R_m values (from −0.484 to 2.18) is practically as wide as that of the log P values (from 0.80 to 3.64) in the octanol–water system. It would be very interesting to show that, with series of drugs more widely differing in their lipophilic character, the extrapolation technique allows the obtaining of ranges of R_m values comparable to those of the log P values.

The relationship between log P and R_m values is described by eq 1 and 2, the latter of which was calculated

		n	r	s	
$\log P_{oct}$	$= 1.392\ (\pm 0.145) + 0.993\ (\pm 0.111)\ R_m$	28	0.960	0.202	(1)
$\log P_{oct}$	$= 1.357\ (\pm 0.149) + 1.013\ (\pm 0.147)\ R_m$	20	0.953	0.192	(2)

without taking into consideration compounds 16–23.

Equations 1 and 2 are very similar and this should support the assumption made for the pK_a values of compounds 16–23.

The slopes of eq 1 and 2 are close to unity. According to Hansch[12,13] this could mean that the stationary phase represented by 5% silicone oil contains the same amount of water as octanol. In a previous work in correlating the R_m values of sulfonamides with the π values determined in an isobutyl alcohol–water system somewhat lower slopes were found. Again this could be in agreement with the Hansch conclusion that with solvents containing much more water than octanol (i.e., isobutyl alcohol) there is a slope of less than unity.[12,13] Similarly, a slope of less than unity can be found in correlating the R_m values of penicillins, as previously obtained in this laboratory,[2] and their log P values determined by Scholtan[14] in an isobutyl alcohol–aqueous buffer system (Table II).

		n	r	s	
$\log P_{i\text{-Bu}}$	$= -0.336 + 0.808\ R_m$	7	0.959	0.130	(3)

It should be pointed out that in calculating the regression equations of the straight lines of Figure 1 the experimental data at 5 and 10% acetone in the mobile phase were not used. This was due to the fact that at these concentrations several compounds showed very short migrations and therefore R_m values out of the range of maximum accuracy. Moreover, by considering only the data between 15 and 45% it was possible to obtain straight lines not very far from parallelism. In fact, the extrapolation from higher acetone concentrations should be considered with confidence only in the case of parallelism among straight lines.

In order to establish the advantages, if any, of the extrapolation technique the experimental R_m values, obtained with increasing acetone concentrations in the mobile phase and corrected for ionization at pH 7.4, were used for calculating eq 4–12. The extrapolation to 0% acetone in the

	n	r	s	
$\log P_{oct} = 1.593\ (\pm 0.158) + 1.207\ (\pm 0.170)\ R_{m\,(5\%\ acetone)}$	28	0.940	0.192	(4)
$\log P_{oct} = 1.710\ (\pm 0.152) + 1.157\ (\pm 0.170)\ R_{m\,(10\%\ acetone)}$	28	0.930	0.250	(5)
$\log P_{oct} = 1.717\ (\pm 0.146) + 1.201\ (\pm 0.170)\ R_{m\,(15\%\ acetone)}$	28	0.941	0.246	(6)
$\log P_{oct} = 1.827\ (\pm 0.139) + 1.228\ (\pm 0.182)\ R_{m\,(20\%\ acetone)}$	28	0.931	0.264	(7)
$\log P_{oct} = 1.883\ (\pm 0.141) + 1.356\ (\pm 0.213)\ R_{m\,(25\%\ acetone)}$	28	0.925	0.277	(8)
$\log P_{oct} = 2.143\ (\pm 0.145) + 1.273\ (\pm 0.259)\ R_{m\,(30\%\ acetone)}$	28	0.888	0.336	(9)
$\log P_{oct} = 2.258\ (\pm 0.133) + 1.653\ (\pm 0.337)\ R_{m\,(35\%\ acetone)}$	28	0.870	0.325	(10)
$\log P_{oct} = 2.626\ (\pm 0.166) + 1.390\ (\pm 0.398)\ R_{m\,(40\%\ acetone)}$	28	0.807	0.430	(11)
$\log P_{oct} = 2.734\ (\pm 0.201) + 1.588\ (\pm 0.539)\ R_{m\,(45\%\ acetone)}$	28	0.766	0.475	(12)

Table II. Log P and R_m Values of Penicillins

Compounds	Log $P_{i\text{-}Bu}$	R_m
Dicloxacillin	1.057	1.63
Cloxacillin	0.752	1.34
Oxacillin	0.588	1.05
Phenethicillin	0.455	1.03
Benzylpenicillin	0.196	0.55
Phenoxymethylpenicillin	0.130	0.89
Ampicillin	−0.229	0.08

mobile phase seems to provide R_m values better correlated with log P values.

The decreasing of the correlation coefficient from eq 4 to eq 12 is due to the fact that at higher acetone concentration all the compounds tend to migrate with the solvent front and therefore have closer R_m values. Moreover, higher acetone concentrations will alter the water structure and therefore the character of the partitioning process. Obviously this could decrease the correctness of the extrapolated R_m values. The correlation coefficient of eq 1 and 2 could support the usefulness of the extrapolation technique. However, the major point would be in the fair parallelism of the straight lines in Figure 1.

The present extrapolated R_m values can also be compared with other chromatographic data. Marcinkiewicz et al.[15,16] had determined the R_m values of many phenols in a chromatographic system where the stationary phase was represented by ethyl oleate and the mobile phase by 25% aqueous ethanol (Table III).

The correlation coefficient provided by eq 13 shows that

	n	r	s	
$R_m = 1.332\ (\pm 0.087) + 1.004\ (\pm 0.150)\ R_{m(Et\ oleate)}$	16	0.961	0.141	(13)

there is a highly significant correlation between the R_m values obtained in the present work and those determined in the ethyl oleate–25% aqueous ethanol system. In particular, the b's and intercepts of eq 1 and 13 are very close. It can be pointed out that the present chromatographic system allows the obtaining of separate R_m values even in the case of halogen-substituted phenols in ortho and para positions. On the other hand, the relationship between π values and ΔR_m values of phenols was also shown by Iwasa et al.,[17] who took advantage of the R_m values obtained in a chromatographic system based on Trigol and diisopropyl ether.[13] By means of the data of Tables I and II, eq 14 was calculated.

	n	r	s	
$R_m = 0.070\ (\pm 0.433) - 1.239\ (\pm 0.813)\ R_{m\,(Trigol)}$	6	0.902	0.225	(14)

Table III. R_m Values of Phenols in Different Chromatographic Systems

Phenols	Ethyl oleate–25% aq EtOH	Trigol–diisopropyl ether
H	−1.063	−0.420
4-Cl	−0.471	−0.585
2-Cl	−0.471	
2-Br	−0.070	
4-Br	−0.070	
4-C_2H_5	−0.376	
4-NO_2		−0.041
4-I	0.204	
2,4-Me_2	−0.250	
3,5-Me_2	−0.444	−0.747
4-Ph	0.556	
2-F	−0.906	
4-F	−0.906	
2-Me	−0.547	
4-*n*-Pr	0.087	
4-*t*-Bu	0.395	
4-OCH_3	−1.070	0.000
4-C_2H_5		−0.796

Even if calculated with only six compounds eq 14 shows a similar relationship between the present R_m values and those of Marcinkiewicz et al.[16] from the Trigol–diisopropyl ether system.

The negative slope of eq 14 is due to the nature of the phases in the Trigol–diisopropyl ether system, where the mobile phase seems to be less hydrophilic than the stationary one. Therefore the most lipophilic compounds tend to migrate with the mobile phase and have lower R_m values. In fact, 4-methoxyphenol, which is the most lipophilic compound in the Trigol–diisopropyl ether system, shows the lowest R_m value in the ethyl oleate–25% aqueous ethanol system.

Structure–Activity Relationships. The structure–activity relationships regarding the antibacterial activity in *Staph. aureus*, the acute toxicity in mice, and the hemolytic activity are described by eq 15–20. In calculating these equations the extrapolated R_m values of Table I were used. However, because of the good correlation coefficients of eq 4–7 one could have used any of the sets of experimental R_m values obtained with 5–20% acetone concentration in the mobile phase.

The introduction of the R_m^2 term into eq 15, 17, and 19 does not improve the correlation coefficient in a significant way.

The log $1/C$ values calculated from eq 15, 17, and 19 are reported in Table IV. The deviation of about $2s$ for com-

Table IV. Data on the Biological Activity of Phenols[a]

No.	Substituent	Hemolytic act. Log 1/C obsd	Hemolytic act. Log 1/C calcd	Acute toxicity in mice Log 1/C obsd	Acute toxicity in mice Log 1/C calcd	Antibacterial act. Log 1/C obsd	Antibacterial act. Log 1/C calcd
1	H	1.236	1.650	2.407	2.439	1.744	2.046
2	4-Cl	2.523	2.487	2.588	2.829	2.699	2.672
3	2-Cl	2.221	2.640	2.738	2.900	2.699	2.786
4	2,4-Cl_2	3.000	2.977	3.028	3.057	3.301	3.038
5	2-Br	2.523	2.529	2.689	2.849	2.522	2.704
6	2-C_2H_5	2.523	2.520	2.851	2.844	2.824	2.697
7	4-C_2H_5	2.523	2.504	2.946	2.837	2.921	2.685
8	4-NO_2	1.921	1.655	2.600	2.442	2.319	2.050
9	4-I	3.000	2.957	3.149	3.048	3.155	3.024
10	2,4-Me_2	2.699	2.435	2.824	2.805	2.522	2.634
11	3,5-Me_2	2.699	2.428	2.894	2.802	2.508	2.628
12	4-Ph	3.301	3.229	3.100	3.175	3.046	3.227
13	2-F	1.886	1.738	2.320	2.481	2.097	2.112
14	4-F	2.221	1.910	2.556	2.560	2.097	2.240
15	2-Me	2.000	2.112	2.638	2.655	2.097	2.392
16	2-Br, 4-Me	2.699	2.345	2.983	2.763	2.678	2.566
17	4-*n*-Pr	2.824	3.020	3.228	3.077	3.046	3.071
18	4-*t*-Bu	3.097	3.181	3.282	3.152	3.187	3.191
19	2-*t*-Bu	3.398	3.468	3.262	3.286	3.398	3.406
20	2-*s*-Bu	3.301	3.398	3.374	3.253	3.398	3.353
21	4-*s*-Bu	3.398	3.503	3.354	3.302	3.522	3.432
22	2-*t*-Bu, 4-Me	3.699	3.581	3.057	3.339	3.522	3.491
23	4-*t*-Bu, 2-Me	3.523	3.653	3.307	3.372	3.398	3.545
24	4-Br	2.824	2.661	2.790	2.910	2.824	2.803
25	2-Cl, 4-NO_2			3.338	3.104		
26	3-OH	0.592	0.917	2.100	2.099	1.521	1.499
27	4-OCH_3			2.267	2.361		
28	2-NO_2	1.618	1.757	2.568	2.489	2.398	2.126

[a] The hemolytic and antibacterial activity of compounds 25 and 27 was not determined.

Hemolysis of rat erythrocytes

	n	r	s	
$\log 1/C = 1.414\ (\pm 0.146) + 1.027\ (\pm 0.111)\ R_m$	26	0.959	0.185	(15)
$\log 1/C = 1.329\ (\pm 0.086) + 1.323\ (\pm 1.326)\ R_m - 0.144\ (\pm 0.148)\ R_m^2$	26	0.965	0.197	(16)

Antibacterial activity in *Staph. aureus*

	n	r	s	
$\log 1/C = 1.870\ (\pm 0.058) + 0.768\ (\pm 0.090)\ R_m$	26	0.956	0.150	(17)
$\log 1/C = 1.873\ (\pm 0.263) + 0.763\ (\pm 0.283)\ R_m + 0.002\ (\pm 0.128)\ R_m^2$	26	0.955	0.170	(18)

Acute toxicity in mice

	n	r	s	
$\log 1/C = 2.330\ (\pm 0.083) + 0.478\ (\pm 0.063)\ R_m$	28	0.928	0.112	(19)
$\log 1/C = 2.318\ (\pm 0.190) + 0.524\ (\pm 0.213)\ R_m - 0.023\ (\pm 0.098)\ R_m^2$	28	0.929	0.134	(20)

pound 16 in eq 15 could be due to the fact that its R_m value was not corrected for ionization.

Hansch et al.[18] for 15 equations correlating the hemolytic activity of a variety of compounds found a mean slope of 0.93 ± 0.17, which is quite close to that of eq 15 (1.03 ± 0.11). In particular the slope of essentially unity in eq 15 shows that the partitioning in the present chromatographic system is closely related to the absorption of phenols by the membrane of the red blood cells, i.e., partitioning between liquid medium and membranes. The best rationalization of the antibacterial activity in *Staph. aureus* is provided by eq 17 which is characterized by a lower value of the slope. In fact, its slope (0.77 ± 0.09) is quite close to that reported for the antibacterial activity of phenols in *M. tuberculosis* (0.78 ± 0.06)[12] or more generally for the toxicity of various antibacterial compounds to gram-positive bacteria (0.73 ± 0.17).[18] The interaction with microorganisms seems to depend on R_m values in a different way. The reason might be in the lower lipid content of a gram-positive organism such as *Staph. aureus*. The partitioning in a different chromatographic system could be a better model of the interaction with microorganisms. However, here the importance of the lipophilic character for the antibacterial activity of phenols is confirmed. This is in agreement with Kaye et al.[19] who have suggested that the antibacterial action of phenols involves disorganization of phospholipid molecules present in bacterial cytoplasmatic membranes.

The relationship between R_m values of phenols and their toxicity to mice is described by eq 19. The slope of 0.48 ± 0.06 is close to that of 0.4 ± 0.1 reported by Hansch[12] as typical of systems less sensitive to drug perturbation. In more complex systems, such as a whole animal, the penetration of drugs to their sites of action results from their movement through various membranes and liquid phases. One of the major factors in regulating this random walk

could be the hydrophobic binding to serum constituents. Hansch[12] found similar slopes in correlating the log P values of penicillin derivatives with their in vivo activity against *Staph. aureus* in mice (−0.46 ± 0.10) and when relating log P values and hypnotic activity in mice of alkylarylureas (0.55 ± 0.09) and 5,5-alkylbarbiturates (0.57 ± 0.21).

Lien et al.,[20] when studying the acute lethal toxicity in mice of N-substituted lactams and *O*-phenyleneureas, also found a rather low slope (0.312).

Conclusion

The present data seem to confirm the usefulness of the R_m values as an expression of the lipophilic character of molecules. In particluar the extrapolation procedure could provide further interesting contributions. In this way the R_m values of several series of chemotherapeutic agents could be compared in the same chromatographic system. The highly significant correlation between R_m and log P values further points out the possibility of relationships between partition data in different systems. The slope of 0.993 in eq 1 indicates the existence of very similar lipophilic characteristics in both systems. Obviously the correctness of the extrapolated R_m values seems to depend on the parallelism of the straight lines extrapolating from the linear range of experimental data. In particular it could be important to point out that the extrapolation technique so far provided R_m values for several series of chemotherapeutic agents in the same standard system. Moreover, when the π or log P values were available equally good correlations were found with the extrapolated R_m values. Finally the results of the structure–activity relationships seem to support the possibility of a classification of drugs according to their mechanism of action at the physicochemical level. The assay of the same series of drugs in different biological systems, in vitro and in vivo, should be carried out intensively in order to get new achievements in this field.

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R_m Values of Steroids as an Expression of Their Lipophilic Character in Structure–Activity Studies

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The chromatographic R_m values of three series of steroids were determined by means of a reversed-phase system. The R_m values at 45% acetone in the mobile phase were shown to be correlated with the partition coefficients in an ether–water system. However, an almost equally good correlation was found when using extrapolated R_m values. The extrapolation technique could provide a standard system. The relationship between biological data and R_m values pointed out the important role of the lipophilic character in regulating the activity of steroids. In particular, the dependence of protein binding absorption and biotransformation on lipophilic character might strongly influence the availability of steroids at the site of action.

The R_m values of testosterone derivatives and corticosteroids had been shown to be related to the Hansch π values and therefore useful in structure–activity studies.[1-4] In series of phenols,[5] penicillins and cephalosporins,[6,7] and steroids[3] the linear relationship between R_m values and acetone concentration in the mobile phase has provided the R_m values extrapolated to 0% acetone in the mobile phase. This was considered to be a standard system, where all the compounds could be compared. The purpose of the present work was to study a larger number of steroids in order to compare their lipophilic character. The possibility of the extrapolation in order to calculate the R_m values at 0% acetone in the mobile phase was taken into consideration. The partition coefficients obtained by Flynn[8] in an ether–water system were compared with the present R_m values. Finally, some data on the interaction of steroids with the erythrocyte membrane and proteins could further point out the usefulness of R_m values in structure–activity studies.