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R_M VALUES OF NAPHTHOLS AND ACETOPHENONES IN STRUCTURE–ACTIVITY STUDIES

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SUMMARY

The R_M values of naphthols obtained in a chromatographic system where the stationary phase consisted of a silica gel G layer impregnated with silicone oil are much more closely related to the log P values in an octanol–water system than the R_M values determined on polyamide layers. Similarly, the R_M values of a series of acetophenones in the silicone system are closely related to their log P values. The equations describing the structure–activity relationship indicate the importance of lipophilic character and halogen substitution in determining the hemolytic activity and the acute toxicity of compounds.

INTRODUCTION

The usefulness of the R_M values as an expression of the lipophilic character of molecules in quantitative structure–activity relationship (QSAR) studies was shown for several series of drugs such as penicillins and cephalosporins[1], testosterone esters[2], phenols[3], steroids[4] and sulphonamides[5]. We now turn our attention to the relationship between the log P values of naphthols and acetophenones and their R_M values. In particular, one aim of the present study was to examine the relationship between structure and biological activity in two series of naphthols and acetophenones.

MATERIALS AND METHODS

Determination of R_M values

The reversed-phase chromatographic technique for the determination of R_M values has been described previously[6,7]. The polar mobile phase was veronal acetate buffer at pH 7.4 in various mixtures with acetone. The stationary non-polar phase consisted of a silica gel G layer impregnated with silicone DC 200 (350 cSt)

$R_F \times 100$ VALUES AND THEIR STANDARD DEVIATIONS FOR NAPHTHOLS AT INCREASING ACETONE CONCENTRATIONS IN THE SILICONE (A) AND POLYAMIDE (B) SYSTEM

Each $R_F \times 100$ value represents the mean of eight determinations.

No.	*Compound*	*System*	*Acetone concentration (%)*							
			25	*30*	*35*	*40*	*45*	*50*	*55*	*60*
1	1,2,3,4-Tetrahydro-1-naphthol	A	43.8 ± 9.8	50.6 ± 6.1	55.5 ± 4.9	65.8 ± 4.9	69.2 ± 6.1	74.5 ± 6.1	79.1 ± 6.1	86.6 ± 6.1
		B	46.6 ± 4.9	48.5 ± 6.1	60.3 ± 4.9	67.4 ± 2.5	72.9 ± 2.5	82.0 ± 1.2	84.1 ± 2.5	91.0 ± 1.2
2	8-Amino-2-naphthol	A	46.3 ± 7.3	55.0 ± 6.1	59.5 ± 8.6	70.8 ± 7.3	76.0 ± 7.3	80.5 ± 6.1	86.6 ± 4.9	89.4 ± 3.7
		B	21.7 ± 1.2	27.8 ± 1.2	37.2 ± 3.7	45.4 ± 4.9	53.4 ± 2.5	66.1 ± 3.7	71.8 ± 3.7	80.1 ± 1.2
3	2-Nitroso-1-naphthol	A		60.9 ± 11.0	75.2 ± 3.7	77.6 ± 8.6	82.5 ± 3.7	85.6 ± 3.7		
		B	24.3 ± 2.5	30.0 ± 3.7	36.6 ± 3.7	42.3 ± 3.7	51.6 ± 3.7	63.0 ± 2.5	69.3 ± 3.7	75.5 ± 3.7
4	2-Amino-3-naphthol	A	42.1 ± 6.1	49.9 ± 6.1	57.8 ± 8.6	67.1 ± 7.3	73.2 ± 7.3	78.3 ± 6.1	83.6 ± 7.3	89.0 ± 3.7
		B	14.6 ± 2.5	20.5 ± 1.2	29.4 ± 2.5	36.5 ± 4.9	46.1 ± 3.7	59.3 ± 3.7	67.3 ± 3.7	76.3 ± 1.2
5	2-Naphthol	A	27.5 ± 6.1	36.6 ± 6.1	46.8 ± 9.8	58.3 ± 4.9	65.2 ± 8.6	71.5 ± 7.3	80.6 ± 7.3	87.0 ± 4.9
		B	12.4 ± 2.5	21.4 ± 4.9	29.3 ± 3.7	39.0 ± 4.9	48.2 ± 3.7	62.6 ± 2.5	69.9 ± 3.7	79.5 ± 2.5
6	1-Naphthol	A	24.5 ± 6.1	32.6 ± 4.9	40.3 ± 7.3	53.6 ± 7.3	62.2 ± 11.0	70.2 ± 8.6	78.1 ± 7.3	85.7 ± 6.1
		B	10.9 ± 1.2	16.5 ± 2.5	26.2 ± 2.5	36.3 ± 4.9	46.5 ± 4.9	61.0 ± 2.5	68.6 ± 2.5	78.3 ± 2.5
7	5,6,7,8-Tetrahydro-2-naphthol	A	20.8 ± 4.9	29.0 ± 4.9	37.2 ± 6.1	49.2 ± 6.1	57.3 ± 9.8	64.3 ± 7.3	73.7 ± 7.3	81.9 ± 7.3
		B	18.0 ± 1.2	24.7 ± 2.5	33.6 ± 3.7	43.5 ± 4.9	52.6 ± 3.7	66.9 ± 1.2	72.5 ± 2.5	81.7 ± 3.7
8	5,6,7,8-Tetrahydro-1-naphthol	A	20.1 ± 4.9	24.9 ± 6.1	32.0 ± 7.3	47.2 ± 7.3	54.9 ± 9.8	63.1 ± 7.3	71.0 ± 6.1	81.8 ± 4.9
		B	17.4 ± 1.2	23.5 ± 2.5	32.5 ± 3.7	42.6 ± 2.5	52.2 ± 4.9	64.7 ± 2.5	71.3 ± 3.7	80.6 ± 3.7
9	2-Methyl-1-naphthol	A	17.1 ± 1.2	28.1 ± 3.7	33.4 ± 7.3	48.1 ± 15.9	55.8 ± 17.2	69.8 ± 7.3	–	–
10	2-Nitro-1-naphthol	A	55.0 ± 9.8	59.2 ± 12.2	74.0 ± 6.1	76.8 ± 11.0	85.0 ± 6.1	87.8 ± 4.9	84.4 ± 6.1	90.5 ± 7.3
		B	15.1 ± 1.2	20.1 ± 3.7	27.6 ± 4.9	28.5 ± 3.7	35.9 ± 3.7	48.1 ± 2.5	58.4 ± 4.9	66.3 ± 3.7
11	1-Bromo-2-naphthol	A	16.1 ± 6.1	21.5 ± 3.7	30.6 ± 6.1	45.2 ± 8.6	54.8 ± 11.0	64.1 ± 7.3	73.7 ± 8.6	84.3 ± 6.1
		B	5.8 ± 1.2	9.8 ± 1.2	16.7 ± 2.5	25.6 ± 1.2	36.1 ± 3.7	51.2 ± 1.2	61.9 ± 2.5	73.0 ± 3.7
12	1-Iodo-2-naphthol	A	11.7 ± 4.9	16.1 ± 2.5	26.5 ± 4.9	41.5 ± 7.3	51.4 ± 8.6	60.0 ± 7.3	72.0 ± 9.8	84.2 ± 6.1
		B	–	7.1 ± 1.2	13.7 ± 2.5	23.5 ± 3.7	37.4 ± 4.9	55.1 ± 2.5	65.7 ± 2.5	75.1 ± 6.1
13	4-Chloro-1-naphthol	A	9.7 ± 3.7	15.0 ± 2.5	22.2 ± 3.7	36.6 ± 3.7	44.3 ± 7.3	58.4 ± 9.8	67.2 ± 9.8	79.6 ± 6.1
		B	–	7.8 ± 0.0	13.6 ± 2.5	22.6 ± 2.5	33.5 ± 3.7	49.4 ± 2.5	61.6 ± 3.7	71.7 ± 2.5
14	6-Bromo-2-naphthol	A	9.8 ± 4.9	15.0 ± 3.7	22.0 ± 6.1	38.3 ± 4.9	47.3 ± 7.3	60.0 ± 9.8	66.3 ± 9.8	81.4 ± 6.1
		B	–	7.2 ± 1.2	13.5 ± 2.5	21.8 ± 2.5	34.4 ± 3.7	48.9 ± 1.2	61.2 ± 2.5	71.7 ± 3.7
15	1-(Pyridin-2-azo)-2-naphthol	A	8.2 ± 2.5	12.8 ± 3.7	20.4 ± 3.7	32.5 ± 8.6	42.8 ± 7.3	54.6 ± 6.1	63.2 ± 11.0	78.9 ± 6.1
		B	–	5.2 ± 1.2	11.5 ± 6.1	16.9 ± 4.9	24.8 ± 3.7	35.6 ± 3.7	48.6 ± 1.2	59.0 ± 2.5
16	1,6-Dibromo-2-naphthol	A	7.0 ± 2.5	10.0 ± 2.5	16.0 ± 2.5	27.2 ± 7.3	39.0 ± 6.1	51.1 ± 6.1	61.8 ± 8.6	77.8 ± 7.3
		B	–	–	7.0 ± 1.2	12.5 ± 2.5	21.5 ± 3.7	32.4 ± 4.9	47.8 ± 1.2	61.0 ± 3.7

from Applied Science Labs. (State College, Pa., U.S.A.). The concentration of acetone in the mobile phase ranged from 25 to 60%. In another chromatographic system, the mobile phase had the same composition, but the stationary phase was a polyamide layer, as already described[8].

Determination of pK_a values

The pK_a values of naphthols were determined by means of a spectrophotometric technique and are reported in Table II. Two wavelengths were chosen at which the dissociated (RO^-) and undissociated (ROH) forms have a different molar absorption. The compounds were assayed at the same concentration. The optical density for each solution at different pH values enabled the relative amounts of RO^- and ROH forms to be determined.

Hemolytic activity determination

The details of the procedure have already been described[2]. A 3.8-ml volume of phosphate-buffered saline was added to 0.2 ml of a rat erythrocytes suspension. Dimethyl sulphoxide solutions of the test compounds were added to the system in 1–10-μl amounts. Tables IV and V report the log ($1/C$) values where C is the molar concentration of each compound provoking a 50% hemolysis.

LD_{50} determination in mice

Albino mice, each weighing 20–25 g, were used. At each dose level eight animals were injected intraperitoneally with a dimethyl sulphoxide solution of the test compound. The results are given in Tables IV and V.

RESULTS

R_M and log P values

Naphthols. The chromatographic work showed that in the system silicone oil–veronal acetate buffer the test compounds did not migrate when the mobile phase was only veronal acetate buffer. The addition of acetone was necessary to obtain suitable R_F values. The $R_F \times 100$ values at increasing acetone concentrations in the mobile phase are reported in Table I. The R_M values calculated from the R_F values of Table I were plotted against the composition of the mobile phase. For each compound there is a range in which the relationship between R_M values and acetone concentration is linear. The equations of these straight lines were used to calculate a theoretical R_M value at 0% acetone concentration in the mobile phase.

The extrapolated R_M values were corrected for their degree of ionization at the pH (7.4) of the mobile phase and are reported in Table II. The log P values in octanol–water, also reported in Table II, were taken or calculated from the literature[9–11]. Although the range of the extrapolated R_M values (0.75–2.50) is somewhat narrower than that of log P values (1.61–4.56), there is a very good correlation between the two sets of values:

	n	r	s	
$\log P_{oct} = 0.928(\pm 0.506) + 1.442(\pm 0.307)R_M$	16	0.938	0.303	(1)
($F = 101.71$, $P < 0.005$)				

The slope of eqn. 1 is not very far from unity. According to Hansch[12] and

TABLE II

R_M AND log P VALUES OF NAPHTHOLS

The R_M values were corrected for ionization.

No.	Compound	pK_a	$\log \frac{K_a + [H^+]}{[H^+]}$	R_M		log P
				Silicone oil	Polyamide	
1	1,2,3,4-Tetrahydro--1-naphthol	—	—	0.75	0.89	2.15
2	8-Amino-2-naphthol	9.38	0.00	0.79	1.40	1.61
3	2-Nitroso-1-naphthol	7.40	0.30	0.85	1.54	2.86
4	2-Amino-3-naphthol	9.30	0.01	0.89	1.69	1.61
5	2-Naphthol	9.58	0.00	1.28	1.80	2.84
6	1-Naphthol	9.39	0.00	1.39	1.94	2.98
7	5,6,7,8-Tetrahydro--2-naphthol	10.39	0.00	1.42	1.60	3.10
8	5,6,7,8-Tetrahydro--1-naphthol	10.40	0.00	1.52	1.59	3.24
9	2 Methyl-1-naphthol	9.76	0.00	1.67	2.35*	3.48
10	2-Nitro-1-naphthol	6.15	1.23	1.72	2.95	3.31
11	1-Bromo-2-naphthol	8.00	0.10	1.86	2.46	3.70
12	1-Iodo-2-naphthol	8.41	0.04	2.06	2.73	3.96
13	4-Chloro-1-naphthol	8.81	0.02	2.08	2.56	3.69
14	6-Bromo-2-naphthol	9.38	0.00	2.08	2.59	3.70
15	1-(Pyridin-2-azo)-2--naphthol	10.00	0.00	2.16	2.55	4.15
16	1,6-Dibromo-2-naphthol	7.79	0.15	2.50	3.11	4.56

* Calculated by adding a ΔR_M of 0.41 for CH_3 to the experimental R_M value of 1-naphthol in the polyamide system. The above ΔR_M value was calculated from the experimental R_M values of sulphadiazine and isosulphamerazine in the same system[8].

Leo *et al.*[13] this could mean that the stationary phase (5% silicone oil) contains the same amount of water as octanol. In a previous paper[4] a slope of 1.459 was found when correlating extrapolated R_M and log K_p values (*i.e.*, partition coefficients in an ether–water system) for a series of 42 steroids.

The $R_F \times 100$ values in the polyamide system at increasing acetone concentrations in the mobile phase are reported in Table I. The linear relationship between the R_M values of naphthols and the acetone concentration allowed the calculation of extrapolated R_M values at 0% acetone concentration in the mobile phase (Table II). The relationship between the R_M values from the silicone and polyamide systems respectively was calculated using R_M values corrected for ionization (Table II):

$$R_{M_{sil\,0\%}} = -0.075(\pm 0.228) + 0.779(\pm 0.104) R_{M_{pol\,0\%}} \quad n = 15 \quad r = 0.901 \quad s = 0.255 \quad (2)$$
$$(F = 56.04,\ P < 0.005)$$

However, eqn. 3 calculated without any correction for ionization shows that such corrections had little effect:

$$R_{M_{sil\,0\%}} = -0.386(\pm 0.263) + 0.924(\pm 0.128) R_{M_{pol\,0\%}} \quad n = 15 \quad r = 0.894 \quad s = 0.291 \quad (3)$$
$$(F = 52.00,\ P < 0.005)$$

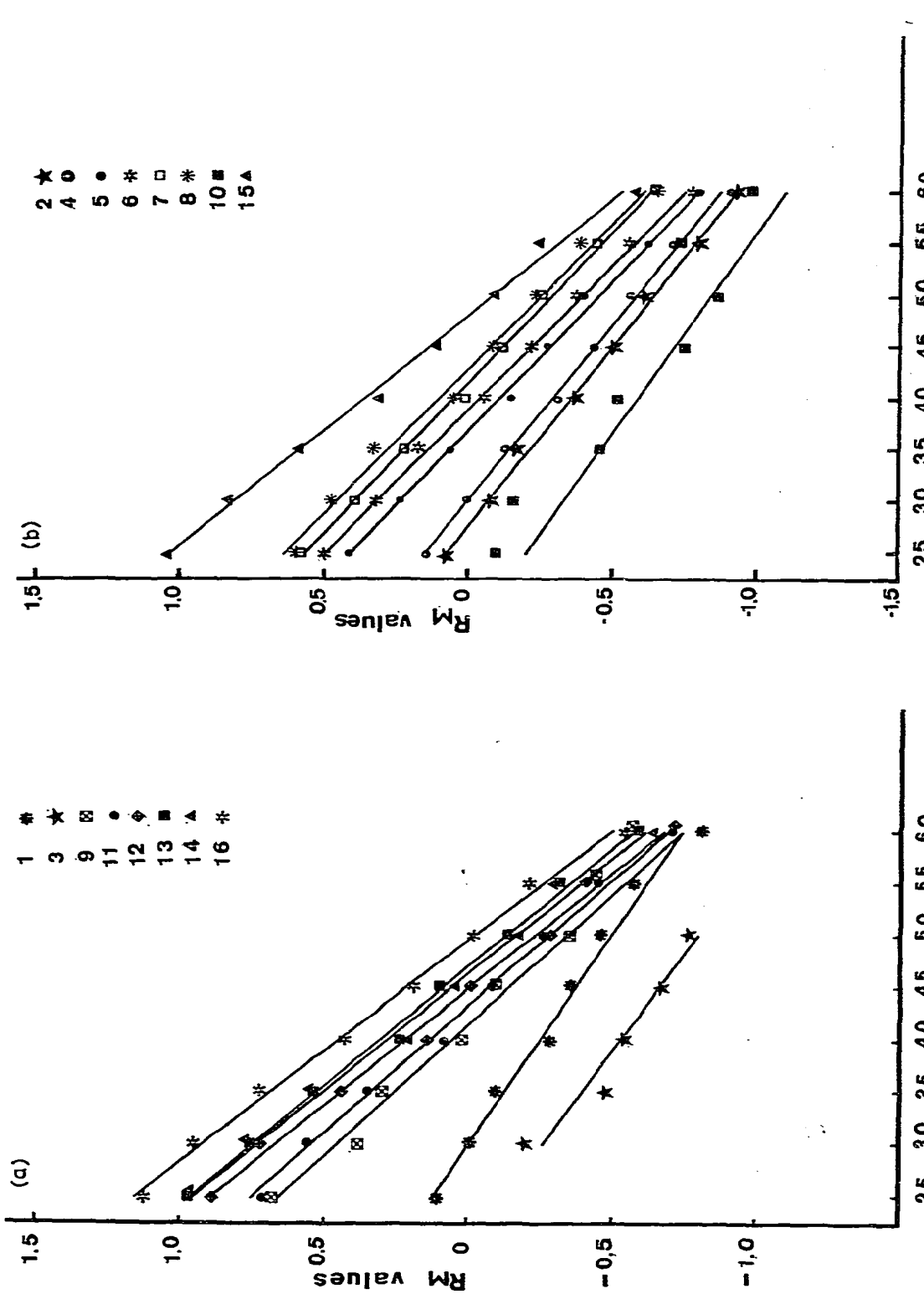
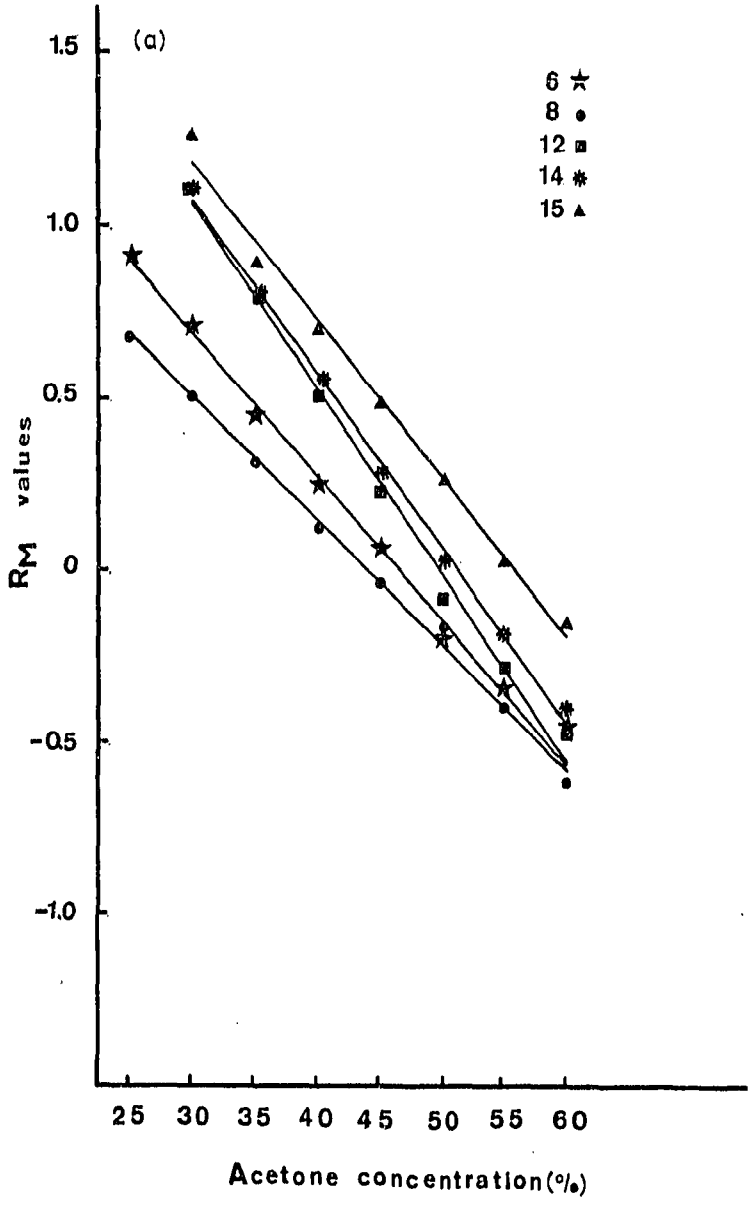
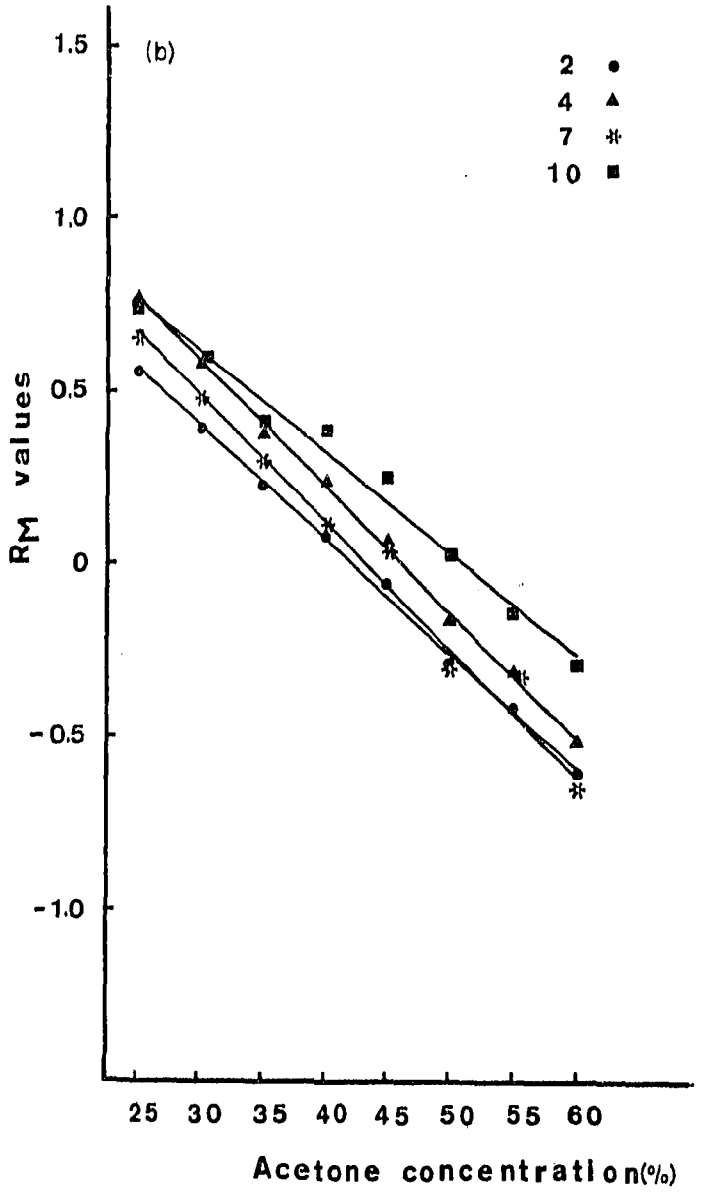


Fig. 1. Relationship between R_M values of naphthols and acetone concentration in the mobile phase. Stationary phase, silica gel G layer impregnated with silicone oil. The straight lines were calculated by means of the least-squares method. The compounds are numbered as in Table I.


(a)
6
8
12
14
15
1.5
1.0
0.5
0
-0.5
-1.0
R_M values
25 30 35 40 45 50 55 60
Acetone concentration(%)


(b)
2
4
7
10
1.5
1.0
0.5
0
-0.5
-1.0
R_M values
25 30 35 40 45 50 55 60
Acetone concentration(%)

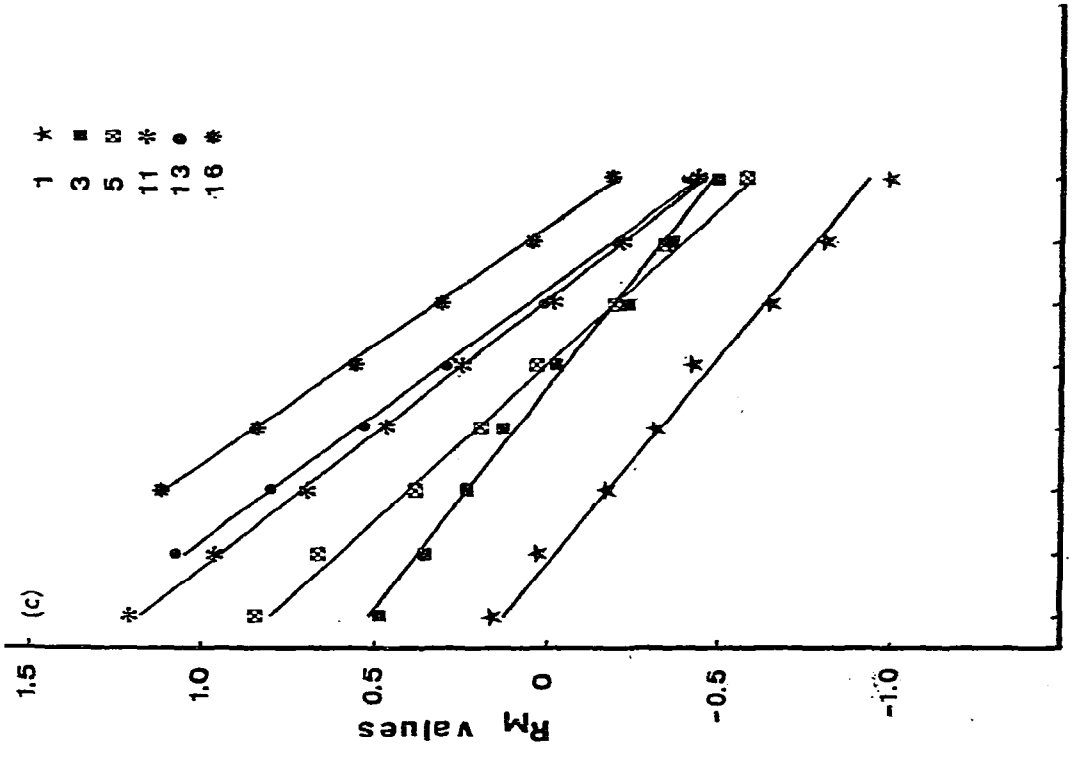


Fig. 2. Relationship between R_M values of naphthols and acetone concentration in the mobile phase with a polyamide layer as the stationary phase. The straight lines were calculated by means of the least-squares method. The compounds are numbered as in Table I.

Eqns. 2 and 3 were calculated with only 15 compounds, since the experimental R_M value of 2-methyl-1-naphthol (9) in the polyamide system was not determined. However an R_M value for compound 9 was calculated as described in Table II and used to obtain a similar equation to eqn. 2:

$$R_{M_{\text{sil }0\%}} = -0.073(\pm 0.220) + 0.776(\pm 0.100) R_{M_{\text{pol }0\%}} \quad (F = 59.94,\ P < 0.005)$$

n	r	s	
16	0.900	0.246	(4)

The relatively low correlation coefficients of eqns. 2 and 4 seem to be due to the different nature of the stationary phase, *i.e.*, to the mechanism by which polyamide separates organic compounds[8]. The different nature of the polyamide layer is also shown by eqn. 1 the relationship between log P values and R_M values from the polyamide system:

$$\log P_{\text{oct}} = 0.918(\pm 0.975) + 1.074(\pm 0.444) R_{M_{\text{pol }0\%}} \quad (F = 26.81,\ P < 0.005)$$

n	r	s	
16	0.811	0.514	(5)

In a previous paper the R_M values of a series of phenols were measured in the system silicone oil–veronal acetate buffer[3]. The ΔR_M values for some alkyl substituents in that series of compounds are reported in Table III and used to calculate ΔR_M values for other alkyl substituents in the present series of naphthols. The π values of Table III were taken from Lien *et al.*[9]. There is a very good correlation between ΔR_M and π values as calculated in Table III:

$$\pi = 0.093(\pm 0.099) + 1.056(\pm 0.054)\,\Delta R_M \quad (F = 1907.70,\ P < 0.005)$$

n	r	s	
11	0.998	0.065	(6)

TABLE III

ΔR_M AND π VALUES OF ALKYL-2-NAPHTHOLS

π Values were taken from ref. 9.

Group	ΔR_M	π
2-Methyl	0.45*	0.50
2,4-Methyl	0.76*	1.00
2-Ethyl	0.85*	1.00
4-*n*-Propyl	1.33*	1.50
4-*n*-Butyl	1.78**	2.00
4-*n*-Amyl	2.23**	2.50
4-*n*-Hexyl	2.68**	3.00
2-*iso*-Butyl	1.70*	1.80
2-*iso*-Amyl	2.15***	2.30
2-*iso*-Hexyl	2.60***	2.80
2-*iso*-Heptyl	3.05***	3.30

* Calculated from the experimental R_M values of phenols (see ref. 3).

** Calculated from the ΔR_M value for the 4-*n*-Pr group by adding a ΔR_M value of 0.45 for each CH_3 group.

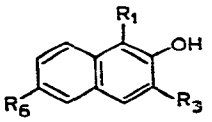
*** Calculated from the ΔR_M value for the 2-*iso*-Bu group by adding a ΔR_M value of 0.45 for each CH_3 group.

Table IV shows the R_M values of a series of alkyl-2-naphthols calculated by taking advantage of the additive property of lipophilic character and using the ΔR_M values of Table III. Also reported are log P values, as well as the log $(1/C)$ values indicating the antibacterial activity *versus Staphylococcus aureus* as taken from Lien *et al.*[9]. There is a very good correlation between the R_M values of alkyl-2-naphthols in Table III and their log P values:

$$\log P_{oct} = 1.482(\pm 0.067) + 1.102(\pm 0.021)R_M \qquad n = 22 \quad r = 0.998 \quad s = 0.062 \qquad (7)$$
$$(F = 6644.64,\ P < 0.005)$$

TABLE IV

R_M AND log P VALUES OF ALKYL-2-NAPHTHOLS



log P values were taken from ref. 9. R_M values were calculated from the experimental R_M value of 2-naphthol by adding the ΔR_M values of Table III. Abbreviations: Me = methyl; Et = ethyl; Pr = propyl; Bu = butyl; Am = amyl; Hex = hexyl; Hep = heptyl.

R_1	R_3	R_6	log P	R_M	*Antibacterial activity*		
					log (1/C) obs.	*log (1/C) calc. (eqn. 6)*	*Δ log (1/C)*
H	H	H	2.84	1.27	2.86	3.13	−0.27
H	H	*iso*-Am	5.14	3.42	4.33	4.60	−0.27
H	H	*iso*-Hex	5.64	3.87	4.66	4.91	−0.25
H	H	*iso*-Hep	6.14	4.32	4.68	5.22	−0.54
H	Me	H	3.34	1.72	3.50	3.44	0.06
H	Et	H	3.84	2.12	3.94	3.71	0.23
H	Pr	H	4.34	2.60	4.25	4.04	0.21
H	Bu	H	4.84	3.07	5.00	4.36	0.64
Me	H	H	3.34	1.72	3.20	3.44	−0.24
Et	H	H	3.84	2.12	3.24	3.71	−0.47
Pr	H	H	4.34	2.60	3.27	4.04	−0.77
Me	H	Me	3.84	2.03	3.94	3.65	0.29
Me	H	Et	4.34	2.57	4.27	4.02	0.25
Et	H	Et	4.84	2.97	4.30	4.29	0.01
Et	H	Pr	5.34	3.45	5.03	4.62	0.41
Et	H	Bu	5.84	3.92	5.06	4.95	0.11
Et	H	Am	6.34	4.35	5.38	5.24	0.14
Et	H	Hex	6.84	4.80	5.19	5.55	−0.36
Me	H	Pr	4.84	3.05	4.60	4.35	0.25
Me	H	Bu	5.34	3.52	5.03	4.67	0.36
Me	H	Am	5.84	3.95	5.06	4.92	0.09
Me	H	Hex	6.34	4.40	5.38	5.27	0.11

It can be seen that the slope (1.10) of eqn. 7 is quite close to unity. Moreover, there is also a very good correlation between activity data and lipophilic character, as expressed by R_M values:

$$\log \frac{1}{C} = 2.257(\pm 0.522) + 0.686(\pm 0.163)R_M \qquad n = 22 \quad r = 0.891 \quad s = 0.358 \qquad (8)$$
$$(F = 77.16,\ P < 0.005)$$

TABLE V

DATA ON THE BIOLOGICAL ACTIVITY OF NAPHTHOL

No.	*Hemolytic activity*			*Acute toxicity to mice*		
	log (1/C) obs.	*log (1/C) calc. (eqn. 10)*	*Δ log (1/C)*	*log (1/C) obs.*	*log (1/C) calc. (eqn. 11)*	*Δ log (1/C)*
1	2.19	2.12	0.07	2.60	2.89	−0.29
2	2.00	2.16	−0.16	2.97	2.91	0.06
3	2.15	2.23	−0.08	3.08	2.95	0.13
4	2.72	2.27	0.45	2.86	2.97	−0.11
5	2.70	2.68	0.02	3.34	3.20	0.14
6	2.49	2.81	−0.32	3.28	3.28	0.00
7	2.99	2.85	0.14	3.37	3.29	0.08
8	3.01	2.95	0.06	3.62	3.36	0.26
9	2.69	3.12	−0.43	—	—	—
10	3.08	3.17	−0.09	3.20	3.48	−0.28
11	3.45	3.32	0.13	3.65	3.56	0.09
12	3.55	3.55	0.00	3.82	3.68	0.14
13	3.57	3.57	0.00	3.66	3.69	−0.03
14	3.77	3.57	0.20	3.71	3.69	0.02
15	3.55	3.65	−0.10	3.71	3.74	−0.03
16	4.15	4.02	0.13	3.80	3.95	−0.15

Eqn. 8 is very similar to that calculated by Lien *et al.*[9] for the same activity data and log *P* values.

Acetophenones. The $R_F \times 100$ values of acetophenones are reported in Table VI. The linear relationship between the experimental R_M values in the silicone oil system and the acetone concentration in the mobile phase allowed the calculation of the extrapolated R_M values in Table VII. There is a very good correlation between chromatographic R_M values and log *P* values in octanol–water as calculated from the literature[9–11] and reported in Table VII:

$$\log P_{oct} = 0.580(\pm 0.182) + 1.898(\pm 0.152)R_M \qquad n = 15 \quad r = 0.961 \quad s = 0.206 \qquad (9)$$
$$(F = 155.93,\ P < 0.005)$$

Structure–activity relationship

Naphthols. Table V shows the biological data on the hemolytic activity and the acute toxicity of naphthols in mice. The structure–activity relationships are described by eqns. 10–13. These were calculated by means of the extrapolated R_M values of Table II. There is a very good correlation between hemolytic activity and R_M values:

$$\log \frac{1}{C} = 1.305(\pm 0.351) + 1.088(\pm 0.219)R_M \qquad n = 16 \quad r = 0.943 \quad s = 0.217 \qquad (10)$$
$$(F = 113.39,\ P < 0.005)$$

The introduction of a R_M^2 term into eqn. 10 did not improve the correlation coefficient significantly.

The slope (1.09) of eqn. 10 is quite close to that previously found when correlating the R_M values and hemolytic activity of phenols[3], and to that found by

Hansch for fifteen equations correlating hemolytic activity and lipophilic character of a variety of compounds[14]. In particular, this shows that the partitioning in the silicone chromatographic system is closely related to the absorption of drugs by the membrane of the erythrocytes. The relationship between R_M values of naphthols and their acute toxicity to mice is described by:

$$\log \frac{1}{C} = 2.438(\pm 0.271) + 0.604(\pm 0.164)R_M \qquad n\ 15\quad r\ 0.910\quad s\ 0.161 \qquad (11)$$

$(F = 62.87,\ P < 0.005)$

Again the introduction of a R_M^2 term into eqn. 11 did not improve the correlation coefficient, and this equation was used to derive the log $(1/C)$ values reported in Table V.

The slope (0.60) of eqn. 11 is quite close to that previously found when correlating R_M values of phenols and acute toxicity to mice. It is also not far from that (0.4 ± 0.1) reported by Hansch[12] as typical of linear equations describing *in vivo* structure–activity relationships. In fact, in more complex systems, such as a whole animal, the penetration of chemicals 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 would be the hydrophobic binding to serum constituents.

Acetophenones. The data regarding the hemolytic activity and the acute toxicity to mice of acetophenones are reported in Table VII. The structure–activity equations show that in both cases the lipophilic character explains less than 16 and 25% of the variability in the log $(1/C)$ data:

Hemolysis

$$\log \frac{1}{C} = 2.259(\pm 0.347) + 0.454(\pm 0.290)R_M \qquad n\ 15\quad r\ 0.398\quad s\ 0.392 \qquad (12)$$

$(F = 2.45,\ P < 0.25)$

Acute toxicity

$$\log \frac{1}{C} = 2.721(\pm 0.371) + 0.650(\pm 0.210)R_M \qquad n\ 15\quad r\ 0.502\quad s\ 0.420 \qquad (13)$$

$(F = 4.39,\ P < 0.10)$

As a further step in the analysis the substituents at the X position (see Table VII) were considered. Among the compounds showing the most evident deviations from the linear relationship between log $(1/C)$ and R_M values in eqns. 12 and 13, five acetophenones were characterized by halogen substitution at X. An indicator variable with a value of 1 was therefore used for the five compounds of Table V bearing an halogen at X:

Hemolysis

$$\log \frac{1}{C} = 1.975(\pm 0.145) + 0.494(\pm 0.117)R_M + 0.714\ (\pm 0.087)I_X \qquad n\ 15\quad r\ 0.934\quad s\ 0.159 \qquad (14)$$

$(F = 41.13,\ P < 0.005,\ t_{R_M} = 4.21,\ t_{IX} = 8.20)$

TABLE VI

$R_F \times 100$ VALUES AND THEIR STANDARD DEVIATIONS FOR ACETOPHENONES AT INCREASING ACETONE CONCENTRATIONS IN THE SILICONE SYSTEM

Each $R_F \times 100$ value represents the mean of 6-8 determinations.

Compound	*Acetone concentration (%)*							
	20	*25*	*30*	*35*	*40*	*45*	*50*	*55*
α-Chloroacetophenone	42.0 ± 4.0	45.4 ± 4.2	52.5 ± 3.0	58.8 ± 7.6	65.8 ± 2.0	70.5 ± 2.2		
4-Fluoroacetophenone	–	–	50.4 ± 4.6	59.8 ± 1.4	64.4 ± 1.9	71.9 ± 3.2	76.6 ± 5.6	81.5 ± 4.7
2-Bromoacetophenone	–	37.6 ± 4.2	44.7 ± 3.9	52.9 ± 4.6	60.9 ± 4.3	68.8 ± 2.0	75.0 ± 5.2	–
α-Chloro-4-fluoroacetophenone	–	37.4 ± 3.0	46.0 ± 5.3	54.7 ± 4.6	61.9 ± 2.9	70.2 ± 4.0	75.9 ± 5.4	–
2-Chloroacetophenone	–	31.6 ± 5.9	41.3 ± 8.2	48.7 ± 6.9	56.9 ± 3.2	64.5 ± 3.9	72.1 ± 6.0	77.5 ± 6.2
4-Chloroacetophenone	25.3 ± 7.5	33.2 ± 3.2	40.6 ± 9.0	48.1 ± 9.2	56.7 ± 13.4	65.5 ± 8.2	–	–
4-Bromoacetophenone	–	28.9 ± 3.0	35.8 ± 4.9	44.6 ± 4.6	53.2 ± 3.5	61.2 ± 4.2	69.6 ± 5.4	–
α,α-Dichloroacetophenone	21.4 ± 6.0	26.2 ± 9.2	34.2 ± 5.4	42.7 ± 4.9	53.6 ± 1.2	59.9 ± 4.5	–	–
α-Bromo-4-chloroacetophenone	19.0 ± 3.0	24.0 ± 2.8	30.6 ± 3.7	41.1 ± 5.6	52.3 ± 1.2	60.3 ± 4.9	–	–
2,4-Dichloroacetophenone	18.7 ± 2.4	22.4 ± 3.2	32.1 ± 4.9	40.8 ± 4.2	52.9 ± 1.9	64.2 ± 11.0	–	–
α,2,4-Trichloroacetophenone	13.7 ± 1.9	17.0 ± 2.0	24.1 ± 3.2	34.7 ± 2.4	44.4 ± 2.9	53.6 ± 5.0	–	–
2,3,4-Trichloroacetophenone	9.9 ± 2.2	13.0 ± 1.9	17.9 ± 1.0	28.3 ± 5.6	41.5 ± 2.2	50.4 ± 6.0	–	–

Acute toxicity

$$\log \frac{1}{C} = 2.417(\pm 0.157) + 0.693(\pm 0.127)R_M + 0.763(\pm 0.094)I_X \quad (F = 45.57,\ P < 0.005,\ t_{R_M} = 5.43,\ t_{I_X} = 8.07)$$

n	r	s	
15	0.940	0.172	(15)

A comparison of eqns. 12–14 and 13–15, respectively, shows the superiority of eqns. 14 and 15 in describing structure–activity relationships. In particular, an analysis of variance shows that the introduction of the I_X term into eqns. 12 and 13 yields a significant improvement in eqns. 14 and 15 respectively. Moreover, collinearity was not shown between independent variables in both eqns. 14 and 15. The positive coefficient obtained with the indicator variable shows that the halogen substitution results in much higher activity. It is interesting that the introduction of an indicator variable for the same set of compounds greatly improved the correlation, both in the case of the hemolytic activity and acute toxicity. On the other hand, this indicates the non-specific nature of such activities.

The positive slope of log $(1/C)$ with R_M indicates the importance of the lipophilic character in determining both hemolysis and acute toxicity.

In a previous paper[3] the hemolytic activity and aute toxicity to mice of a series of phenols was investigated and correlated with R_M values. The data regarding phenols, naphthols and acetophenones were then combined, employing the indicator variable I_X with a value of 1 for five acetophenones:

Hemolysis

$$\log \frac{1}{C} = 1.443\ (\pm 0.069) + 0.992(\pm 0.049)R_M + 0.687(\pm 0.102)I_X \quad (F = 221.88,\ P < 0.005,\ t_{R_M} = 20.39,\ I_X = 6.72)$$

n	r	s	
57	0.944	0.218	(16)

Acute toxicity

$$\log \frac{1}{C} = 2.360(\pm 0.064) + 0.574(\pm 0.046)R_M + 0.955(\pm 0.099)I_X \quad (F = 118.50,\ P < 0.005,\ t_{R_M} = 12.55,\ t_{I_X} = 9.63)$$

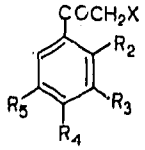
n	r	s	
58	0.901	0.211	(17)

CONCLUSIONS

The present work seems to confirm the usefulness of the chromatographic technique in determining partition data for quantitative structure–activity studies. In particular, by means of the extrapolation technique, the R_M values of different series of compounds can be calculated in a standard system silicone oil–water. This is supported by the fact that, so far, the extrapolated R_M values have been found to be very well correlated with the log P or π values. On the other hand, the practical advantages of the chromatographic technique over the direct measurement of the partition coefficients, mostly when dealing with highly insoluble compounds, have been clearly indicated by Boyce and Milborrow[15].

TABLE VII

LIPOPHILIC CHARACTER AND BIOLOGICAL ACTIVITY OF ACETOPHENONES



Compound	*Structure*					R_M	*log P*	I_X	*Hemolytic activity*		*Acute toxicity to mice*	
	X	R_2	R_3	R_4	R_5				*log (1/C) obs.*	*log (1/C) calc. (eqn. 14)*	*log (1/C) obs.*	*log (1/C) calc. (eqn. 15)*
2-Fluoroacetophenone	H	F	H	H	H	0.559*	1.83	0	2.237	2.251	2.742	2.805
α-Chloroacetophenone	Cl	H	H	H	H	0.598	1.97	1	3.288	2.985	3.672	3.595
4-Fluoroacetophenone	H	H	H	F	H	0.726	1.89	0	2.284	2.334	2.980	2.920
2-Bromoacetophenone	H	Br	H	H	H	0.926	2.47	0	2.290	2.433	3.020	3.059
α-Chloro-4-Fluoroacetophenone	Cl	H	H	F	H	0.941	2.28	1	3.219	3.154	4.089	3.832
2-Chloroacetophenone	H	Cl	H	H	H	1.034	2.27	0	2.408	2.486	2.992	3.134
4-Chloroacetophenone	H	H	H	Cl	H	1.050	2.51	0	2.531	2.494	3.241	3.145
4-Bromoacetophenone	H	H	H	Br	H	1.148	2.71	0	2.480	2.542	3.200	3.213
α,α-Dichloroacetophenone	Cl_2	H	H	H	H	1.199	2.36	1	3.080	3.282	3.600	4.011
α-Bromo-4-Chloroacetophenone	Br	H	H	Cl	H	1.328	3.11	1	3.090	3.346	4.256	4.101
2,4-Dichloroacetophenone	H	Cl	H	Cl	H	1.414	3.20	0	2.610	2.674	3.329	3.397
2,5-Dichloroacetophenone	H	Cl	H	H	Cl	1.414**	3.31	0	2.750	2.674	3.480	3.397
3,4-Dichloroacetophenone	H	H	Cl	Cl	H	1.457***	3.55	0	2.888	2.695	3.344	3.427
α,2,4-Trichloroacetophenone	Cl	Cl	H	Cl	H	1.554	3.59	1	3.547	3.457	4.180	4.257
2,3,4-Trichloroacetophenone	H	Cl	Cl	Cl	H	1.821	4.24	0	2.980	2.875	3.850	3.679

* Calculated by subtracting from the experimental R_M value for 4-fluoroacetophenone the difference of 0.167 between the R_M values for 4-fluoro- and 2-fluorophenol (see ref. 3).

** Assumed to be equal to that of 2,4-dichloroacetophenone.

*** Calculated by adding to the experimental R_M value for 4-chloroacetophenone the ΔR_M value for the 3-Cl group as obtained from 2,4-dichloroacetophenone and 2,3,4-trichloroacetophenone.

The correlation coefficient provided by both eqn. 16 and 17 is very good. When considering that the R_M values for phenols, naphthols and acetophenones were determined in different times, this could provide good evidence of the importance of R_M values as a standard measure of the lipophilic character of compounds.

What is the meaning of the importance of halogen substitution at the X position in the acetophenones? An indicator variable at the X position could account for a lowered electron density near the carbonyl carbon atom. Hermann *et al.*[16] found that for a series of acetophenones a lowered electron density at that position was related to their improved efficiency as substrates in enzymatic reactions with rabbit kidney reductase. In the present case, where non-specific activity is involved in determining both hemolysis and acute toxicity, the lowered electron density at the X position could be related to an increased protein binding.

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