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R_M AND log P VALUES OF 5-NITROIMIDAZOLES

M. C. GUERRA*, A. M. BARBARO, G. CANTELLI FORTI, M. T. FOFFANI and G. L. BIAGI
Istituto di Farmacologia, Università di Bologna, Bologna (Italy)
P. A. BOREA
Istituto di Farmacologia, Università di Ferrara, Ferrara (Italy)
and
A. FINI
Istituto di Chimica degli Intermedi, Università di Bologna, Bologna (Italy)
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SUMMARY

The chromatographic R_M values of a series of nitroimidazoles and their log P values were determined in view of a study of their structure–activity relationships as mutagenic agents. The equations describing the relationship between R_M and log P values show a low correlation coefficient. The introduction of the molar refractivity of the R_1 and R_2 groups yields a significant improvement in the correlation coefficient.

The molar refractivity could be an expression of the adsorption activity of the silica gel layer.

INTRODUCTION

The nitroimidazoles play an important role in chemotherapy, mainly for their antibacterial, antitrichomonal and antiamoebic activity[1,2]. Recent toxicological studies revealed that many of these compounds have mutagenic or carcinogenic activity[1].

Structure–activity relationships demonstrated the importance of physico-chemical parameters in determining the biological activity of nitroimidazoles. The influence of the lipophilic character and electronegativity of the R_1 groups on the antischistosomal and antiprotozoal activity has been discussed[3,4]. A relationship between antibacterial activity and electro-reduction was shown by Chien and Mizuba[5]. The frontier electron density is important for the protein binding of metronidazole derivatives[6].

The purpose of this work was to study the relationship between the chromatographic R_M values for a series of 5-nitroimidazoles and their log P values with a view to studying their structure–activity relationships as mutagenic agents.

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EXPERIMENTAL

Determination of ionization constants

The pK_a values of 5-nitroimidazoles were determined by means of a spectrophotometric and/or potentiometric technique in water at 25°C[7,8].

In the spectrophotometric method, two wavelengths were chosen at which the dissociated and undissociated forms have different molar absorptivities. The compounds were assayed at the same concentration by means of a Perkin-Elmer 124 double-beam spectrophotometer. The absorbance of each solution at different pH values enabled the relative amounts of dissociated and undissociated forms to be determined. As $IMH^+ \overset{K_1}{\rightleftharpoons} IM \overset{K_2}{\rightleftharpoons} IM^-$, the pK'_1 or pK'_2 values could be obtained[9].

The potentiometric titrations were carried out by means of an Orion 601 potentiometer. The ionization constants reported in Table I are referred to the highest basic or acidic fraction in each compound.

Determination of R_M values

The reversed-phase chromatographic technique for the determination of R_M values has been described previously[10,11]. The polar mobile phase was ammonium chloride buffer (1 *M*) of pH 9.0, alone or in various mixtures with methanol. With compound 14 the mobile phase was sodium acetate–Veronal buffer of pH 3.6. The non-polar stationary phase was a silica gel G F_{254} layer impregnated with a 5% (v/v) solution of silicone oil [silicone DC 200 (350 cSt) from Applied Science Labs. (State College, PA, U.S.A.)] in diethyl ether. The concentration of methanol in the mobile phase ranged from 5 to 30%. In other chromatographic systems the mobile phase had the same composition but the stationary phase was a silica gel G F_{254} layer impregnated with a 5% (v/v) solution of squalane, undecane or liquid paraffin (Merck, Darmstadt, G.F.R.) in light petroleum.

The 5-nitroimidazoles were dissolved in methanol or ethanol (1 mg/ml) and 1–5 µl volumes of solution were spotted on the plates in random locations. The developed plates were dried and sprayed with an alkaline solution of potassium permanganate. Most of the compounds were also visible by their fluorescence when the silica gel G f_{254} plates were viewed under an ultraviolet lamp. Finally, the compounds could be detected by spraying the plates with a 1.5% solution of titanium(III) chloride in 10% acetic acid and heating at 80°C for 20 min in order to reduce the nitro group[12]. The plates were then sprayed with diazotized sulphanilic acid[13].

Determination of partition coefficients

The octanol/water partition coefficients were determined according to the classical procedure[14]. The aqueous layer was ammonium chloride buffer (1 *M*) of pH 9.0. The partition coefficient of compound 14 was determined with aqueous sodium acetate–Veronal buffer (0.14 *M*) of pH 4.6. The concentration of the compounds in the octanol and/or aqueous layer was determined by UV measurement with a Perkin-Elmer 124 double-beam spectrophotometer.

RESULTS

Ionization constants

The ionization constants of the test compounds are reported in Table I, together with literature data[7,15].

The substituent groups R_1 and R_2 do not have a substantial effect on the basicity of the N-3 atom in the imidazole ring. In fact, the pK'_1 values do not differ very much from the pK'_1 values of 1-methyl-5-nitroimidazole (2.12) or 2-methyl-5-nitroimidazole (2.73) as given in or calculated from the literature[7]. However, the pK'_1 values of compounds 10, 13 and 20 are much higher, owing to the presence of basic moieties in their side-chain. On the other hand, the lower pK'_1 values of compounds 12, 17 and 18 are due to the presence of a strong electron-withdrawing R_1 or R_2 group. The pK'_2 values are referred to the ionization of the N-1 atom of compounds 1 and 2 or the carboxyl group in the side-chain of compound 14.

R_M values

The chromatographic work showed that in the system 5% silicone oil–ammonium chloride buffer most of the test compounds migrated when the mobile phase was only buffer. However, the addition of methanol was necessary to obtain suitable R_F values for compound 15. The R_M values were plotted against the composition of the mobile phase. For each compound there was a range where a linear relationship between R_M values and methanol concentration applied. The equations of these straight lines were used to calculate a theoretical R_M value at 0% methanol in the mobile phase (Table II). The very close correlation between the extrapolated and the experimental R_M values at 0% methanol from Table II shows the validity of the extrapolation technique (eqn. 1).

	n	r	s	
R_M (5% sil.) (exptl.) = $-0.024 + 1.018\ R_M$ (5% sil.) ($a = R_M$)	21	0.999	0.032	(1)

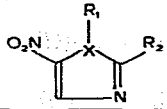
The R_M values of 21 compounds measured at different methanol concentrations when the stationary phase was squalane, undecane or liquid paraffin were used to calculate the extrapolated R_M values reported in Table II. Eqns. 2, 3 and 4 show a very good correlation between the extrapolated R_M values ($a = R_M$) on silicone-impregnated layers and those on squalane, undecane or liquid paraffin-impregnated layers.

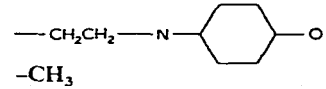
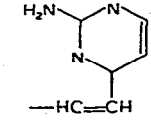
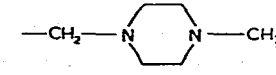
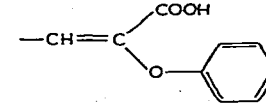
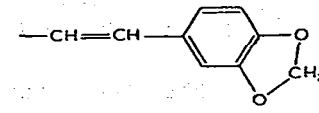
	n	r	s	
R_M (5% undecane) = $0.389 + 0.971\ R_M$ (5% sil.)	21	0.949	0.207	(2)
R_M (5% squalane) = $0.438 + 1.070\ R_M$ (5% sil.)	21	0.941	0.249	(3)
R_M (5% paraffin) = $0.481 + 0.848\ R_M$ (5% sil.)	21	0.927	0.221	(4)

The data in Table II and the intercepts of eqns. 2, 3 and 4 show that in the undecane, squalane or liquid paraffin systems the R_M values are higher than in the silicone system. This could be due to a stronger interaction with the support and/or to a higher solubility in squalane, undecane or liquid paraffin than in silicone oil. How-

TABLE I

IONIZATION CONSTANTS AND LOG *P* VALUES OF 5-NITROIMIDAZOLES

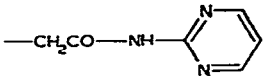
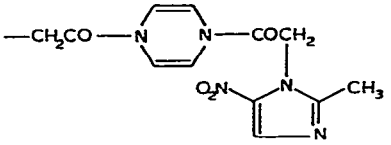
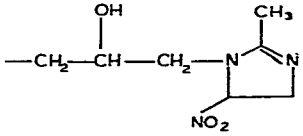
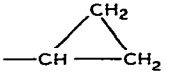


No.	*Compound*	*Structure (X = N)*	
		R_1	R_2
1	5-Nitroimidazole	–H	–H
2	2-Methyl-5-nitroimidazole	–H	$-CH_3$
3	1-Ethanol-2-methyl-5-nitro-imidazole (Metronidazole)	$-CH_2CH_2OH$	$-CH_3$
4	1-Methyl-2-(1-methylethyl)-5-nitroimidazole (Ipronidazole)	$-CH_3$	$-CH(CH_3)CH_3$
5	1-Methyl-2-formyl-5-nitro-imidazole	$-CH_3$	–CHO
6	1-(2-Ethylcarbamothiotic acid O-methyl ester)-2-methyl-5-nitroimidazole (Carnidazole)	$-CH_2CH_2NHC(=S)OCH_3$	$-CH_3$
7	1-[2-(Ethylsulphonyl)ethyl]-2-methyl-5-nitroimidazole (Tinidazole)	$-CH_2CH_2SO_2CH_2CH_3$	$-CH_3$
8	1-[α-Chloromethyl)ethanol]-2-methyl-5-nitroimidazole (Ornidazole)	$-CH(CH_2Cl)CH_2OH$	$-CH_3$
9	1[Methyl-2-(methanol carbamate)]-5-nitroimidazole (Ronidazole)	$-CH_3$	$-CH_2COONH_2$
10	1-(2-N-Morpholinylethyl)-5-nitroimidazole (Nimorazole)		–H
11	1-Methyl-2-hydroxymethyl-5-nitroimidazole	$-CH_3$	$-CH_2OH$
12	1-Methyl-2-(2-amino-4-ethinyl-pyrimidine)-5-nitroimidazole (Azanidazole)	$-CH_3$	
13	DA 3804	$-CH_3$	
14	DA 3831	$-CH_3$	
15	DA 3832	$-CH_2CH_2OH$	
16	DA 3838	$-CH_2-CH(OH)-CH_2OH$	$-CH_3$

Empirical formula	*Molecular weight*	pK'_1	pK'_2	*log P (observed)*	*log P (calc.)*	$\Sigma MR_{1,2}$
$C_3H_3N_3O_2$	113.8		9.37 (9.20[7])	−0.16	−0.17[a]	0.206
$C_4H_5N_3O_2$	127.10	(2.73)	8.79	0.49	0.39[b]	0.668
$C_6H_9N_3O_3$	171.16	(2.55[7]) 2.38		−0.10	−0.11[c]	1.749
$C_7H_{11}N_3O_2$	169.18	2.73		1.06	1.49[d]	2.061
$C_5H_5N_3O_3$	155.11	1.60		−0.69	−0.69[e]	1.253
$C_8H_{12}N_4O_3S$	244.27	2.08		0.90	0.88[f]	3.802
$C_8H_{13}N_3O_4S$	247.26	1.82		−0.36	−0.30[g]	3.306
$C_7H_{10}N_3O_3Cl$	219.63	2.27		0.60	0.18[h]	2.695
$C_6H_8N_4O_4$	200.16	1.41 (1.2[15])		−0.38	0.70[i]	2.365
$C_9H_{14}N_4O_3$	266.23	5.00		0.07	0.06[j]	3.343
$C_5H_7N_3O_3$	157.12	2.04		−0.03	−1.07[k]	1.284
$C_{10}H_{10}N_6O_2$	246.23	0.26		0.85	0.56[l]	5.071
$C_{10}H_{17}N_5O_2$	239.29	7.60		−0.32	0.00[m]	3.684
$C_{13}H_{11}N_3O_5$	289.25		3.81	−1.00	−1.50[n]	5.024
$C_{14}H_{13}N_3O_5$	303.27	2.73		2.03	2.23[o]	5.260
$C_7H_{11}N_3O_4$	201.20	2.95		−0.63	−0.68[p]	2.362

(Continued on p. 98)

TABLE I *(continued)*

No.	*Compound*	*Structure (X = N)*	
		R_1	R_2
17	DA 3839		$-CH_3$
18	DA 3840		$-CH_3$
19	DA 3853		$-CH_3$
20	DA 3854	$-CH_2CH_2OCH_2CH_2-N(CH_2CH_3)_2$	$-CH_3$
21	MY 40/20	$-CH_2-CH_2-SO_2-CH_3$	$-CH_3$
22	DA 3851	$-CH_3$	

[a] $\log P_{No.1} = \log P(\text{imidazole}) + \pi(NO_2) = -0.08 - 0.09 = -0.17$; $\log P$(imidazole) was taken from ref. 20 and $\pi(NO_2)$ from ref. 20 by subtracting the log P values of 2-nitropyridine (No. 1785) and 3-nitroquinoline (No. 6712).

[b] $\log P_{No.2} = \log P_{No.1} + \pi(CH_3) = -0.17 + 0.56 = 0.39$; see ref. 20 from which a π value of 0.56 was taken for the CH_3 group.

[c] See refs. 19 and 20.

[d] $\log P_{No.4} = \log P_{No.1} + \pi(CH_3) + \pi[CH(CH_3)CH_3] = -0.17 + 0.13 + 1.53 = 1.49$. See ref. 20. A π value of 0.13 for the CH_3 group attached to nitrogen was calculated by subtracting the log P values of pyridine (No. 1871) and quinolone (No. 6824) from the log P values of N-methylpyridone (No. 3541) and N-methylquinolone (No. 7964), respectively. A π value of 1.53 was taken for the isopropyl group.

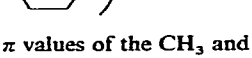
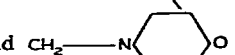
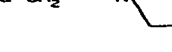
[e] $\log P_{No.5} = \log P_{No.1} + \pi(CH_3) + \pi(CHO) = -0.17 + 0.13 - 0.65 = -0.69$. See ref. 20 for the π value of the CHO group.

[f] $\log P_{No.6} = \log P_{No.2} + \pi(CH_2CH_2NHC(=S)COCH_3) = 0.39 + 0.49 = 0.88$. A π value of 0.49 for the $CH_2CH_2NHC(=S)COCH_3$ group was obtained in the following way. From ref. 20, $\log P(NH_2-C(=O)-CH_3) - \log P(NH_2-C(=O)-NH_2) = -0.66 - (-1.09) = 0.43$. Therefore, $\pi(NH_2-C(=S)-COCH_3) = \log P(NH_2-C(=S)-NH_2) + 0.43 = -1.06 + 0.43 = -0.63$. Finally, $\pi(CH_2CH_2-NH-C(=S)-OCH_3) = \pi[(CH_3)_2] - 0.63 = 1.12 - 0.63 = 0.49$.

[g] See refs. 20 and 21.

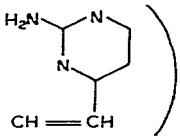
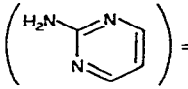
[h] $\log P_{No.8} = \log P_{No.2} + \pi[CH(CH_2Cl)CH_2OH] = 0.39 + \pi(CH_2CH_2Cl) + \pi(CH_2OH) = 0.39 + 0.82 - 1.03 = 0.18$.

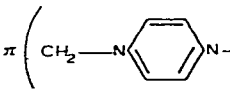
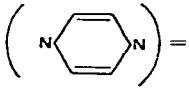
[i] $\log P_{No.9} = \log P_{No.1} + \pi(CH_3) + \pi(CH_2COONH_2) = -0.17 + 0.13 - 0.66 = -0.70$. See ref. 20 for the π value of CH_2COONH_2.

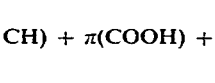
[j] $\log P_{No.10} = \log P_{No.1} + \pi$  $= \log P_{No.1} + \pi(CH_3) + \pi$  $= -0.17 + 0.56 - 0.33 = 0.06$. See ref. 20 for the π values of the CH_3 and  groups.

Empirical formula	*Molecular weight*	pK'_1	pK'_2	*log P (observed)*	*log P (calc.)*	$\Sigma MR_{1,2}$
$C_{10}H_{10}N_6O_3$	262.23	0.96		−0.30	−0.67[q]	3.941
$C_{16}H_{20}N_8O_6$	402.36	1.00		−0.70	−0.54[r]	7.739
$C_{11}H_{14}N_6O_5$	310.27	2.19		0.31	0.83[s]	5.166
$C_{12}H_{22}N_4O_3$	270.34	5.07		−0.38	0.51[t]	5.086
$C_7H_{11}N_3SO_4$	233.24	2.04		−0.35	−0.82[u]	2.841
$C_7H_9N_3O_2$	167.16	2.56		1.00	1.10[v]	1.918

[k] $\log P_{No.11} = \log P_{No.1} + \pi(CH_3) + \pi(CH_2OH) = -0.17 + 0.13 - 1.03 = -1.07$. A π value of 0.13 was taken for the CH_3 group in the 1-position; see ref. 15 for the π value of the CH_2OH group.

[l] $\log P_{No.12} = \log P_{No.1} + \pi(N\text{–}CH_3) + \pi$  $= \log P_{No.1} + \pi(N\text{–}CH_3) + \pi(CH{=}CH) +$ π  $= -0.17 + 0.13 + 0.82 - 0.22 = 0.56$. See ref. 20 for the π values of ethynyl and 2-amino-pyrimidine groups.

[m] $\log P_{No.14} = \log P_{No.1} + \pi(N\text{–}CH_3) + \pi$  $= \log P_{No.1} + 3\pi(N\text{–}CH_3) +$ π  $= -0.17 + 0.39 - 0.22 = 0.00$.

[n] $\log P_{No.16} = \log P_{No.1} + \pi(N\text{–}CH_3) + \pi(CH{=}CH) + \pi(COOH) + \pi$  $= -0.17 + 0.13 + 0.82 - 4.36 + 2.08 = -1.50$.

[o] $\log P_{No.17} = \log P_{No.3} - \pi(CH_3) + \log P$(1,2-methylenedihydroxybenzene) $+ \pi(CH{=}CH) = -0.11 - 0.56 + 2.08 + 0.82 = 2.23$.

[p] See ref. 20: $\pi(CH_2\text{–}\underset{\displaystyle OH}{\underset{|}{CH}}\text{–}CH_2OH) = \log P$ 10.75 $+ \pi$(OH aliphatic) $= 0.05 - 1.12 = -1.07$. Therefore $\log P_{No.19} = \log P_{No.2} - 1.07 = 0.39 - 1.07 = -0.68$.

[q] See ref. 15: $\log P_{No.20} = \log P_{No.2} + \log P$(pyrimidine) $+ \log P(NH_2COCH_3) = 0.39 - 0.40 - 0.66 = -0.67$.

[r] See ref. 20: $\log P_{No.21} = \log P_{No.2} + \log P_{No.2} + \log P$(pyrazine) $+ 2\pi(COCH_3) = 0.39 + 0.39 - 0.22 - 1.10 = -0.54$.

[s] See ref. 20: $\log P_{No.22} = \log P_{No.2} + \log P_{No.2} + \pi$(isopropyl) $= 0.39 + 0.39 + 0.05 = 0.83$.

[t] See ref. 20: $\log P_{No.23} = \log P_{No.2} + \pi(CH_3\text{–}CH_2\text{–}O\text{–}CH_2) + \pi(CH_2\text{–}N(C_2H_5)_2) = 0.39 - 0.24 + 0.36 = 0.51$.

[u] See ref. 20.

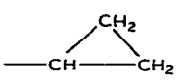
[v] $\log P_{No.26} = \log P_{No.1} + \pi(N\text{–}CH_3) + \pi$  $= -0.17 + 0.13 + 1.14 = 1.10$.

TABLE II

R_M VALUES OF 5-NITROIMIDAZOLES

*Compound No.**	*Silica gel G F_{254} impregnated with 5% silicone oil*			$a = R_M$			R_M *(0% sil.)*
	R_M *(exptl)*	*Eqn. from TLC*		*Undecane*	*Squalane*	*Liquid paraffin*	
		$a = R_M$	b				
1	−0.19	−0.19	−0.01	0.22	0.23	0.27	−0.41
2	0.26	0.26	−0.01	0.46	0.47	0.55	0.01
3	0.08	0.08	−0.02	0.42	0.53	0.60	−0.31
4	0.72	0.72	−0.02	1.31	1.37	1.36	0.14
5	−0.26	−0.16	−0.02	0.08	0.15	0.18	−0.66
6	0.79	0.81	−0.02	1.48	1.61	1.35	−0.14
7	0.33	0.35	−0.02	0.86	1.04	1.06	−0.14
8	0.32	0.33	−0.02	0.98	0.93	1.12	−0.38
9	−0.11	−0.07	−0.01	0.39	0.60	0.68	−0.44
10	0.97	0.97	−0.03	1.39	1.95	1.47	0.23
11	−0.15	−0.14	−0.01	0.15	0.28	0.28	−0.50
12	1.28	1.31	−0.03	1.66	2.02	1.42	−0.86
13	1.44	1.44	−0.02	1.64	1.91	1.57	0.95
14	0.69	0.69	−0.03	1.45	1.29	1.19	0.60
15	1.68	1.68	−0.04	2.04	2.23	1.85	1.16
16	−0.18	−0.13	−0.02	0.30	0.18	0.13	0.02
17	0.96	0.95	−0.02	1.00	1.11	1.15	0.54
18	1.58	1.58	−0.04				0.50
19	1.08	1.11	−0.03	1.48	1.49	1.30	0.00
20	1.60	1.56	−0.03	1.56	1.58	1.57	1.19
21	−0.30	−0.30	−0.01	−0.23	−0.26	−0.19	−0.72
22	0.66	0.68	−0.02	1.14	1.27	1.33	0.03

* See Table I.

ever, the slopes of eqns. 2, 3 and 4 are very close and therefore show that the hydrophobic characteristics of the test chromatographic systems are very similar.

Mercier[16] and Hulshoff and Perrin[17] pointed out the adsorption activity of the silica gel layer towards the compounds investigated. In addition, Bird and Marshall[18] pointed out that silica gel can alter the pH of buffers. In fact, in the present experiments the interaction with the stationary phase is shown by the R_M values measured on unimpregnated silica gel G F_{254} layers and reported in Table II. However, the correlation between the R_M values measured on silica gel G F_{254} layers impregnated with 5% silicone oil and the R_M values measured on unimpregnated silica gel G F_{254} layers, as shown by eqn. 5, is not very high:

$$R_M\ (5\%\ \text{sil.}) = 0.484 + 1.031\ R_M\ (0\%\ \text{sil.}) \qquad n = 22 \quad r = 0.893 \quad s = 0.306 \qquad (5)$$

This indicates that the addition of silicone oil to the stationary phase is able in some way to alter the partitioning process between the stationary phase and the mobile phase.

log P values

The log P values of test compounds are reported in Table I, together with log P values taken from the literature[19–21] or calculated according to Hansch and Leo[20]. The relatively low correlation coefficient of eqn. 6 agrees with the finding of Lin *et al.*[22], who observed substantial differences between the calculated and experimental log P values of nitroimidazoles:

$$\log P \text{ (exptl.)} = 0.054 + 0.736 \log P \text{ (calcd.)} \qquad n = 22 \quad r = 0.895 \quad s = 0.336 \qquad (6)$$

Relationship between log P and R_M values

The equation describing the relationship between the R_M values with 5% silicone oil and the log P values shows a very low correlation coefficient:

$$R_M \text{ (5\% sil.)} = 0.576 + 0.297 \log P \qquad n = 22 \quad r = 0.331 \quad s = 0.641 \qquad (7)$$
$$(F = 2.453;\ P < 0.25)$$

As a further step in the analysis, the molar refractivity summed over the R_1 and R_2 groups was considered (Table I):

$$R_M \text{ (5\% sil.)} = -0.334 + 0.291\ \Sigma MR_{1,2} \qquad n = 22 \quad r = 0.800 \quad s = 0.408 \qquad (8)$$
$$(F = 35.60;\ P < 0.005)$$

Moreover, the introduction of the log P term into eqn. 8 yielded the equation

$$R_M \text{ (5\% sil.)} = -0.364 + 0.304 \log P + 0.292\ \Sigma MR_{1,2} \qquad n = 22 \quad r = 0.869 \quad s = 0.345 \qquad (9)$$
$$(F = 29.23;\ P < 0.005;\ t \log P = 2.978;\ t\ \Sigma MR_{1,2} = 7.071)$$

A comparison of eqns. 7, 8 and 9 shows the superiority of eqn. 9. In particular, an analysis of variance shows that the introduction of the log P term into eqn. 8 yields a significant improvement in eqn. 9. The lipophilic character expressed by the log P values and the molar refractivity of the R_1 and R_2 groups explain 75% of the variability in the R_M data. Although this is not a very high correlation coefficient, eqn. 9 shows that the molar refractivity could be an expression of the adsorption activity of the silica gel layer. When eqns. 7, 8 and 9 were calculated by using the R_M values at 0% silicone, eqns. 10, 11 and 12 were obtained. The correlation coefficient of eqn. 12 is much lower than that of eqn. 9.

$$R_M \text{ (0\% sil.)} = 0.101 + 0.163 \log P \qquad n = 22 \quad r = 0.210 \quad s = 0.576 \qquad (10)$$
$$(F = 0.92;\ \text{n.s.}^*)$$

* n.s. = not significant.

	n	r	s	
R_M (0% sil.) $= -0.569 + 0.212\ \Sigma MR_{1,2}$ ($F = 16.68$; $P < 0.005$)	22	0.674	0.435	(11)
R_M (0% sil.) $= -0.585 + 0.168 \log P + 0.213\ MR_{1,2}$	22	0.708	0.426	(12)

($F = 9.56$; $P < 0.005$; $t \log P = 1.334$; $t\Sigma MR_{1,2} = 4.176$)

In particular, the introduction of the log P term into eqn. 11 did not improve the correlation coefficient of eqn. 12 significantly. All this is obviously a consequence of the relatively low correlation coefficient of eqn. 5 between the R_M values with 5% silicone oil and those with 0%. However, it also shows that the presence of the silicone oil exerts an important influence.

In conclusion, the results confirm the usefulness of this thin-layer chromatographic (TLC) technique as a general procedure for the determination of R_M values. In contrast to the 1-octanol shake-flask partition coefficients, the TLC procedure can tolerate impurities, requires little material and gives rapid results. However, the poor correlation between the R_M and log P values indicates that most of the 5-nitroimidazoles deviate from the linear relationship.

Unger *et al.*[23] pointed out that only small, basic, unhindered pyridines deviated from the agreement between shake-flask and reversed-phase high-performance liquid chromatographic procedures, presumably because of binding to residual silanol sites. As the 5-nitroimidazoles are small, basic molecules, their deviation could be due to a similar kind of phenomenon, *viz.*, interaction with the silica gel G layer. Finally, a series of experiments in our laboratory have shown the usefulness of the present R_M values in studying the relationship between the structure and mutagenic activity of 5-nitroimidazoles.

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