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Note

R_M values, retention times and octanol–water partition coefficients of a series of 5-nitroimidazoles

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In recent years there has been increasing interest in comparing different procedures for measuring the lipophilicity of drugs. In particular, reversed-phase high-performance liquid chromatography (HPLC) has been presented as an alternative to both reversed-phase TLC and direct partitioning[1–5]. In particular, Hulshoff and Perrin[1] compared reversed-phase HPLC and TLC procedures. Unger and co-workers,[2,3] showed that the log (relative retention times) are highly correlated to log (partition coefficients) obtained from the classical shake-flask procedure. Caroon *et al.*[5] determined the 1-octanol distribution coefficients of a series of 2-substituted imidazoles by HPLC.

The purpose of this work was to compare the partition coefficients of a series of 5-nitroimidazoles evaluated by means of HPLC with the R_M and log P values previously measured[6].

EXPERIMENTAL

The nitroimidazole derivatives were obtained from commercial sources and drug companies. The DA and MY compounds were generous gift from Carlo Erba and Midy, respectively. The compounds used are listed in Table I. Analytical-reagent grade solvents were used.

Chromatography was performed on a Waters 6000 A chromatograph using a μBondapak C_{18} column (300 × 3.9 mm I.D.) (Waters), packed with silica gel (particle size 10 μm) with a C_{18} chemically bonded non-polar stationary phase. A UV detector (Waters Model 440) at 313 nm and Hamilton 802 chromatographic syringes (25 μl) were also used.

The nitroimidazoles were separated using methanol–water (40:60) as the mobile phase at a flow-rate of 1 ml/min. Compounds DA 3831 and DA 3804 were eluted with 40% methanol buffer at pH 3.8 and 7.6, respectively. Samples were dissolved in methanol (1 mg/ml) and applied to the column in 5-μl volumes. All solutions were first filtered to reduce contamination.

The experiments were performed at room temperature (20–22°C). The retention

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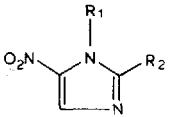
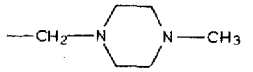
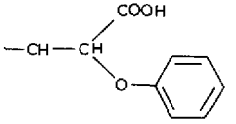
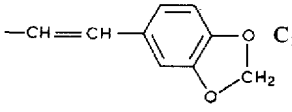
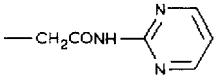
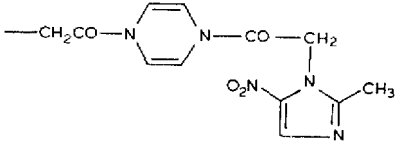
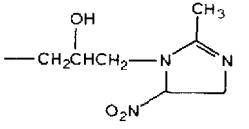
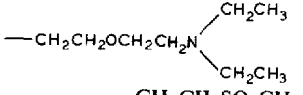
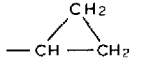
TABLE I

RETENTION TIMES, R_M VALUES AND LOG P VALUES OF 5-NITROIMIDAZOLES



Compound	*Structure*		*Empirical formula*	*Molecular weight*	*Log k'*	R_M	*Log P*	$\Sigma MR_{1,2}$
	R_1	R_2						
5-Nitroimidazole	-H	-H	$C_3H_3N_3O_2$	113.08	0.00	−0.19	−0.16	0.206
2-Methyl-5-nitroimidazole	-H	$-CH_3$	$C_4H_5N_3O_2$	127.10	0.141	0.26	0.49	0.668
1-Ethanol-2-methyl-5-nitro-imidazole (metronidazole)	$-CH_2CH_2OH$	$-CH_3$	$C_6H_9N_3O_3$	171.16	−0.067	0.08	−0.10	1.749
1-Methyl-2 (1-methylethyl)-5-nitroimidazole (ipronidazole)	$-CH_3$	$-CH(CH_3)CH_3$	$C_7H_{11}N_3O_2$	169.18	0.450	0.72	1.06	2.061
1-Methyl-2-formyl-5-nitro-imidazole	$-CH_3$	-CHO	$C_5H_5N_3O_3$	155.11	−0.067	−0.16	−0.69	1.253
1-(2-Ethyl carbamothioic acid O-methyl ester)-2-methyl-5-nitroimidazole (carnidazole)	$-CH_2CH_2NHCOCH_3$ (‖ S)	$-CH_3$	$C_8H_{12}N_4O_3S$	244.27	0.465	0.81	0.90	3.802
1-[2-(Ethylsulphonyl) ethyl]-2-methyl-5-nitroimidazole (tinidazole)	$-CH_2CH_2SO_2CH_2CH_3$	$-CH_3$	$C_8H_{13}N_3O_4S$	247.26	−0.125	0.35	−0.36	3.306
1-[(α-Chloromethyl) ethanol]-2-methyl-5-nitroimidazole (Ornidazole)	$-CH(CH_2Cl)CH_2OH$	$-CH_3$	$C_7H_{10}N_3O_3Cl$	219.63	0.199	0.33	0.60	2.695
1-[Methyl-2-(methanol carba-mate)]-5-nitroimidazole (ronidazole)	$-CH_3$	$-CH_2COONH_2$	$C_6H_8N_4O_4$	200.16	0.141	−0.07	−0.38	2.365
1-(2-N-Morpholinylethyl)-5-nitroimidazole (nimorazole)	$-CH_2CH_2-N$ O	-H	$C_9H_{14}N_4O_3$	266.23	0.188	0.97	0.07	3.343
1-Methyl-2-hydroxymethyl-5-nitroimidazole	$-CH_3$	$-CH_2OH$	$C_5H_7N_3O_3$	157.12	−0.110	−0.14	−0.03	1.284
1-Methyl-2-(2-amino-4-ethinyl pyrimidine)-5-nitroimidazole azanidazole)	$-CH_3$	H_2N, N, N, $-CH=CH$	$C_{10}H_{10}N_6O_2$	246.23	0.423	1.31	0.85	5.071

DA 3804	$-CH_3$		$C_{10}H_{17}N_5O_2$	239.29	0.014	1.44	−0.33	3.684
DA 3831	$-CH_3$		$C_{13}H_{11}N_3O_5$	289.25	−0.199	0.69	−1.00	5.024
DA 3832	$-CH_2CH_2OH$		$C_{14}H_{13}N_3O_5$	303.27	0.659	1.68	2.03	5.260
DA 3838	$-CH_2CHCH_2OH$ (OH)	$-CH_3$	$C_7H_{11}N_3O_4$	201.20	−0.199	−0.13	−0.63	2.362
DA 3839		$-CH_3$	$C_{10}H_{10}N_6O_3$	262.23	−0.115	0.95	−0.30	3.941
DA 3840		$-CH_3$	$C_{16}H_{20}N_8O_6$	402.36	0.028	1.58	−0.70	7.739
DA 3853		$-CH_3$	$C_{11}H_{14}N_6O_5$	310.27	0.199	1.11	0.31	5.166
DA 3854		$-CH_3$	$C_{12}H_{22}N_4O_3$	270.34	0.021	1.56	−0.38	5.086
MY 40/20	$-CH_2CH_2SO_2CH_3$	CH_3	$C_7H_{11}N_3SO_4$	233.24	−0.097	−0.30	−0.35	2.841
DA 3851	$-CH_3$		$C_7H_9N_3O_2$	167.16	0.455	0.68	1.00	1.918

times were expressed as log capacity factor (k') (Table I), where $k' = (t_x - t_0)/t_0$. The determination of R_M and log P values and dissociation constants was carried out as described previously[6].

RESULTS AND DISCUSSION

The relationship between log k' and log P in the octanol–water system is described by the equation

$$\log k' = 0.082 + 0.306 \log P \qquad (n = 22, r = 0.921, s = 0.098) \qquad (1)$$

($F = 112.22$; $P < 0.005$; t log $P = 10.594$), which shows a fairly high correlation coefficient (n is the number of data points, r is the correlation coefficient, s is the standard deviation. The F test shows the significance of each term.) On the other hand, in a previous paper[1] we investigated the relationship between the chromatographic R_M values and the log P values finding a very low correlation coefficient. In fact, using the extrapolated R_M values in Table I we obtained

$$R_M = 0.590 + 0.292 \log P \qquad (n = 22, r = 0.331, s = 0.630) \qquad (2)$$

($F = 2.46$; $P < 0.25$; t log $P = 1.567$). When considering the molar refractivity summed over the R_1 and R_2 groups, the following equation was obtained:

$$R_M = -0.308 + 0.287\, \Sigma MR_{1,2} \qquad (n = 22, r = 0.804, s = 0.397) \qquad (3)$$

($F = 36.50$; $P < 0.005$; $t\, \Sigma MR_{1,2} = 6.041$). Moreover, introduction of the log P term into eqn 3 yielded a significant improvement:

$$R_M = -0.338 + 0.299 \log P + 0.288\, \Sigma MR_{1,2} \qquad (n = 22, r = 0.872, s = 0.335) \qquad (4)$$

($F = 30.20$; $P < 0.005$; t log $P = 3.017$; $t\, \Sigma MR_{1,2} = 7.191$).

The molar refractivity was considered as an expression of the binding of nitroimidazoles to the silica gel layer. In a similar way we obtained the equations

$$\log k' = 0.039 + 0.021\, \Sigma MR_{1,2} \qquad (n = 22, r = 0.160, s = 0.248) \qquad (5)$$

($F = 0.53$; not significant), and

$$\log k' = 0.009 + 0.306 \log P + 0.022\, \Sigma MR_{1,2} \qquad (n = 22, r = 0.937, s = 0.090) \qquad (6)$$

($F = 68.05$; $P < 0.005$; t log $P = 11.494$; $t\, \Sigma MR_{1,2} = 2.112$).

Although eqn. 5 is not significant, an analysis of variance showed that the introduction of the $\Sigma MR_{1,2}$ term into eqn. 1 yields a significant improvement in eqn. 6. However, eqns. 5 and 6 show that the molar refractivity as an expression of the binding to the stationary phase in HPLC is much less important. At this point one

would expect a relatively low correlation between log k' and R_M values. In fact, eqn. 7 shows a very low correlation coefficient:

$$R_M = 0.480 + 1.236 \log k' \qquad (n = 22, r = 0.467, s = 0.590) \qquad (7)$$

($F = 5.58$; $P < 0.05$; $t \log k' = 2.362$). The introduction of the $\Sigma MR_{1,2}$ term yielded the equation

$$R_M = -0.346 + 0.925 \log k' + 0.267 \Sigma MR_{1,2} \qquad (n = 22, r = 0.875, s = 0.331) \qquad (8)$$

($F = 30.92$; $P < 0.005$; $t \log k' = 3.102$; $t \Sigma MR_{1,2} = 6.650$), showing a significant improvement and emphasizing again the role of the binding to the stationary phase.

In conclusion, assuming that the log P values are the best measure of lipophilicity, this work appears to show that the R_M values of nitroimidazoles represent a measure both of lipophilic and polar character. On the other hand, the log k' values are better correlated with the log P values even if the significance of $\Sigma MR_{1,2}$ term in eqn. 6 shows that the interaction with the stationary phase also plays a role in HPLC.

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