Relationship between Lipophilic Character and Urinary Excretion of Nitroimidazoles and Nitrothiazoles in Rats

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Many processes are involved in the renal excretion of drugs, but very little is known about their quantitative structure–activity relationship. The relationship between urinary excretion and lipophilic character of a series of nitroimidazoles and nitrothiazoles was studied. The unmetabolized forms of the drugs were detected in the urine by means of UV and HPLC procedures. The urinary excretion of unmetabolized forms is parabolically related with the log P, as an expression of lipophilic character of molecules.

In recent years there has been a considerable interest in the study of the mutagenic activity of nitroimidazoles.[1] In previous reports we have investigated the mutagenic activity in vitro of a series of nitroimidazoles and nitrothiazoles and its relationship with physicochemical parameters.[2-5] The mutagenic activity was shown to be influenced by both the R_m value, as an expression of the lipophilic character of the molecules, and an interaction term taking into account the positive effect exerted by substituents characterized by higher molar refractivity and capable of hydrogen bonding.[5] More recently we have determined the urinary excretion of some of the above compounds in their unchanged and metabolic forms by means of a HPLC technique.[6]

In an attempt to clarify the role played by the lipophilic character of drugs in the very complex process of renal excretion,[7] we have investigated the relationship between the lipophilic character and the urinary excretion of the unchanged forms of a series of 26 nitroheterocyclic compounds. The present paper describes the results of that work.

Results

Relationship between Log BR and Log P Values. The amounts of the unchanged form of each drug were detected in the urine samples collected at 0–18, 18–36, 36–54, and 54–72 h after the treatment. The total 18, 36, 54, and 72 h urinary excretion of the unchanged form of each drug was then calculated and the data reported in Table II as log (percent × 10) of the administered dose. As a first step in our work we detected the unchanged form of 11 compounds in urine by means of both the UV and HPLC technique. The very good correlation coefficient of eq 1 seems to justify the use in the main part of our work

$$\log BR_{HPLC} = -0.352\ (\pm 0.149) + 1.136\ (\pm 0.067) \log BR_{UV} \quad (1)$$

$$n = 11 \quad r = 0.985 \quad s = 0.063 \quad F = 290.68 \quad P < 0.005$$

of the only UV procedure for the detection of the unchanged forms in urine. The log BR_{UV} values showed that the unchanged forms of 25 test compounds were present in the urine collected at 0–18 h. In particular, for compounds **26**, **10**, and **13** the unmetabolized forms excreted in the 18-h period accounted for 48.6%, 43.6%, and 41.7%, respectively, of the administered dose. On the other hand, for **14**, **22**, **4**, and **25** the unmetabolized forms excreted in the same time accounted only for 2.5%, 4.1%, 4.3% and 4.6%, respectively, of the administered dose. The only exception was represented by **15** for which it was not possible to detect its unchanged form in the 0–18-h urine. The HPLC technique also did not show any peak with the retention time of compound **15**, and as shown in a previous paper,[2] the urine collected in the first 18 h did not demonstrate any mutagenic activity in the Ames test, which could be related to the absence of any active form of **15**. The lack of detectable traces of **15** in urine in the first 18 h after application could be due to higher biotransformation in the liver and/or higher renal reabsorption. This could be related to the fact that **15** shows the highest log P value in the series reported in Table I. However, the lipophilic character seemed to influence the excretion of all the test compounds. The relationship between the log P values of the compounds and their log BR values is described by eq 2 and 3.

18 hours

$$\log BR_{UV} = 2.240\ (\pm 0.069) - 0.217\ (\pm 0.119) \log P \quad (2)$$

$$n = 25 \quad r = 0.356 \quad s = 0.347 \quad F = 3.35 \quad P < 0.10$$

$$\log BR_{UV} = 2.570\ (\pm 0.042) + 0.050\ (\pm 0.054) \log P - 0.964\ (\pm 0.089)\ (\log P)^2 \quad (3)$$

$$n = 25 \quad r = 0.928 \quad s = 0.141 \quad F = 68.37 \quad P < 0.005$$

The introduction of the $(\log P)^2$ term into eq 2 significantly improved the correlation coefficient of eq 3, showing a quadratic regression between lipophilic character and urinary excretion. Obviously, because of the lack in the 18-h sample of urine of the unchanged form of **15**, eq 2 and 3 were calculated for only 25 compounds (Figure 1). The ideal log P_0, i.e. the lipophilic character determining the maximum of the urinary excretion, was calculated from eq 3 and found to be log P_0 = 0.026 (0.079 and −0.055 being the 95% confidence limits). On the basis of eq 3, compound **15** with a log P = 2.03 should have a log BR of −1.30, which means a very low excretion percentage (0.05%). This might explain the lack of any detectable amount of **15** in the 18-h urine sample. Since 11 compounds had been

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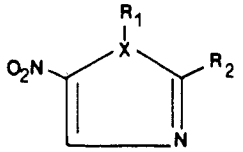
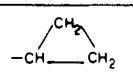
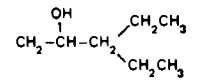
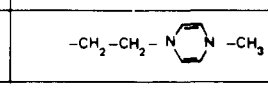
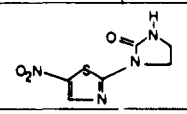
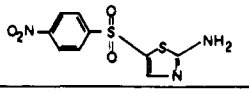
Table I. Structural Formulas and Physicochemical Parameters of the Nitroheterocyclic Compounds



Compound		Structure			Empirical formula	Molecular weight	log k′	R_m	log P
No.	Name	X	R_1	R_2					
1	5-Nitroimidazole	N	–H	–H	$C_3H_3N_3O_2$	113.08	0.000	–0.19	–0.16
2	2-Methyl-5-nitroimidazole	N	–H	$-CH_3$	$C_4H_5N_3O_2$	127.10	0.141	0.26	0.49
3	1-Ethanol-2-methyl-5-nitro-imidazole (Metronidazole)	N	$-CH_2CH_2OH$	$-CH_3$	$C_6H_9N_3O_3$	171.16	–0.067	0.08	–0.10
4	1-Methyl-2 [1-methylethyl)-5--nitroimidazole (Ipronidazole)	N	$-CH_3$	$-CH(CH_3)CH_3$	$C_7H_{11}N_3O_2$	169.18	0.450	0.72	1.06
5	1-Methyl-2-formyl-5-nitro-imidazole	N	$-CH_3$	–CHO	$C_5H_5N_3O_3$	155.11	–0.067	–0.16	–0.69
6	1-(2-ethyl-carbamothioic acid O-methyl ester)-2-methyl-5--nitroimidazole (Carnidazole)	N	$-CH_2CH_2NHCOCH_3$ (S)	$-CH_3$	$C_8H_{12}N_4O_3S$	244.27	0.465	0.81	0.90
7	1-[2-(ethylsulfonyl)-ethyl]-2--methyl-5-nitroimidazole (Tinidazole)	N	$-CH_2CH_2SO_2CH_2CH_3$	$-CH_3$	$C_8H_{13}N_3O_4S$	247.26	–0.125	0.35	–0.36
8	1-[(α-chloromethyl)-ethanol]-2--methyl-5-nitroimidazole (Ornidazole)	N	$-CH(CH_2Cl)CH_2OH$	$-CH_3$	$C_7H_{10}N_3O_3Cl$	219.63	0.199	0.33	0.60
9	1-[Methyl-2-(methanol-carbamate)]5-nitroimidazole (Ronidazole)	N	$-CH_3$	$-CH_2CONH_2$	$C_6H_8N_4O_4$	200.16	0.141	–0.07	–0.38
10	1-(2-N-morpholinylethyl)-5-nitroimidazole (Nimorazole)	N	$-CH_2-CH_2-$ N O	–H	$C_9H_{14}N_4O_3$	266.23	0.188	0.97	0.07
11	1-Methyl-2-hydroxymethyl-5--nitroimidazole	N	$-CH_3$	$-CH_2OH$	$C_5H_7N_3O_3$	157.12	–0.110	–0.14	–0.03
12	1-Methyl-2-(2-amino-4-ethinyl--pyrimidine)-5-nitroimidazole (Azanidazole)	N	$-CH_3$	H_2N, N, N, –CH=CH	$C_{10}H_{10}N_6O_2$	246.23	0.423	1.31	0.85
13	(DA 3804)	N	$-CH_3$	$-CH_2-$ N N $-CH_3$	$C_{10}H_{17}N_6O_2$	239.29	0.014	1.44	–0.33
14	(DA 3831)	N	$-CH_3$	–CH=C(COOH)–O–	$C_{13}H_{11}N_3O_6$	289.25	–0.199	0.69	–1.00
15	(DA 3832)	N	$-CH_2-CH_2OH$	–CH=CH– O, O, CH_2	$C_{14}H_{13}N_3O_5$	303.27	0.659	1.68	2.03
16	(DA 3838)	N	$-CH_2-CH(OH)-CH_2OH$	$-CH_3$	$C_7H_{11}N_3O_4$	201.20	–0.199	–0.13	–0.63
17	(DA 3839)	N	$-CH_2CO-NH-$	$-CH_3$	$C_{10}H_{10}N_6O_3$	262.23	–0.115	0.95	–0.30
18	(DA 3840)	N	$-CH_2-CO-$ N N $-CO-CH_2$, O_2N, CH_3	$-CH_3$	$C_{16}H_{20}N_8O_6$	402.36	0.028	1.58	–0.70
19	(DA 3853)	N	$-CH_2-CH(OH)-CH_2-$ N, CH_3, N, NO_2	$-CH_3$	$C_{11}H_{14}N_6O_5$	310.27	0.199	1.11	0.31

Table I (Continued)

Compound		Structure			Empirical formula	Molecular weight	log k'	R_m	log P
20	(DA 3854)	N	$-CH_2-CH_2-O-CH_2-CH_2-N(CH_2CH_3)(CH_2CH_3)$	$-CH_3$	$C_{12}H_{22}N_4O_3$	270.34	0.021	1.56	−0.38
21	(MY 40/20)	N	$-CH_2-CH_2-SO_2-CH_3$	$-CH_3$	$C_7H_{11}N_3SO_4$	233.24	−0.097	−0.30	−0.35
22	(DA 3851)	N	$-CH_3$		$C_7H_9N_3O_2$	167.16	0.455	0.68	1.00
23	(DA 3829)	N		$-CH_3$	$C_{11}H_{20}N_4O_3$	256.30	0.058	0.96	−0.08
24	(DA 3915)	N		$-H$	$C_{10}H_{13}N_5O_2$	235.25	0.021	−0.30	−0.20
25	1-(5-nitro-2-thiazolyl)-2-imidazolidinone (Niridazole)				$C_6H_7N_4O_3S$	215.20	0.383	0.59	0.92
26	2-amino-5(p-nitrophenyl sulfonyl)-thiazole				$C_9H_7N_3O_4S_2$	285.30	−0.049	--	−0.43

detected by means of the HPLC technique in the urine collected in the first 18 h after application, eq 4 and 5 have been calculated with those log BR_{HPLC} values.

$$\log BR_{HPLC} = 2.226\ (\pm 0.074) - 0.417\ (\pm 0.122) \log P \quad (4)$$

$n = 11$ $r = 0.752$ $s = 0.240$ $F = 11.73$
$P = <0.05$

$$\log BR_{HPLC} = 2.469\ (\pm 0.069) - 0.069\ (\pm 0.104) \log P - 0.788\ (\pm 0.175)\ (\log P)^2 \quad (5)$$

$n = 11$ $r = 0.936$ $s = 0.136$ $F = 28.44$
$P < 0.005$

As one could have expected on the basis of eq 1, eq 4 and 5, despite the lower number of compounds, are fairly similar to eq 2 and 3. The log P_0 value of −0.044 from eq 5 is very close to that of eq 3. On the other hand, eq 4 and 5 are similar to eq 6 and 7 calculated with the UV data for the same 11 compounds.

$$\log BR_{UV} = 2.264\ (\pm 0.069) - 0.342\ (\pm 0.113) \log P \quad (6)$$

$n = 11$ $r = 0.710$ $s = 0.222$ $F = 9.17$
$P < 0.05$

$$\log BR_{UV} = 2.491\ (\pm 0.062) - 0.018\ (\pm 0.093) \log P - 0.733\ (\pm 0.158)\ (\log P)^2 \quad (7)$$

$n = 11$ $r = 0.930$ $s = 0.110$ $F = 25.78$
$P < 0.005$

In conclusion eq 4–7 seem to point out again the agreement between the data obtained with either the HPLC technique or the UV procedure.

As a further step in our work we turned our attention to the data describing the cumulative excretion of the test compounds over 36, 54, and 72 h. In particular it was rather interesting to compare the excretion percentages in the first 18 h with the cumulative data from the urine collected over 36 h. In fact, the excretion of **14** increased from 2.5% to 19% of the administered dose; moreover, it was possible to show a urinary recovery of 3.4% for **15**. Therefore from 18 to 36 h the largest relative increments in urinary excretion seemed to involve both the most hydrophilic and the most lipophilic compound. On the contrary, the data obtained from the urine collected over 54- and 72-h periods did not show any further significant increase in the excretion percentages. From any practical point of view, the unchanged form of the test compounds seems to be totally excreted in the first 36 h after administration. The relationships between the log P and log BR_{UV} values from the urine collected over 36, 54, and 72 h are described by eq 8–13. Since the 18-h urine sample did not show any detectable amount of **15**, eq 8–13 were first calculated without that compound in order to allow a better comparison with eq 2–3 (Figure 1).

36 hours

$$\log BR_{UV} = 2.490\ (\pm 0.044) - 0.257\ (\pm 0.076) \log P \quad (8)$$

$n = 25$ $r = 0.577$ $s = 0.222$ $F = 11.51$
$P < 0.005$

$$\log BR_{UV} = 2.661\ (\pm 0.045) - 0.119\ (\pm 0.058) \log P - 0.499\ (\pm 0.096)\ (\log P)^2 \quad (9)$$

$n = 25$ $r = 0.838$ $s = 0.151$ $F = 25.98$
$P < 0{,}005$ $\log P_0 = -0.119$

54 hours

$$\log BR_{UV} = 2.508\ (\pm 0.045) - 0.259\ (\pm 0.077) \log P \quad (10)$$

$n = 25$ $r = 0.572$ $s = 0.227$ $F = 11.20$
$P < 0.005$

$$\log BR_{UV} = 2.678\ (\pm 0.047) - 0.121\ (\pm 0.061) \log P - 0.501\ (\pm 0.100)\ (\log P)^2 \quad (11)$$

$n = 25$ $r = 0.828$ $s = 0.159$ $F = 23.99$
$P < 0.005$ $\log P_0 = -0.121$

72 hours

$$\log BR_{UV} = 2.512\ (\pm 0.046) - 0.254\ (\pm 0.079) \log P \quad (12)$$

$n = 25$ $r = 0.557$ $s = 0.232$ $F = 10.32$
$P < 0.005$

$$\log BR_{UV} = 2.687\ (\pm 0.048) - 0.113\ (\pm 0.062) \log P - 0.510\ (\pm 0.103)\ (\log P)^2 \quad (13)$$

$n = 25$ $r = 0.822$ $s = 0.163$ $F = 22.86$
$P < 0.005$ $\log P_0 = -0.111$

Table II. Urinary Excretion of Nitroheterocyclic Compounds over 18-, 36-, 54-, and 72-h Periods (Log BR[a] ± SE)

Compd no.	HPLC analysis		UV analysis								Wavelength (nm)
	0-18 h		0-18 h		0-36 h		0-54 h		0-72 h		
	%	log BR	%	log BR	%	log BR	%	log BR	%	log BR	
1	-	-	33.8	2.529 ± 0.11	39.9	2.601 ± 0.03	46.1	2.664 ± 0.03	46.1	2.664 ± 0.03	295
2	-	-	29.8	2.474 ± 0.09	38.1	2.581 ± 0.01	40.6	2.609 ± 0.01	42.2	2.626 ± 0.01	310
3	25.5	2.407 ± 0.05	30.5	2.484 ± 0.10	49.2	2.692 ± 0.02	49.2	2.692 ± 0.02	49.2	2.692 ± 0.02	320
4	2.5	1.398 ± 0.10	4.3	1.633 ± 0.05	9.1	1.960 ± 0.07	9.1	1.960 ± 0.07	9.1	1.960 ± 0.07	320
5	10.4	2.023 ± 0.09	10.6	2.025 ± 0.04	29.9	2.477 ± 0.02	29.9	2.477 ± 0.02	29.9	2.477 ± 0.02	310
6	6.5	1.819 ± 0.02	6.7	1.826 ± 0.08	21.5	2.332 ± 0.04	23.1	2.364 ± 0.03	23.1	2.364 ± 0.03	320
7	27.3	2.436 ± 0.02	29.4	2.468 ± 0.11	66.3	2.822 ± 0.01	66.3	2.822 ± 0.01	66.3	2.822 ± 0.01	315
8	11.7	2.068 ± 0.06	13.2	2.121 ± 0.13	29.6	2.471 ± 0.01	36.9	2.567 ± 0.02	42.1	2.625 ± 0.02	320
9	27.2	2.495 ± 0.11	28.3	2.452 ± 0.05	57.4	2.759 ± 0.02	60.0	2.778 ± 0.01	60.0	2.778 ± 0.01	310
10	-	-	43.6	2.639 ± 0.05	52.4	2.719 ± 0.07	52.4	2.719 ± 0.07	52.4	2.719 ± 0.07	305
11	-	-	32.6	2.513 ± 0.02	56.8	2.754 ± 0.01	60.6	2.782 ± 0.01	64.9	2.812 ± 0.01	310
12	11.3	2.054 ± 0.05	13.8	2.142 ± 0.04	16.0	2.202 ± 0.03	16.0	2.202 ± 0.03	16.0	2.202 ± 0.03	380
13	-	-	41.7	2.620 ± 0.06	45.5	2.658 ± 0.01	45.5	2.658 ± 0.01	45.5	2.658 ± 0.01	320
14	-	-	2.5	1.400 ± 0.14	19.0	2.280 ± 0.04	21.7	2.337 ± 0.03	21.7	2.337 ± 0.03	355
15	-	-	-	-	3.4	1.529 ± 0.01	3.4	1.529 ± 0.01	3.4	1.529 ± 0.01	320
16	-	-	14.3	2.155 ± 0.10	64.3	2.808 ± 0.02	66.4	2.822 ± 0.03	66.4	2.822 ± 0.03	325
17	35.4	2.549 ± 0.07	37.1	2.569 ± 0.07	38.9	2.590 ± 0.03	42.1	2.701 ± 0.01	42.1	2.701 ± 0.01	325
18	-	-	14.3	2.155 ± 0.06	18.6	2.270 ± 0.02	18.6	2.270 ± 0.02	18.6	2.270 ± 0.02	325
19	15.7	2.196 ± 0.02	18.2	2.260 ± 0.11	22.8	2.358 ± 0.03	22.8	2.358 ± 0.03	22.8	2.358 ± 0.03	325
20	-	-	23.4	2.369 ± 0.07	28.5	2.455 ± 0.04	28.5	2.455 ± 0.04	28.5	2.455 ± 0.04	325
21	24.8	2.394 ± 0.06	25.2	2.401 ± 0.05	27.9	2.446 ± 0.02	28.0	2.448 ± 0.02	28.0	2.448 ± 0.02	322
22	-	-	4.1	1.613 ± 0.04	12.9	2.113 ± 0.03	12.9	2.113 ± 0.03	12.9	2.113 ± 0.03	338
23	-	-	36.4	2.561 ± 0.09	51.7	2.713 ± 0.02	51.7	2.713 ± 0.02	51.7	2.713 ± 0.02	327
24	-	-	17.0	2.230 ± 0.07	31.3	2.496 ± 0.02	31.3	2.496 ± 0.02	31.3	2.496 ± 0.02	315
25	-	-	4.6	1.663 ± 0.08	7.3	1.861 ± 0.03	7.3	1.861 ± 0.03	7.3	1.861 ± 0.03	375
26	-	-	48.6	2.687 ± 0.05	64.7	2.811 ± 0.02	64.7	2.811 ± 0.02	64.7	2.811 ± 0.02	335

a log BR is log (% x 10) of the administered dose

The introduction of the $(\log P)^2$ term into eq 8, 10, and 12 significantly improved the correlation coefficient and therefore eq 9, 11, and 13 still showed a parabolic relationship between the lipophilic character of test compounds and their urinary excretion.

The somewhat lower correlation coefficients of eq 9, 11, and 13, when compared with that of eq 3, can be explained by considering the relationship between the correlation coefficient, r, and the standard error, s. In fact, $r^2 = 1 - [s^2(n - 3)/\text{Dev } y]$, where Dev y is the sum of squares of the deviations of the y values from their mean. Since in eq 9, 11, and 13 the s values are very close to that of eq 3, the lower r values of eq 9, 11, and 13 must be due to their smaller Dev y. This is a consequence of the narrower range of the log BR values at 36, 54, or 72 h, which is reflected also in the broadening of the parabolas, as shown by the lower coefficients of the $(\log P)^2$ term in eq 9, 11, and 13. All this seems to be due to the higher excretion of the most hydrophilic and lipophilic compounds, which takes place from 18 to 36 h. The log P_0 values corresponding to the maxima of the curves described by eq 9, 11, and 13 are respectively –0.119, –0.121, –0.111 and therefore very close to the log P_0 of 0.026 for eq 3. As one might have expected the optimal lipophilic character for the urinary excretion in rats of the test compounds is a constant.

Finally eq 8, 10, and 12 are very similar, as are eq 9, 11, and 13, pointing out that after the first 36 h there are not significant changes. The overall picture did not seem to be modified in a relevant way when eq 8–13 were recalculated with the addition of **15**.

36 hours

$$\log \text{BR}_{\text{UV}} = 2.479\ (\pm 0.042) - 0.325\ (\pm 0.065) \log P \quad (14)$$

$$n = 26 \quad r = 0.716 \quad s = 0.229 \quad F = 25.29 \quad P < 0.005$$

$$\log \text{BR}_{\text{UV}} = 2.578\ (\pm 0.043) - 0.143\ (\pm 0.069) \log P - 0.234\ (\pm 0.059) (\log P)^2 \quad (15)$$

$$n = 26 \quad r = 0.843 \quad s = 0.180 \quad F = 28.31 \quad P < 0.005 \quad \log P_0 = -0.305$$

54 hours

$$\log \text{BR}_{\text{UV}} = 2.496\ (\pm 0.046) - 0.329\ (\pm 0.066) \log P \quad (16)$$

$$n = 26 \quad r = 0.713 \quad s = 0.234 \quad F = 24.81 \quad P < 0.005$$

$$\log \text{BR}_{\text{UV}} = 2.596\ (\pm 0.045) - 0.145\ (\pm 0.070) \log P - 0.237\ (\pm 0.061) (\log P)^2 \quad (17)$$

$$n = 26 \quad r = 0.839 \quad s = 0.185 \quad F = 27.45 \quad P < 0.005 \quad \log P_0 = -0.306$$

72 hours

$$\log \text{BR}_{\text{UV}} = 2.500\ (\pm 0.047) - 0.327\ (\pm 0.068) \log P \quad (18)$$

$$n = 26 \quad r = 0.702 \quad s = 0.240 \quad F = 23.31 \quad P < 0.005$$

$$\log \text{BR}_{\text{UV}} = 2.604\ (\pm 0.046) - 0.138\ (\pm 0.072) \log P - 0.244\ (\pm 0.062) (\log P)^2 \quad (19)$$

$$n = 26 \quad r = 0.834 \quad s = 0.189 \quad F = 26.33 \quad P < 0.005 \quad \log P_0 = -0.283$$

One might point out that compound **15** provokes a shift to the left of the parabolas, which is described by the lower log P_0 values of eq 15, 17, and 19, and a further decrease of the coefficients of their $(\log P)^2$ terms. In conclusion, the unchanged forms of the compounds characterized by log P values close to the ideal lipophilic character seem to be almost totally excreted in the first 18 h after application. Both the most hydrophilic and the most lipophilic compounds are excreted more slowly, and a period of 36 h is necessary for the total excretion of their unchanged forms. As a consequence, the parabolas calculated with the data from the 36-, 54-, and 72-h urine are less skewed than that from the 18-h urine. An interesting point seems to arise from eq 20, which was calculated with the data from over just the second 18 h, i.e. by subtracting in Table II the log BR values of the 0–18-h from those of the 0–36-h period.

$$\Delta \log \text{BR}_{\text{UV}} = 0.116\ (\pm 0.048) - 0.175\ (\pm 0.062) \log P + 0.438\ (\pm 0.102) (\log P)^2 \quad (20)$$

$$n = 25 \quad r = 0.685 \quad s = 0.162 \quad F = 9.70 \quad P < 0.005$$

The correlation coefficient is rather low. However, eq 20 seems to point out a reversed parabolic relationship, which is in agreement with the higher excretion of the most hydrophilic and lipophilic compounds, which takes place from 18 to 36 h.

Relationship between Log BR and R_m Values. Attempts to correlate the log BR values with R_m were much less successful than the correlations involving log P. The low correlation coefficients are explained by the very low correlation between the chromatographic R_m values and the log P, as shown in a previous paper with 22 compounds of the same series.[3] In the previous paper, a significant improvement in the equation relating R_m and log P values could be achieved by the introduction of an $\sum MR_{1,2}$ term as an expression of the molar refractivity of the R_1 and R_2 groups.[3] It was suggested that the molar refractivity could be related to the adsorption activity of the silica gel layer. Unger et al. had pointed out that small, basic, unhindered pyridines deviated from the agreement between shake-flask and reversed-phase HPLC procedure, presumably because of binding to residual silanol sites.[14] As the 5-nitroimidazoles are small, basic molecules, their deviation could be due to a similar kind of interaction with the silica gel. Therefore, at least for the present series of compounds, the R_m values could be an expression of both the lipophilic and polar character of the molecules. It is interesting to note that the R_m values, while useless in the present pharmacokinetic study, had been shown to play a significant role in studying the relationship between mutagenic activity and physicochemical parameters of 20 nitroimidazoles of the same series.[5]

Relationship between Log BR and Log k' Values. The log k' values of Table I allowed the calculation of eq 21 and 22 with the log BR values from the 18-h urine. In particular, eq 22 showed a somewhat better correlation

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(9) Biagi, G. L.; Gandolfi, O.; Guerra, M. C.; Barbaro, A. M.; Cantelli-Forti, G. *J. Med. Chem.* **1975**, *18*, 868.
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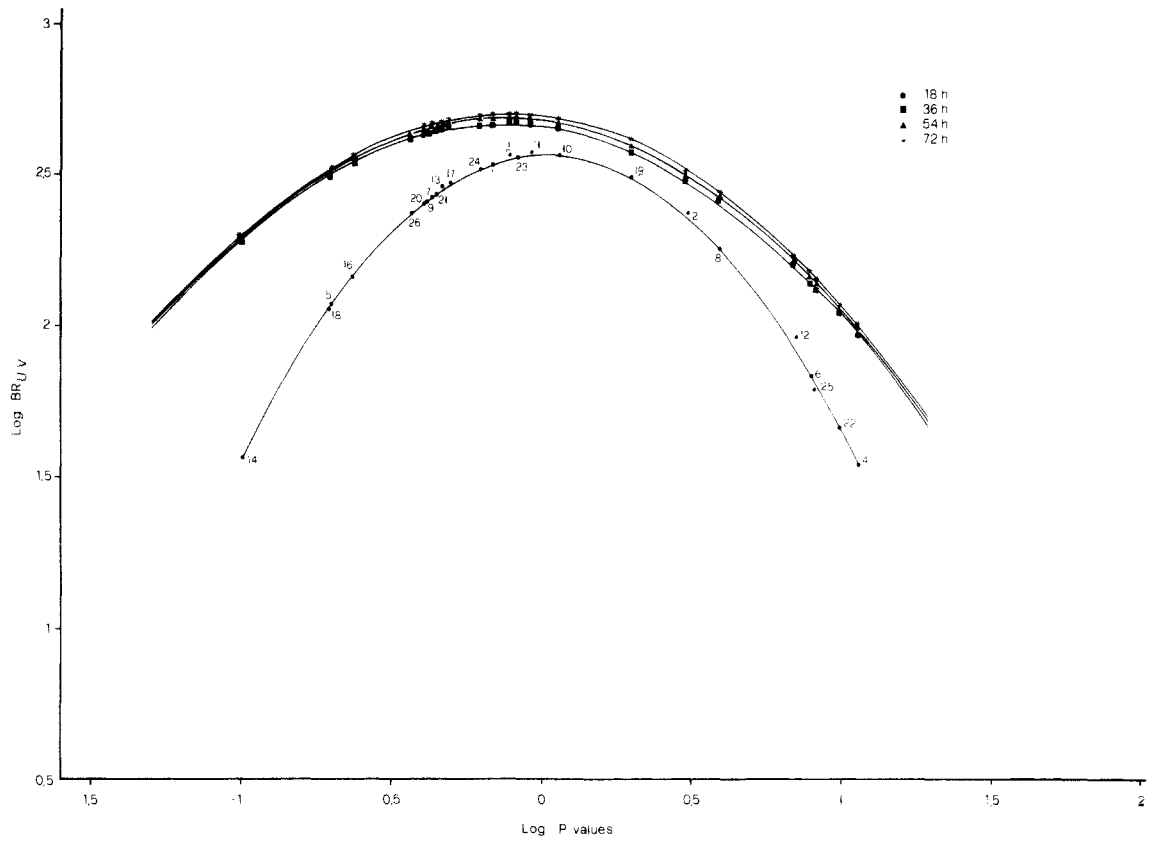

Figure 1. Relationship between urinary excretion and log P values of nitroimidazoles and nitrothiazoles.

when compared with the equations correlating log BR and R_m values. In fact, eq 23 describing the relationship between log k' and log P values shows a fairly high correlation coefficient. In a previous paper it was suggested that this might indicate that in HPLC the interaction with the stationary phase as expressed by the molar refractivity of the R_1 and R_2 groups is much less important than in TLC.[4] However the correlation coefficient of eq 22, when compared with that of eq 3, is still much lower. This seems to be mainly due to the narrower range of the log k' values in Table I as shown also by the very high coefficient of the $(\log P)^2$ term in eq 22.

$$\log BR_{UV} = 2.305\ (\pm 0.072) - 0.769\ (\pm 0.325) \log k' \quad (21)$$
$$n = 25 \quad r = 0.442 \quad s = 0.334 \quad F = 5.60$$
$$P < 0.05$$

$$\log BR_{UV} = 2.437\ (\pm 0.065) + 0.823\ (\pm 0.471) \log k' - 5.418\ (\pm 1.350)\ (\log k')^2 \quad (22)$$
$$n = 25 \quad r = 0.732 \quad s = 0.259 \quad F = 12.69$$
$$P < 0.005$$

$$\log k' = 0.084\ (\pm 0.018) + 0.317\ (\pm 0.031) \log P \quad (23)$$
$$n = 25 \quad r = 0.905 \quad s = 0.091 \quad F = 104.23$$
$$P < 0.005$$

Discussion

Excretion of drugs by the kidney involves several major processes, i.e. passive glomerular filtration, active tubular secretion of organic acids and bases, passive reabsorption of undissociated molecules, and active reabsorption. The amount of unchanged drug entering the tubular lumen by glomerular filtration is dependent on the filtration rate (renal blood flow), the degree of plasma protein binding, the extent of biotransformation in the liver and the size of the drug molecule. The nature of the filtering apparatus allows the free passage of compounds with a molecular weight of 5000 or less. However, since so many processes are involved in the renal excretion of drugs, very little is known about their quantitative structure–action relationship.[15] Lien showed a parabolic relationship between partition coefficient and renal excretion of probenecid analogues.[15] However, he pointed out that in several other investigations no satisfactory correlations have been reported.

Since our compounds have molecular weights of less than 500, practically all the free drug in the plasma will be filtered. Therefore, for our series of compounds the percent of the dose recovered from the urine in the unchanged form after ip administration should involve the following major processes: absorption from the site of inoculation, biotransformation in the liver, plasma protein binding, and tubular reabsorption. Because of their dissociation constants, the present test compounds should be in the undissociated form at the pH of the tubular urine.[3] However, while the lipophilic character plays a role in each of these processes, the parabolic relationship described by eq 3 cannot be easily explained. In fact, the ideal lipophilic character could ultimately provoke a higher renal excretion of the unchanged form by fulfilling one or more of the following conditions: higher absorption from the site of inoculation, lower biotransformation in the liver, lower degree of plasma protein binding, and/or tubular reabsorption.

A detailed analysis of each of the above pharmacokinetic processes should be necessary in order to show the influence of the lipophilic character of each of them and therefore the real meaning of our equation. On the other hand, the percent of the administered dose that has not been excreted unchanged over 18 h could have been excreted as metabolites or still be in the animal in unchanged

(15) Lien, E. J. In "Drug Design"; Ariens, E. J., Ed.; Academic Press: New York, 1975; Vol. 5, pp 81–132.

and/or metabolized form. The data from the urine collected over 36, 54, or 72 h seem to add some useful information. A comparison of the data from the 36-h urine with those from 18 h shows that the most lipophilic and hydrophilic compounds are excreted more slowly. The slower excretion of the most hydrophilic compounds could be due to their slower absorption from intraperitoneal site of administration, and that of the most lipophilic compounds could be due to their higher plasma protein binding and/or tubular reabsorption. Moreover, since the 54- and 72-h parabolas are very similar to the 36-h parabola, the unchanged forms seem to be totally excreted in the first 36 h. The percent of the administered dose that has not been excreted unchanged over 72 h should have been excreted as metabolites or still be in the animal in metabolized form. Finally, it could be very interesting to ascertain if the ideal lipophilic character shown by the present parabolic equations might help in designing compounds aimed to the treatment of urinary tract diseases.

Experimental Section

Chemicals. The drugs under study, obtained from commercial sources and drug companies, are listed in Table I. The DA and MY compounds were generous gift from Carlo Erba and Midy, respectively. Their purity was ascertained by thin-layer chromatography (TLC). All other chemicals and solvents were of reagent grade.

Animal Experiments. Female Sprague–Dawley rats (200 ± 20 g) were maintained on a laboratory chow diet. The test compounds were administered ip in Me_2SO solutions (50 μmol mL^{-1} kg^{-1}). Me_2SO is the solvent of choice for compounds insoluble in water and is considered to be without any significant mutagenic effect.[8] Preliminary control experiments were carried out in order to rule out possibility of toxic effects of Me_2SO at concentration of 1 mL/kg in rats.[9] Each test compound was administered to a group of three animals. The urine of each animal was collected with use of metal metabolic cages as 0–18, 18–36, 36–54, and 54–72 h fractions after the treatment. The water bottle was placed outside the cage and within reach of the rats so that water drained from the bottle would not mix with urine. The urine excreted was collected in a test tube submerged in an ice bath. The urine was centrifuged, and the clear supernatant fluid was filtered through a 0.22-μm Millipore filter and immediately used for the UV or HPLC analyses. All operations were carried out under yellow light, and all chemicals and biological fluids were protected from exposure to direct light in order to avoid any photochemical reactions.

Detection of Test Compounds in Urine. (a) UV Analysis. The urinary concentration of the unchanged form of each drug was detected by means of UV analysis at the appropriate wavelength reported in Table II, using a Perkin-Elmer 124 double-beam spectrophotometer. Three to five different volumes of each urine sample were diluted to 2.5 mL with water in the UV cuvette and read against a blank. This was prepared with equivalent volumes of urine collected from the same animal before the treatment. Finally the concentration of the unmodified forms was determined by means of a standard curve for each compound.

(b) High-Performance Liquid Chromatography (HPLC). One milliliter of urine was spiked with internal standard and extracted as described by Brodie et al.[10] The residue was dissolved in 200 μL of methanol and an aliquot (10–20 μL) was injected into the chromatograph. Samples of predose urine (1 mL) were spiked with the test compound and taken through the excretion procedures. Reversed-phase chromatography was performed with use of a mobile phase of 50% methanol in water. The mobile phase flow rate was 1 mL/min. The chromatograph consisted of a Waters M6000A pump (Waters Association, Northwich, U.K.) coupled to a Waters 480 Lambda-Max variable-wavelength spectrophotometer at 313 nm. Injection was made by Hamilton 802 chromatographic syringes (25 μL) via a Waters U6K, universal injector; the column was a Waters μBondapak C_{18} (300 × 3.9 mm i.d.) (Waters), packed with silica gel (particle size 10 μm) with a C_{18} chemically bonded nonpolar stationary phase. The HPLC technique has been described.[6]

Physicochemical Parameters. (a) Determination of Partition Coefficients (Log *P* Values). The octanol/water partition coefficients were determined according to the classical procedure.[3,11] The aqueous layer was ammonium chloride buffer (1 M) of pH 9.0. The partition coefficient of 14 was determined with aqueous sodium acetate–barbital buffer (0.14 M) of pH 3.6. The concentration of the compounds in octanol and/or aqueous layer was determined by UV measurement with a Perkin-Elmer 124 double-beam spectrophotometer.

(b) Determination of R_m Values. The reversed-phase chromatographic technique for the determination of R_m values had been described previously.[3,12,13] The polar mobile phase was ammonium chloride buffer (1 M) of pH 9.0, alone or in various mixtures with methanol. With 14 the mobile phase was sodium acetate–barbital buffer of pH 3.6. The nonpolar stationary phase was a silica gel GF_{254} layer impregnated with a 5% (v/v) solution of silicone oil [silicone DC 200 (350 cSt) from Applied Science Labs. (State College, PA)] in diethyl ether. The concentration of methanol in the mobile phase ranged from 5% to 30%. The compounds were dissolved in methanol or ethanol (1 mg/mL) and 1–5-μL volumes of solutions were spotted on the plates in random locations. The developed plates were dried and sprayed with an alkaline solution of potassium permanganate. Most of compounds were also visible by their fluorescence when the silica gel GF_{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. The plates were then sprayed with diazotized sulfanilic acid.

(c) Determination of HPLC Retention Times (Log k' Values). Chromatography had been performed by using the HPLC equipment described above.[4] The nitroimidazoles were separated with use of 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 times were expressed as log capacity factor (k') (Table I), where $k' = (t_x - t_0)/t_0$.

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Registry No. 1, 3034-38-6; 2, 696-23-1; 3, 443-48-1; 4, 14885-29-1; 5, 4750-57-6; 6, 42116-76-7; 7, 19387-91-8; 8, 16773-42-5; 9, 7681-76-7; 10, 6506-37-2; 11, 936-05-0; 12, 62973-76-6; 13, 54387-29-0; 14, 74550-88-2; 15, 74550-86-0; 16, 62580-80-7; 17, 74550-89-3; 18, 80348-51-2; 19, 74550-92-8; 20, 74550-90-6; 21, 30746-54-4; 22, 74550-87-1; 23, 74550-94-0; 24, 74571-56-5; 25, 61-57-4; 26, 39565-05-4.