Nitroimidazole Derivatives. Relationship between Structure and Antitrichomonal Activity

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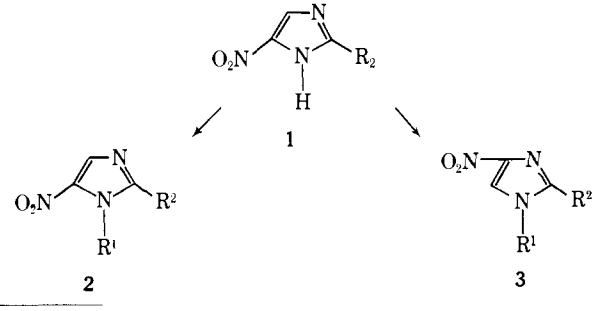
The *in vivo* trichomonacidal properties of some nitroimidazoles have been determined and are reported. Biological activity is associated with the 1-alkyl-5-nitroimidazole nucleus and is strongly influenced by the partition coefficient. Steric effects and possible metabolic effects are considered. Four new nitroimidazoles are described which are at least five times as potent as metronidazole.

Trichomoniasis is a parasitic infection of the genitourinary tract caused by *Trichomonas vaginalis*. Treatment of clinical trichomoniasis was revolutionized by the discovery in 1959 of the antitrichomonal activity of certain nitroimidazole derivatives.[1] Since that time, metronidazole (1-β-hydroxyethyl-2-methyl-5-nitroimidazole)[2] has gained wide acceptance for the systemic treatment of trichomoniasis.[3] We undertook a study of a series of nitroimidazole derivatives in order to determine the structural features which control biological activity, our ultimate goal being the design of a superior antitrichomonal compound.

Imidazole derivatives are readily prepared according to the Maquenne procedure.[4] Nitration of imidazoles yields the 4- (5-) nitroimidazoles,[5] which can be alkylated to produce either the 1-alkyl-4-nitro or 1-alkyl-5-nitro derivatives depending upon the conditions employed. Alkylation of a nitroimidazole in the presence of a base yields a 1-alkyl-4-nitro derivative (**3**), whereas alkylation under neutral or acidic conditions generally gives a 1-alkyl-5-nitroimidazole (**2**) as the major product. Substituents at C-2 of the 4- (5-) nitroimidazole (**1**) exert a steric influence on the direction of N-alkylation. When R^2 is large (*e.g.*, *t*-butyl), alkylation occurs at the least hindered position so that even under neutral or acidic conditions appreciable quantities of the 1-alkyl-4-nitroimidazole (**3**) derivative is produced. A series of nitroimidazoles obtained by these methods has been described in the recent literature.[6] The imidazoles **1–3** can be easily distinguished by their characteristic ultraviolet absorption spectra shown in Table I.



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2

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TABLE I

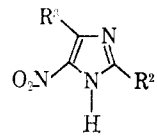
Compd	λ, mμ (ε) 0.01 *N* HCl		0.01 *N* NaOH	
1	295	(2060)	358–360	(5500)
2	278–280	(5800)	311	(9200)
3	303	(6700)	303	(6700)
1-β-Cyanoethyl-5-nitroimidazoles	305	(6400)	309–311	(8820)

Evaluation of the antitrichomonal properties of nitroimidazoles was carried out in mice inoculated intraperitoneally with 500,000 viable *Trichomonas foetus* organisms. Commencing 24 hr after inoculation the infected animals were treated with three daily oral doses of the test compound. The delay in treatment is to facilitate the establishment of the infecting organism which in untreated mice produces a significant and consistent pathological pattern. Infected mice develop a marked swelling of the abdomen within 3 days, and at autopsy, usually 24 hr after the final drug treatment, the abdominal cavity of infected control animals is filled with a viscous white fluid containing numerous trichomonads and leukocytes. Activity of test compounds is based on their ability to prevent the described pathology and eliminate the parasite. The relative potency of a series of compounds after assessing the minimum effective dose (MED) for each candidate is based on results from several trials. The MED is defined as the lowest oral dose of the test compound which clears 100% of the infected mice when administered daily on 3 consecutive days. Tables II–V include physical, chemical, and biological data from studies with various nitroimidazole derivatives. Antitrichomonal activity was observed for those compounds listed in Tables III and IV; other nitroimidazole derivatives were devoid of *in vivo* antitrichomonal activity even at high dosage.

Simple 1-alkyl-5-nitroimidazoles are weak bases (conjugate acid, pK = 2.13).[7] Protonation occurs only in strong acid solutions so that at physiological pH these compounds will exist in the uncharged form. When R^1, R^2, and R^3 (**5**) are varied through a range for simple alkyl substituents, we would expect little modification of the pK of the nitroimidazole nucleus, and this is supported by the close similarity of the ultraviolet absorption spectra in acidic solutions. The 1-β-hydroxyethyl-5-nitroimidazoles have the same absorption spectra as the simple 1-alkyl derivatives, although it is possible to differentiate these compounds in 2 *M* H_2SO_4 (metronidazole conjugate acid, pK =

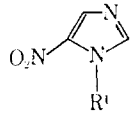
(1) Flagyl®.
(2) C. Cosar, L. Julou, and M. Bonazet, *Ann. Inst. Pasteur*, **96**, 238 (1959).
(3) H. Beckman, Yearbook of Drug Therapy 1963–1964. Year Book Publishers, Inc., Chicago, Ill., 1964, p 383.
(4) Maquenne, *Ann. Chim.* (Paris), [6] **24**, 525 (1891); H. J. Lucas and E. R. Kennedy, *Org. Syn.*, **22**, 65 (1942).
(5) R. G. Fargher and F. L. Pyman, *J. Chem. Soc.*, **115**, 217 (1919).
(6) C. Cosar, C. Crisan, R. Horclois, R. M. Jacob, J. Robert, S. Tehelitcheff, and R. Vaupré, *Arzneimittel-Forsch.*, **16**, 23 (1966).
(7) G. G. Gallo, C. R. Pasqualucci, P. Radaelli, and G. C. Lancini, *J. Org. Chem.*, **29**, 862 (1964).

Table II



No.	R^2	R^3	Mp, °C	Formula	% calcd C	% calcd H	% calcd N	% found C	% found H	% found N	K (isooctane)	MED, mg/kg
1[13]	H	H	312–313	$C_3H_3N_3O_2$	31.86	2.67	37.16	31.92	2.80	37.31	...	>200
2[13]	CH_3	H	250–253	$C_4H_5N_3O_2$	...	...	33.06	...	...	33.10	0.034	>200
3[13]	C_2H_5	H	141–145	$C_5H_7N_3O_2$	42.55	5.00	29.78	42.58	5.16	29.51	0.41	>200
4[13]	n-C_3H_7	H	161–163	$C_6H_9N_3O_2$	46.44	5.85	27.08	46.57	5.77	27.15	...	>200
5[2]	i-C_3H_7	H	180	$C_6H_9N_3O_2$	46.44	5.85	27.08	46.31	5.88	27.28	0.055	>200
6	n-C_4H_9	H	130–131	$C_7H_{11}N_3O_2$	49.69	6.55	24.84	49.95	7.07	24.56	...	>200
7	i-C_4H_9	H	170–171	$C_7H_{11}N_3O_2$	49.69	6.55	24.84	49.82	6.73	24.61	...	>200
8	t-C_4H_9	H	250–251	$C_7H_{11}N_3O_2$	49.69	6.55	24.84	49.71	6.30	24.90	...	>200
9[14]	H	CH_3	253	$C_4H_5N_3O_2$	37.80	3.97	33.06	38.08	3.93	32.92	0.025	>200
10[15]	CH_3	CH_3		$C_5H_7N_3O_2$							...	>200
11	C_2H_5	CH_3	183–185	$C_6H_9N_3O_2$	...	...	27.08	...	...	27.01	...	>200

2.55).[7] The 1-β-cyanoethyl-5-nitroimidazoles seem to be slightly less basic than the simple 1-alkyl derivatives, and they are not completely protonated in 0.01 N HCl. This is apparent from observations of the ultraviolet absorption spectra. 4- (5-) Nitroimidazoles (**1**) are considerably more acidic than the corresponding 1-alkyl derivatives and form yellow sodium salts in aqueous alkali. This difference in pK may account for the lack of biological activity of the 4- (5-) nitroimidazoles (**1**), but the various 1-alkly-5-nitromidazoles considered in this article fall within a narrow range of pK such that we would not be able to differentiate between them or correlate their biological properties with their pK values.



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The biological testing data for a large number of nitroimidazole derivatives are compiled in Tables II and V. Compounds which require a regimen greater than 200 mg/kg to control experimental trichomoniasis in mice are considered to be biologically ineffective. The simplest structural unit common to all of the biologically active nitroimidazole derivatives is the 1-alkyl-5-nitroimidazole nucleus (**4**). Therefore, this unit contains the necessary molecular architecture for *intrinsic* antitrichomonal activity. This implies that the biologically inactive 1-alkyl-5-nitroimidazoles possess intrinsic activity but fail to demonstrate this property *in vivo* because they did not arrive at the site of action within the host or parasite, or because steric effects of the ring substituents adversely affected the fit to the drug receptor. Our analysis of the data suggests that the ring substituents (R^1, R^2, R^3) have a profound effect on transport phenomena (absorption, distribution, excretion), fit to the receptor, and metabolism. These conclusions were not obvious from a cursory study of the various nitroimidazole derivatives. It was necessary first to divide the test compounds into categories to permit study of the effects of each substituent group upon the various parameters which influence *in vivo* biological activity.

In the first series of 5-nitroimidazoles studied, R^3 was H, R^2 was maintained constant, and R^1 was varied. A plot of the *in vivo* activity of **2** (R^2 = H; R^1 = lower alkyl) presented as log (1/MED) against log K (isooctane–aqueous buffer pH 7.0) is shown in Figure 1. Similar plots for **2**, R^2 = CH_3 and R_2 = $CH(CH_3)_2$, are shown in Figures 2 and 3. The shapes of the curves in these cases are similar, passing through a peak of optimum activity at a certain range of K, followed by a plateau of diminished activity and eventual loss of all biological potency when R^1 is n-octyl. If the partition coefficient (K) were the only important physical property influencing the *in vivo* activity of these compounds, we should have observed a bell-shaped distribution

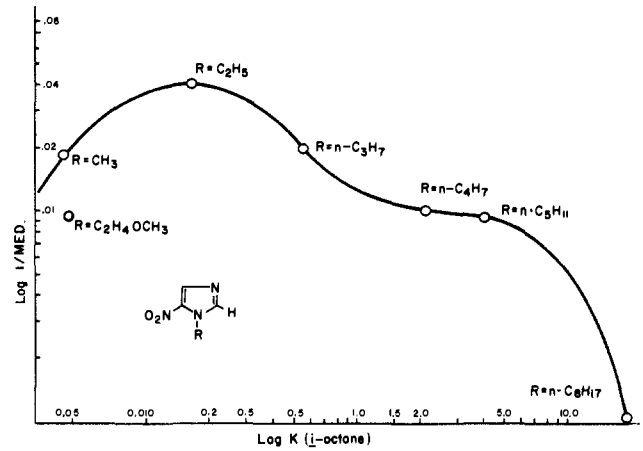


Figure 1.

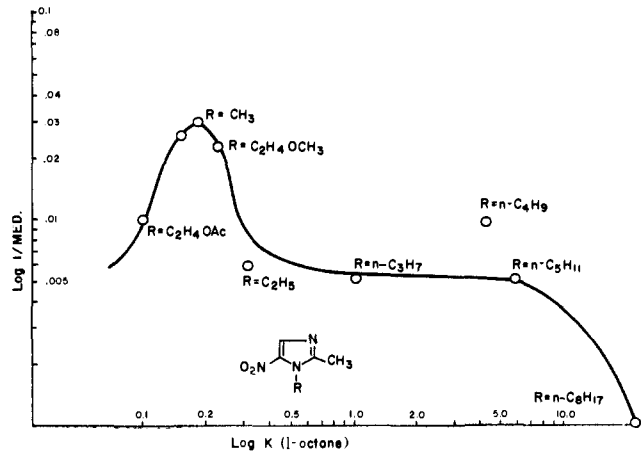
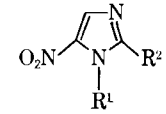


Figure 2.

TABLE III



No.	R^1	R^2	Form	Mp, °C	Formula	% calcd C	% calcd H	% calcd N	% calcd Cl or S	% found C	% found H	% found N	% found Cl or S	K (isooctane)	MED,[c] mg/kg	CD_{50},[c] mg/kg
12	CH_3	H	Base	57–58	$C_4H_5N_3O_2$	37.80	3.97	33.06		38.11	4.16	32.80		0.04 (0.91[a])	50	
13[2]	C_2H_5	H	*p*-$CH_3C_6H_4SO_3H$	174–175	$C_{12}H_{15}N_3O_5S$			13.42				13.66		0.15	25	
14[2]	$(CH_2)_2CH_3$	H	*p*-$CH_3C_6H_4SO_3H$	173–174	$C_{13}H_{17}N_3O_5S$			12.84				12.85		0.55	50	
15[2]	$(CH_2)_3CH_3$	H	*p*-$CH_3C_6H_4SO_3H$	168	$C_{14}H_{19}N_3O_5S$			12.31				12.57		2.15	100	
16[2]	$(CH_2)_4CH_3$	H	*p*-$CH_3C_6H_4SO_3H$	170–172	$C_{15}H_{21}N_3O_5S$			11.82				11.99		3.80	200	
17[2]	$(CH_2)_7CH_3$	H	*p*-$CH_3C_6H_4SO_3H$	168–169	$C_{18}H_{27}N_3O_5S$			10.57				10.29		18.10	>200	
18[2]	CH_2CH_2OH	H	HBr	Gum	$C_5H_8BrN_3O_3$									...	>200	
19	$CH_2CH_2OCH_3$	H	Base	Oil	$C_6H_9N_3O_3$			24.55				24.76		0.04	100	
20	CH_2CH_2CN	H	Base	55–58	$C_6H_6N_4O_2$	43.37	3.64	33.73		43.40	3.71	33.80		(0.39[a])	200	100
21[d,16]	CH_3	CH_3	Base	136–139	$C_5H_7N_3O_2$			29.78				29.78		0.16 (1.4[a])	50	28
22[2]	CH_2CH_2	CH_3	HCl	147	$C_6H_{10}ClN_3O_2$			21.94				21.86		0.33	200	
23[2]	$(CH_2)_2CH_3$	CH_3	HCl	151–153	$C_7H_{12}ClN_3O_2$			20.44				20.61		1.05	200	
24[2]	$(CH_2)_3CH_3$	CH_3	HCl	150–151	$C_8H_{14}ClN_3O_2$			19.14				19.37		4.3	100	
25[2]	$(CH_2)_4CH_3$	CH_3	HCl	160–162	$C_9H_{16}ClN_3O_2$			17.98				18.05		6.0	200	
26[2]	$(CH_2)_7CH_3$	CH_3	HCl	155–158	$C_{12}H_{22}ClN_3O_2$			15.23				15.21		24.2	>200	
27[1]	CH_2CH_2OH	CH_3	Base	158–160	$C_6H_9N_3O_3$									0.01	100	
28	$CH_2CH_2OCH_3$	CH	Base	Oil	$C_7H_{11}N_3O_3$	45.40	5.99	22.69		45.55	6.08	22.30		0.25	50–100	
29	CH_2CH_2CN	CH_3	Base	50	$C_7H_8N_4O_2$	46.66	4.48	31.1		46.71	4.55	31.22		0.1 (0.67[a])	21	14.7
30	$CH_2CH_2OCOCH_3$	CH_3	Base	78–80	$C_8H_{11}N_3O_4$			19.71				19.69		0.10	100	
31	CH_3	C_2H_5	Base	80–81	$C_6H_9N_3O_2$			27.08				27.02		0.30 (2.52[a])	25	17.5
32	CH_2CH_2OH	C_2H_5	Base	87–89	$C_7H_{11}N_3O_3$			22.69				22.77		...	100	
33	CH_2CH_2CN	C_2H_5	Base	118–120	$C_8H_{10}N_4O_2$	49.48	5.19	28.85		49.69	5.27	28.75		0.18 (1.75[a])	21	11.5
34	CH_3	$CH(CH_3)_2$	Base	62–63	$C_7H_{11}N_3O_2$	49.69	6.55	24.84		49.81	6.55	24.63		1.12 (4.5[a])	12.5–21.0	9.8
35	CH_2CH_3	$CH(CH_3)_2$	Picrate	118	$C_{14}H_{16}N_6O_9$			20.38				20.45		3.5[b]	50	...
36	$(CH_2)_2CH_3$	$CH(CH_3)_2$	Picrate	138–139	$C_{15}H_{18}N_6O_9$			19.71				19.60		10.1[b]	50	...
37	$(CH_2)_3CH_3$	$CH(CH_3)_2$	Picrate	172–173	$C_{16}H_{20}N_6O_9$			19.09				19.23		14.5[b]	50	
38	$(CH_2)_4CH_3$	$CH(CH_3)_2$	Picrate	174	$C_{17}H_{22}N_6O_9$			18.50				18.72		30.0[b]	100	
39	$(CH_2)_7CH_3$	$CH(CH_3)_2$	Picrate	110	$C_{20}H_{28}N_6O_9$			16.93				16.99		42.0[b]	>200	
40	CH_2CH_2OH	$CH(CH_3)_2$	Base	102–104	$C_8H_{13}N_3O_3$	48.23	6.58	21.0		48.49	6.68	20.91		0.035	100	
41	CH_2CH_2CN	$CH(CH_3)_2$	*p*-$CH_3C_6H_4SO_3H$	206	$C_{16}H_{20}N_4O_5S$	50.52	5.30	14.72	8.43	50.56	5.52	14.40	8.31	(3.53)[a]	21	13.5
42	CH_3	$(CH_2)_2CH_3$	HCl	195	$C_7H_{12}ClN_3O_2$	40.90	5.85	20.45	17.29	40.75	5.86	20.50	17.24	1.0 (5.0[a])	100	
43	CH_2CH_2CN	$(CH_2)_2CH_3$	*p*-$CH_3C_6H_4SO_3H$	182–183	$C_{16}H_{20}N_4O_5S$	50.52	5.30	14.72	5.43	50.74	5.51	14.94	8.30	(3.67[a])	100	67
44	CH_3	$(CH_2)_3CH_3$	HCl	190–193	$C_8H_{14}ClN_3O_2$	43.70	6.43	19.14	16.20	43.86	6.69	19.04	16.68	3.56 (6.15[a])	100	
45	CH_2CH_2CN	$(CH_2)_3CH_3$	*p*-$CH_3C_6H_4SO_3H$	196	$C_{17}H_{22}N_4O_5S$	51.77	5.62	14.21	8.15	51.71	5.66	14.27	7.96	(5.30[a])	100	83
46	CH_3	$CH_2CH(CH_3)_2$	HCl	190–192	$C_8H_{14}ClN_3O_2$	43.70	6.43	19.14	16.20	44.01	6.50	19.10	16.15	3.14 (6.06[a])	25	11.5
47	CH_2CH_2CN	$CH_2CH(CH_3)_2$	*p*-$CH_3C_6H_4SO_3H$	210	$C_{17}H_{22}N_4O_5S$	51.77	5.62	14.21	8.15	51.84	5.62	14.25	8.05	(5.06[a])	85	53
48	CH_3	$C(CH_3)_3$	HCl	192	$C_8H_{14}ClN_3O_2$	43.70	6.43	19.14	16.20	44.15	6.54	18.84	15.34	3.10 (5.95[a])	50	

[a] *K* for 1-octanol–phosphate buffer pH 7.2. [b] Values obtained by use of the free base. [c] Expressed in terms of the free base. [d] Dimetridazole, Emtryl®.

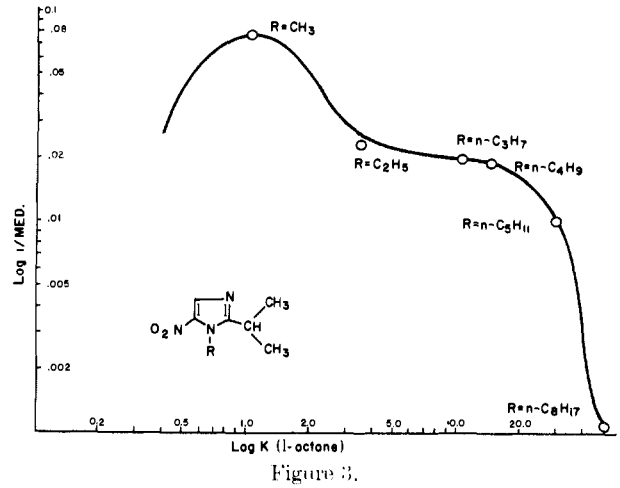
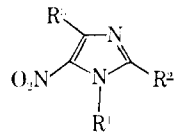


Figure 3.

curve, and all of the points in Figures 1–3 should fit onto one plot. The fact that these compounds deviate from this theoretical curve suggests that modification of the ring substituents has introduced other variable factors together with change of the partition coefficients.

The plateaus of diminished activity observed in Figures 1–3 are probably the results of steric effects of R^1 which crowd the nitro group that seems to be necessary for biological activity. This idea is reinforced by study of the compounds in Table IV. These compounds have two alkyl substituents which flank the nitro group and only the first member of each series (**5**, $R^1 = R^3 = CH_3$) has trichomonacidal properties; all other compounds where R^1 is larger than methyl are devoid of this activity. Thus, for optimum biologi-



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cal activity the nitro group should not be crowded, and to this purpose R^1 should be kept as small as possible. When R^3 is methyl, we are restricted to a methyl substituent for R^1, and the lipophilic properties can only be controlled by modification of R^2. However, changes of partition coefficient did not result in improved biological potency for this series of derivatives (Table IV) so we deduce that the steric factor is of over-riding significance. This parallels our observations for nitroimidazoles at the plateaus of Figures 1–3 where, for example, increase of K from 0.3 to 8.0 did not change the biological response. It is significant that the biologically inactive compounds **17** and **16** (R^1 = octyl, R^2 = H; and R^1 = octyl, $R^2 = CH_3$) of Table III have lower K values than **38** (R^1 = pentyl, R^2 = isopropyl), and yet **38** is as active as metronidazole in this test. Thus, the inactivity of **17** and **26** cannot be due to lipophilicity alone, but is probably the result of the steric requirements of the octyl group.

From the data presented in Table III and in Figure 2, one would predict that 1-β-hydroxyethyl-2-methyl-5-nitroimidazole is too hydrophilic to have good biological activity [metronidazole has K (isooctane) of 0.01 which places it off the scale of Figure 2]. However, despite its extremely low partition coefficient it has 1/MED of 0.01 indicating good biological potency.

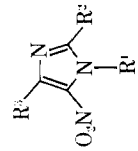
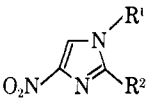


Table IV[a]

No.	R^1	R^2	R^3	Form	Mp, °C	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Calcd, % Cl	Found, % C	Found, % H	Found, % N	Found, % Cl	MED, mg/kg	A (isooctane)
40[b]	CH_3	H	CH_3	Base	57	$C_4H_7N_3O_2$	42.55	5.00	29.78		42.58	5.07	28.75		100	0.17
50	CH_3CH_2	H	CH_3	*p*-$CH_3C_6H_4SO_3H$	160	$C_{12}H_{17}N_3O_5S$			12.84				12.86		>200	0.5
51	$(CH_3)_2CH$	H	CH_3	*p*-$CH_3C_6H_4SO_3H$	148–150	$C_{13}H_{19}N_3O_5S$			12.31				12.50		>200	1.9
52	$(CH_3)_3CH_2$	H	CH_3	*p*-$CH_3C_6H_4SO_3H$	166–167	$C_{14}H_{21}N_3O_5S$			11.82				11.79		>200	6.75
53	$(CH_3)_4CH_2$	H	CH_3	*p*-$CH_3C_6H_4SO_3H$	169	$C_{15}H_{23}N_3O_5S$			11.38				11.47		>200	8.25
54	$(CH_3)_2CH_2$	H	CH_3	HCl	120–123	$C_7H_{12}ClN_3O_2$	56.33	8.67	16.43		56.51	8.68	16.38		>200	26.0
55	CH_2CH_2OH	H	CH_3	HBr	20–25	$C_6H_{10}BrN_3O_3$			16.66				16.74		>200	
56	$CH_2CH_2OCH_3$	H	CH_3	Base	Oil	$C_7H_{11}N_3O_3$	45.40	5.99	22.69		45.41	6.25	22.58		>200	0.31
57	CH_2CH_2CN	H	CH_3	Base	142–144	$C_7H_8N_4O_2$	46.66	4.48	31.10		46.71	4.42	31.15		>200	
58	CH_3	CH_3	CH_3	HCl	185–187	$C_6H_{10}ClN_3O_2$	37.60	5.27	21.90	18.55	37.57	5.40	21.96	18.33	100	
59	CH_3	C_2H_5	CH_3	Base	Oil	$C_7H_{11}N_3O_2$			24.84				25.20		100	0.75
60	CH_2CH_3	C_2H_5	CH_3	Base	Oil	$C_8H_{13}N_3O_2$			22.94				22.71		>200	3.4
61	$(CH_3)_2CH$	C_2H_5	CH_3	Base	Oil	$C_9H_{15}N_3O_2$			21.31				22.63		>200	9.15
62	$(CH_3)_3CH_2$	C_2H_5	CH_3	HCl	135–137	$C_{10}H_{18}ClN_3O_2$	48.49	7.32	16.96	14.32	48.52	7.08	16.80	14.35	>200	17.1
63	$(CH_3)_4CH_2$	C_2H_5	CH_3	HCl	150	$C_{11}H_{20}ClN_3O_2$	50.49	7.70	16.06	13.55	50.57	7.72	16.13	13.55	>200	28.5
64	$(CH_3)_2CH_2$	C_2H_5	CH_3	HCl	Gum	$C_{14}H_{26}ClN_3O_2$									>200	32
65	CH_2CH_2OH	C_2H_5	CH_3	HBr	167	$C_8H_{14}BrN_3O_3$	34.28	5.02	14.98		34.34	4.91	14.80		>200	0.39
66	$CH_2CH_2OCH_3$	C_2H_5	CH_3	Base	Oil	$C_9H_{15}N_3O_4$			19.71				19.99		>200	1.89

TABLE V



No.	R^1	R^2	Form	Mp, °C	Formula	% calcd C	% calcd H	% calcd N	% found C	% found H	% found N	MED, mg/kg
76[2]	ClH_3	CH_3	Base	183–184	$C_5H_7N_3O_2$	42.55	5.00	29.78	42.73	5.22	29.60	>200
68[2]	CH_2CH_2OH	CH_3	Base	129–130	$C_6H_9N_3O_3$	42.10	5.30	24.55	42.15	5.41	25.02	>200
69[2]	CH_3	$CH(CH_3)_2$	Base	132–133	$C_8H_{13}N_3O_2$	52.44	7.15	22.94	52.50	7.15	22.86	>200
70[2]	CH_3	H	Base	134–135	$C_4H_5N_3O_2$	37.80	3.97	33.06	37.81	3.66	33.15	>200

The same is true for the 1-β-hydroxyethyl-2-isopropyl-5-nitroimidazole and for the corresponding 2-ethyl analog. One probable metabolic product of metronidazole is the O-acetyl derivative, which has the same MED as metronidazole and fits quite closely to the experimental curve in Figure 2. Of course, this does not constitute evidence for the *in vivo* metabolism of metronidazole to produce more lipophilic, active derivatives; rather it serves to point out an apparent anomaly concerning the 1-β-hydroxyethyl-5-nitroimidazoles in general.

In order to examine the hypothesis that a small alkyl substituent is required at R^1, we compared a series of 1-methyl-5-nitroimidazoles, where the partition coefficient is varied by change of R^2. The observed relationship is shown in Figure 4. Optimum biological

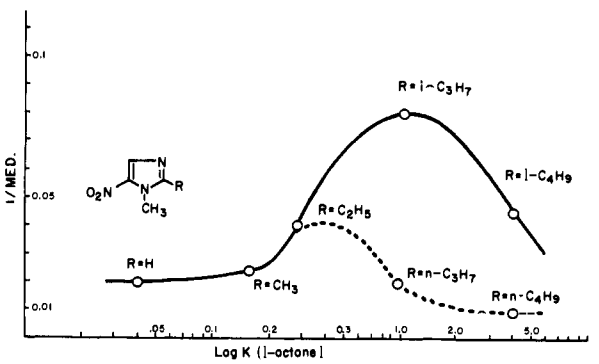


Figure 4.

activity is associated with a branched side-chain at C-2 and a K value of about 1.0, but for both the straight-chain and the branched-chain homologs the plots resemble bell-shaped, normal distribution curves. From such data we conclude that partition coefficient is of prime importance for good biological potency, but that the size of R^1 must be kept small. The pronounced difference between straight-chain and branched-chain alkyl substituents at C-2 is probably associated with drug metabolism. It is known that 1,2-dimethyl-5-nitroimidazole is metabolized by oxidation at the C-2 substituent to produce an alcohol, which is further oxidized to the carboxylic acid.[8] Substitution at C-2 will modify this metabolic process and change the drug half-life, thus 1-methyl-2-isopropyl-5-nitroimidazole should have a longer half-life than the 2-*n*-propyl analog and this, in part, might account for the superior *in vivo* activity of the 2-isopropyl-5-nitroimidazole derivatives. A similar relationship is observed for the 2-butyl derivatives, but both the branched and unbranched 1-methyl-2-butyl-5-nitroimidazoles tend to be too lipophilic to have really good antitrichomonal activity.

All of these observations can be conveniently summarized by superimposing Figure 1–3 upon Figure 4, as shown in Figure 5. The most obvious feature of this presentation is that 1-methyl-2-isopropyl-5-nitroimidazole has the greatest biological potency and its partition coefficient is close to 1.0, which suggests that this is the ideal partition coefficient for good biological activity in this particular system. If we consider the series where R^2 = methyl we observe that as K increases there is an increase in biological activity followed by a depression (when R^1 = Et, Pr, Bu, and pentyl) and finally all activity is lost when R^1 = octyl. The partition coefficient of 1-propyl-2-methyl-5-nitroimidazole is very close (K = 1.05) to the value we consider necessary for optimum *in vivo* antitrichomonal activity (K = 1.0, as for 1-methyl-2-isopropyl-5-nitroimidazole). Nevertheless, 1-*n*-propyl-2-methyl-5-nitroimidazole fails to show good antitrichomonal activity because the 1-propyl substituent imposes a severe steric effect which drastically reduces biological potency. If we reduce the size of R^1 but keep K close to 1.0, we would expect an increase in biological potency and this is observed for 1-ethyl-2-ethyl-5-nitroimidazole. Further reduction of the size of R^1 results in still better antitrichomonal activity as observed for 1-methyl-2-*n*-propyl-5-nitroimidazole. In the case of these three compounds K was essentially constant and we changed only the size of R^1, and the increase in potency is thus attributed to the reduction in size of this group. The final point on the vertical line AX is 1-methyl-2-isopropyl-5-nitroimidazole, and for this substance the improved potency must be due to modification of metabolism or fit of the drug. A similar set of compounds would be 1-butyl-2-methyl-, 1-propyl-2-ethyl-, 1-ethyl-2-isopropyl-, and 1-methyl-2-isobutyl-5-nitroimidazole, but all of these compounds are too lipophilic (K = *ca.* 3.0) to have really good antitrichomonal properties. It is clear that lengthening of R^1 in most cases introduced severe steric effects long before we were able to obtain the ideal K value for optimum biological activity (all compounds to the left of the vertical line AX). Compounds to the right of this line are too lipophilic and/or too sterically hindered to be good trichomonacides.

The line BX links a series of derivatives with equal steric effects at R^1, and potency increases with increased values of K. A parallel line links the 1-ethyl derivatives.

The major purpose of this research was to derive some basis for the logical design of a drug. We were quite successful, and were able to predict biological activity of compounds with good accuracy. Many of the points shown in Figure 5 were predicted and then

(8) G. K. Law, G. P. Mansfield, D. F. Muggleton, and E. W. Parnell, *Nature*, **197**, 1034 (1963).

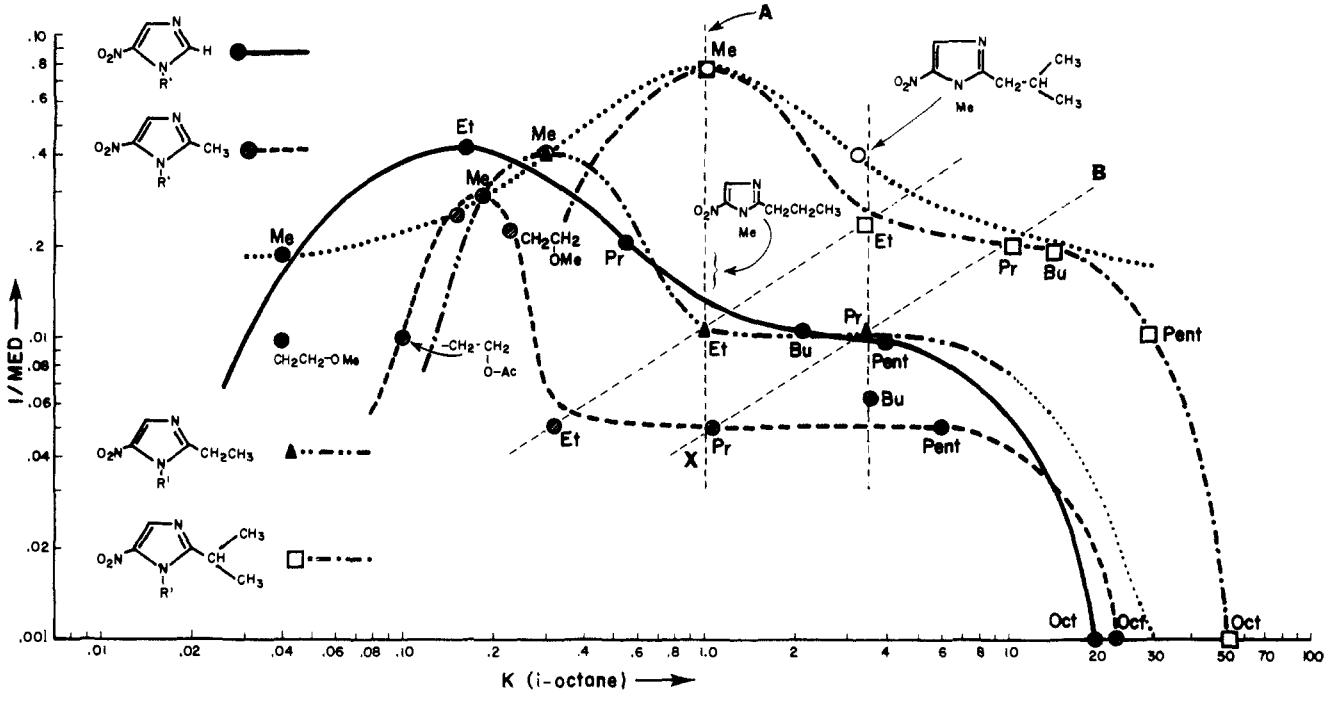


Figure 5.

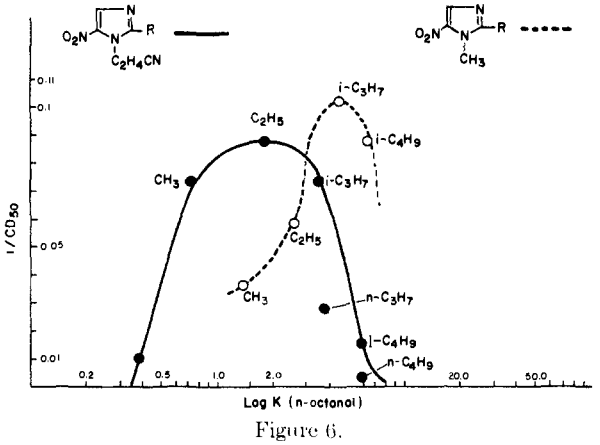


Figure 6.

established by experiment. The utility of such techniques is without question. For example, we can calculate the K value for 1-pentyl-2-ethyl-5-nitromidazole to be 14–16 (isooctane–pH 7), and the MED would be about 150 mg/kg. This compound would have no advantages over 1-methyl-2-isopropyl-5-nitroimidazole.

In another series, the nitroimidazoles (**2**, R^1 = β-cyanoethyl) proved to be too insoluble in isooctane to permit accurate measurements of K in this solvent. Partition coefficients for these compounds are reported for 1-octanol–aqueous buffer pH 7.0, and the relationship between CD_{50} (curative dose is that level which affords protection to 50% of the treated mice) and K (1-octanol) is shown in Figure 6. It was not possible to differentiate between the 2-methyl, 2-ethyl, and 2-isopropyl analogs on the basis of MED (Table III) but a plot of the CD_{50} of these compounds against K gave a symmetrical bell-shaped curve, with the 2-ethyl derivatives at the apex. For comparison, a plot of CD_{50}/K (octanol) for some 1-methyl-2-alkyl-5-nitroimidazoles is included in Figure 6. We observed for the 1-β-cyanoethyl-2-alkyl-5-nitroimidazoles, that a branched-chain alkyl substituent at C-2 affords greater biological potency than the corresponding straight-chain analog. This result is in accord with our observations in the 1-methyl-2-alkyl-5-nitroimidazole series (Figure 4). The 1-methyl-substituted series is slightly more active than the 1-β-cyanoethyl compounds, but the reason is not immediately clear. It is possible that the cyanoethyl group is a trifle too large and crowds the nitro group, or it might cause some modification of the electronic structure of the nitroimidazole nucleus. The ultraviolet absorption spectra of the 1-β-cyanoethyl-5-nitroimidazoles are different from the spectra of the simple alkyl derivatives, which strongly suggest some interaction between the cyano group and the nitroimidazole chromophore. However, the difference in biological potency between these two classes of compounds is too small to be significant. It is more important that four of these nitroimidazoles (**29**, **33**, **34**, **41**, Table III) have five times the trichomonacidal activity of metronidazole.

Experimental Section

Imidazoles.—The following imidazoles were obtained from commercial sources, or were synthesized according to published procedures: imidazole,[9] 2-methylimidazole,[9] 2-ethylimidazole,[4] 4-methylimidazole,[10] 2-*n*-propylimidazole,[4] 2-isopropylimidazole,[4] 2,4-dimethylimidazole,[11] 2-ethyl-4-methylimidazole,[12] 2-*n*-butylimidazole,[4] 2-isobutylimidazole,[4] and 2-*t*-butylimidazole.[4]

4- (5-) Nitroimidazoles.—Nitroimidazoles were prepared from the parent imidazole by nitration according to published procedures.[5,13,14] Data for various nitroimidazoles are shown in Table II.

1-Methyl-5-nitroimidazoles were prepared from the appropriate 4- (5-) nitroimidazole and dimethyl sulfate using the procedure of Pyman.[15,16] Other 1-alkyl-5-nitroimidazoles were

(9) Houdry Process Chemical Co., Philadelphia, Pa.
(10) A. Windaus and F. Knoop, *Ber.*, **38**, 1167 (1905).
(11) A. Windaus, *ibid.*, **39**, 3887 (1906).
(12) K and K Laboratories, Jamaica, N. Y.
(13) R. G. Fargher and F. L. Pyman, *J. Chem. Soc.*, **115**, 234 (1919).
(14) A. Windaus, *Ber.*, **42**, 762 (1909).
(15) F. L. Pyman, *J. Chem. Soc.*, **121**, 2621 (1922).
(16) V. K. Bhagwat and F. L. Pyman, *ibid.*, **127**, 1832 (1925).

prepared from the 4- (5-) nitroimidazole and the appropriate alkyl *p*-toluenesulfonate.[17,18]

General Procedure.—2-Isobutyl-4- (5-) nitroimidazole (2.9 g, 0.017 mole) and the β-cyano-*p*-toluenesulfonate (7.72 g, 0.034 mole) were combined and heated to 135° for 12 hr. The cooled mixture was extracted with water, and the aqueous extract was washed once with a small volume of $CHCl_3$ which was discarded. Addition of alkali to pH 9.0 gave a precipitate of the 1-β-cyanoethyl-2-isobutyl-5-nitrolmidazole which was extracted out with $CHCl_3$, washed once with water, dried (Na_2SO_4), and evaporated to yield 1.65 g (43%) of the desired compound which was then recrystallized as the *p*-toluenesulfonate salt (mp 211°) from 2-propanol.

1-Alkyl-4-nitroimidazoles were obtained by essentially the same procedure described by Cosar, *et al.*[2]

1-β-Hydroxyethyl-2-methyl-4-nitroimidazole (68) is best obtained by heating an ethanolic solution of 2-methyl-4- (5-) nitroimidazole with an excess (2 *M*) of ethylene oxide in the presence of a catalytic amount of NaOH. The desired product is obtained in quantitative yield by evaporation of the solvent, followed by recrystallization from ethyl acetate.

Acknowledgment.—We are greatly indebted to Dr. R. L. Wagner and his group for the microanalyses.

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(18) S. Tchelitcheff, U. S. Patent 3,065,133 (1962); R. M. Jacob, G. L. Regnier, and C. Cristan, U. S. Patent 2,944,061 (1960); Societe des Usines Chimique Rhone-Poulenc, British Patent 837,838 (1960).

Anthelmintic Quaternary Salts. Thiacyanines and Hemithiacyanines

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Comparison of the activities of analogs of dithiazinine (III) against gastrointestinal nematodes of sheep showed that the pentamethine chain is essential. Activity may be retained when the 6 position is substituted with alkyl or alkoxy groups, but substitution with electron-withdrawing groups produces inactive compounds. Analogs with activity comparable to dithiazinine tended to be more toxic to mice than dithiazine itself. Two members of a series of hemithiacyanines containing the 4-dimethylamino-1,3-butadienyl group were very active in inhibiting *Ascaris suum* larval migration in mice and in swine and were more effective than dithiazinine in preventing liver pathology due to migratory ascariasis.

Since the initial discovery of anthelmintic activity in cyanine dyes,[1] a number of compounds containing the conjugated amidinium ion characteristic of the cyanines have found use as both veterinary and clinical anthelmintics. Dithiazinine (III), the most potent member of this class of anthelmintics, is effective against a wide range of gastrointestinal nematodes,[2,3] but its use is severely limited by its toxicity. Because of its gastrointestinal side effects, the clinical application of dithiazinine is now restricted to cases of strongyloidiasis and severe trichuriasis.

The structure–activity–toxicity relationships of some structural analogs of dithiazinine were examined with the purpose of discovering compounds with comparable anthelmintic potency but with reduced mammalian toxicity. The structural features which were varied included the length of the polymethine chain, the replacement of the 3-methyl group by ethyl, and the introduction of substituents on the 6 position of the benzothiazole ring. In addition, a group of compounds in which one of the benzothiazole residues was replaced by a dimethylamino group (hemithiacyanines) was also prepared.

The most convenient method for the preparation of the thiadicarbocyanines (pentamethinethiacyanines) was the treatment of 2-methyl-3-alkylbenzothiazolium iodides with 1-methyl-1,2-dihydro-2-iminopyrimidine hydroiodide[4] in the presence of triethylamine.

The 2-methylbenzothiazolium salts were prepared by alkylation of 2-methylbenzothiazoles, which were themselves obtained by the oxidation of substituted thioacetanilides.[5,6]

The monomethine,[7] trimethine,[8] and heptamethine[9] analogs of dithiazinine were prepared by known methods. The hemithiacyanines, which included compounds with 2-dimethylaminovinyl and 4-dimethylamino-1,3-butadienyl substituents, were prepared by procedures which have been described by Brooker and his collaborators.[10]

Biological Properties.—Comparison of the first four compounds in Table I shows that acute toxicity in mice increases sharply with increasing length of the polymethine chain joining the benzothiazole residues. Thus the heptamethine analog IV is 2000 times more toxic than the monomethine analog I. However, only the pentamethine III (dithiazinine) showed appreciable anthelmintic activity. The trimethine analog II was totally inactive while the other two were only weakly active.

(1) L. G. S. Brooker and L. A. Sweet, *Science*, **105**, 496 (1947).

(2) A. C. Cuckler and K. C. Mezey, *Arzneimittel-Forsch.*, **16**, 411 (1966).

(3) M. C. McCowen, M. E. Callender, and M. C. Brandt, *Am. J. Trop. Med. Hyg.*, **6**, 894 (1957).

(4) D. J. Fry, J. D. Kendall, and A. J. Morgan [British Patent 870,633 (June 14, 1961)] described this compound, which is obtained by treating 2-aminopyrimidine with methyl iodide, as a quaternary salt. The correct structure was established by Brown and his collaborators, who have studied the fission of the pyrimidine ring in compounds of this type: D. J. Brown and J. S. Harper, *J. Chem. Soc.*, 1276 (1963), and earlier papers.

(5) A Nederlof, *Bull. Soc. Chim. Belges*, **68**, 148 (1959).

(6) B. Naiman and M. Bogert, *J. Am. Chem. Soc.*, **57**, 1660 (1935).

(7) G. M. Oksengendler, *Zh. Obshch. Khim.*, **23**, 135 (1953); *Chem. Abstr.*, **48**, 672 (1954).

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(9) N. I. Fisher and F. M. Hamer, *ibid.*, 189 (1933).

(10) L. G. S. Brooker, F. L. White, G. H. Keyes, C. P. Smyth, and P. F. Oesper, *J. Am. Chem. Soc.*, **63**, 3192 (1941).