(*p*-aminobenzamido)pyridine by the phosphorazo[2] method gave the free base II ($R^1 = NO_2$).

Biological Testing.—Full details of the test procedure have been given previously.[2,3] The legends in Table III correspond to test groups of six animals which have been inoculated with 10^5 L1210 cells intraperitoneally; dosage has been once daily intraperitoneally at the indicated figure for 5 days starting 24 hr after tumor inoculation. Weight change refers to difference in weight at days 1 and 8. Survivors' column lists those animals surviving as long or longer than controls in the group. Compounds which have been tested from a toxic (evidenced by death or marked weight loss) to a nontoxic dose level and have given no increase in life span are classified as negative and so designated in the tables of analytical data. Full details of testing for these negative compounds have not been given.

Acknowledgments.—We are greatly indebted to Miss L. Armiger and her capable assistants for performance of the many biological tests. This work was supported by the Auckland Division, Cancer Society of New Zealand (Inc.).

Potential Antileukemic and Immunosuppressive Drugs. Preparation and *in Vitro* Pharmacological Activity of Some Benzo-2,1,3-oxadiazoles (Benzofurazans) and Their N-Oxides (Benzofuroxans)

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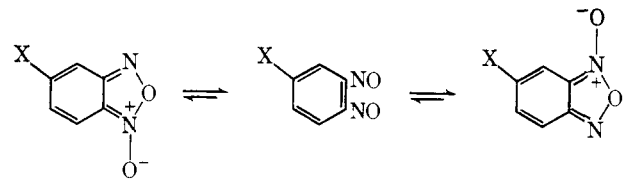
Eight methods for synthesizing various benzo-2,1,3-oxadiazoles (benzofurazans) and their N-oxides (benzofuroxans) are described. The activity of benzofurazans and benzofuroxans as *in vitro* inhibitors of RNA synthesis in sheep lymphocytes is given. Optimal drug activity *in vitro* was exhibited by 4-nitrobenzofurazan and 4-nitrobenzofuroxan and their 7-thio or 7-phenoxy derivatives. These compounds readily formed Meisenheimer complexes with nucleophiles and their drug action was abolished by preincubation with aliphatic thiols, *e.g.*, glutathione. It is concluded that the most active compounds reacted with key intracellular thiol groups. The 4-nitro group and furazan ring were essential for optimal drug activity.

In the course of testing some analogs of the purines and the antiarthritic drug, 2,3-bis(*p*-methoxyphenyl)-indole,[1] as inhibitors of lymphocyte metabolism *in vitro*,[2] we discovered that 4-nitrobenzo-2,1,3-oxadiazole[3] was a powerful inhibitor of nucleic acid and protein biosynthesis in many types of animal cells but with an especially toxic effect upon the metabolism of leukocytes *in vitro*.[4] This report summarizes our exploration of the structure–activity relationship for suppressing the incorporation of tritiated uridine into sheep lymphocyte RNA by benzo-2,1,3-oxadiazoles and their N-oxides. For convenience in distinguishing these two series of compounds, we shall hereafter refer to them by their alternative names, namely, benzofurazans and benzofuroxans (the N-oxide).

Benzofuroxan itself (I, X = H) has been shown by low-temperature nmr studies to be a rapidly equilibrating system, the transformation between the 1- and 3-oxide structure probably proceeding *via* *o*-dinitrosobenzene (II, X = H) as an intermediate.[5a,b] In the case of benzofuroxans substituted in the 5 position, the amount of each tautomer present in solution is dependent on the nature of X. When X is an electron donor, structure I is more abundant, while an electron-accepting group favors structure III.[5c] 4-Nitrobenzofuroxan exists in one form (oxide in the 1 position)



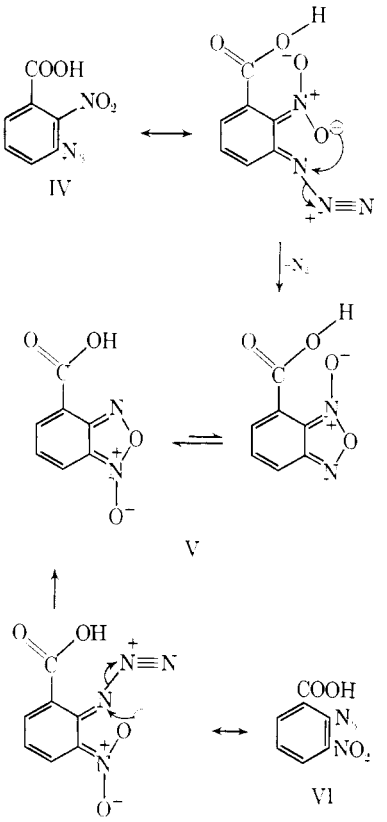
I II III

at all temperatures.[5a,c] For a recent review of benzofuroxans see ref 6.

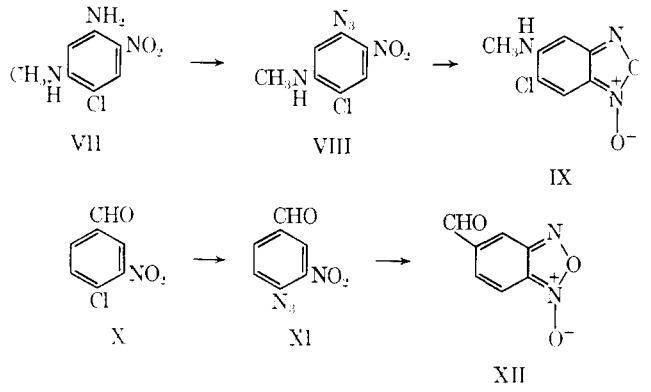
Preparation of Compounds.—Benzofuroxans were prepared by pyrolysis of the appropriately substituted *o*-nitrophenyl azides (method A) (see Experimental Section for details of each method) or hypohalite oxidation of substituted *o*-nitroanilines (method B). Method A was generally preferred to B because the oxidant used in the latter method may disrupt the furoxan ring[7] or displace a substituent. For example, alkaline hypochlorite reacts with 2,4-dinitroaniline to give 5-chloro-4-methoxybenzofuroxan instead of the expected 5-nitrobenzofuroxan.[8a] *o*-Nitrophenyl azides are readily obtained from *o*-nitroanilines either by diazotization and treatment with aqueous sodium azide, or by nucleophilic displacement of halogeno or nitro groups with sodium azide in dimethyl sulfoxide.[9] Treatment of 2-bromo-3-nitrobenzoic acid with sodium azide in dimethyl sulfoxide gave 2-azido-3-nitrobenzoic acid, previously only obtained from 2-bromo-3-nitrobenzoic acid by conversion to the amino compound

(1) Indoxol. E. M. Glenn, B. J. Bowman, W. Kooyers, T. Koslowske, and M. L. Myers, *J. Pharmacol. Exp. Ther.*, **155**, 157 (1967).
(2) M. W. Whitehouse, *J. Pharm. Pharmacol.*, in press.
(3) P. Drost, *Ann. Chem.*, **307**, 49 (1899).
(4) M. W. Whitehouse and P. B. Ghosh, submitted for publication.
(5) (a) R. K. Harris, A. R. Katritzky, S. Øksne, A. S. Bailey, and W. G. Paterson, *J. Chem. Soc.*, 197 (1963); (b) G. Englert, *Z. Anal. Chem.*, **181**, 447 (1961); (c) A. J. Boulton, A. R. Katritzky, M. J. Sewell, and B. Wallis, *J. Chem. Soc., B*, 914 (1967). (d) F. B. Mallory, S. L. Manatt, and C. S. Wood, *J. Am. Chem. Soc.*, **87**, 5433 (1965).
(6) A. J. Boulton and P. B. Ghosh, *Advan. Heterocyclic Chem.*, in press.
(7) A. G. Green and F. M. Rowe, *J. Chem. Soc.*, **103**, 2023 (1913).
(8) (a) F. B. Mallory and S. P. Varimbi, *J. Org. Chem.*, **28**, 1656 (1963); (b) F. B. Mallory, C. S. Woods, and M. Hurwitz, *ibid.*, **29**, 2605 (1964); (c) F. B. Mallory, *Org. Syn.*, **37**, 1 (1957).
(9) A. J. Boulton, P. B. Ghosh, and A. R. Katritzky, *J. Chem. Soc., C*, 971 (1966).

followed by diazotization and treatment with sodium azide.[10] Attempts to convert this very stable azide to 4-carboxybenzofuroxan (V) gave only poor yields of the expected product, due to the extensive charring which occurred during the pyrolytic ring closure. In contrast, 3-azido-2-nitrobenzoic acid (IV), prepared from 3-amino-2-nitrobenzoic acid, evolved nitrogen quite smoothly at 170–180° in sulfolane to give 4-carboxybenzofuroxan.

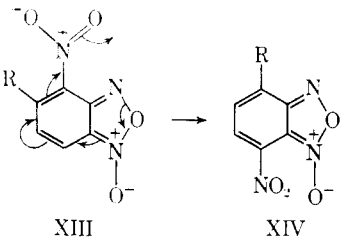


1-Amino-4-chloro-5-methylamino-2-nitrobenzene[11] (VII) was diazotized and converted to the azide VIII which lost nitrogen on refluxing in glacial acetic acid to give 6-chloro-5-methylaminobenzofuroxan (IX). 4-Chloro-3-nitrobenzaldehyde[12] (X) reacted with sodium azide in dimethyl sulfoxide to give the azide (XI) which afforded 5-formylbenzofuroxan (XII) by heating under reflux in acetic acid.

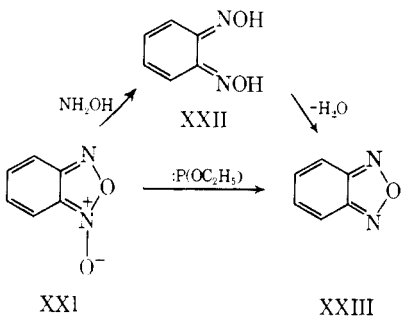


(10) A. J. Boulton, P. B. Ghosh, and A. R. Katritzky, *J. Chem. Soc., B*, 1004 (1966).
(11) I. Molnar, *Helv. Chim. Acta*, **46**, 1780 (1963).
(12) H. H. Hodgson and H. G. Beard, *J. Chem. Soc.*, 24 (1927).

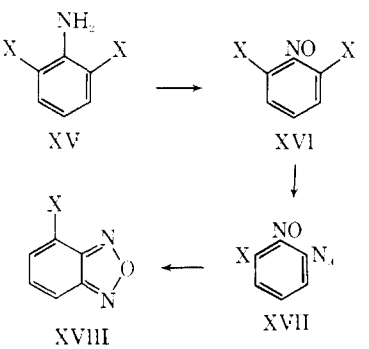
Benzofuroxan and 5- or 7-substituted benzofuroxans readily nitrate in the 4 position[13,14] (method C). The 5-substituted 4-nitrobenzofuroxans (XIII, R = Cl, CH_3) rearrange on heating above their melting points to the sterically less hindered 4-substituted 7-nitro isomers[15] (XIV, R = Cl, CH_3) (method D); however, when X is an electron-releasing group the rearrangement occurs spontaneously at room temperature.[16]



Benzofuroxans were converted to benzofurazans either by deoxygenation with triethyl phosphite[17] (method E) or reduction with alkaline hydroxylamine to the quinone dioxime (XXII) which readily dehydrated on boiling with alkali[18] (method F).



4-Substituted benzofurazans were more conveniently prepared by the reaction of 2,6-dihalogeno nitrosobenzenes with sodium azide in dimethyl sulfoxide[19] (method G). The required 2,6-dihalogenonitrosobenzenes (XVI) were readily obtained from 2,6-dihalogenoanilines (XV) by oxidation with peracetic acid.[20]

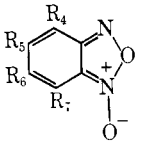


The intermediate nitroso azides (XVII) cannot be isolated generally; however, in the preparation of 4,6-dibromobenzofurazan from 2,4,6-tribromonitrosobenzene some 2-azido-4,6-dibromonitrosobenzene was

(13) R. J. Gaughran, J. P. Picard, and I. V. R. Kaufman, *J. Am. Chem. Soc.*, **76**, 2233 (1954).
(14) P. Drost, *Ann. Chem.*, **313**, 303 (1900).
(15) A. J. Boulton and A. R. Katritzky, *Rev. Chim.* (Bucharest), **7**, 691 (1962).
(16) P. B. Ghosh, submitted for publication.
(17) J. H. Boyer and S. E. Ellzey, *J. Org. Chem.*, **26**, 4684 (1961).
(18) T. Zincke and P. Schwarz, *Ann. Chem.*, **307**, 28 (1899).
(19) A. J. Boulton, P. B. Ghosh, and A. R. Katritzky, *Tetrahedron Letters*, 2887 (1966).
(20) R. P. Bayer and R. R. Holmes, *J. Am. Chem. Soc.*, **82**, 3454 (1960).

TABLE I

BENZO-2,1,3-OXADIAZOLE N-OXIDES TESTED IN APPROXIMATE DESCENDING ORDER OF BIOLOGICAL ACTIVITY



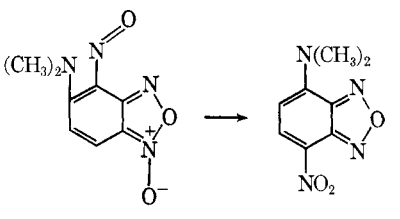
Substituent							% inhib for drug concn (μM) of				
R_4	R_5	R_6	R_7	Prepn	Mp, °C	Ref	5	20	100	250	500
NO_2	H	H	NMePh	H	186	16	75	85	...	...	
NO_2	H	H	SPh	H	196	16	30	95	...	...	
NO_2	H	H	Cl	C + D	140	15	30	95	...	...	
NO_2	Cl	H	H	C	78	15	25	85	...	...	
NO_2	H	H	H	C	143	3	25	90	...	...	
NO_2	H	H	NMe_2	H	145	16	35	70	...	...	
NO_2	H	H	$HNCH_2Ph$	H	168	16	25	65	...	...	
NO_2	Cl	Cl	H	C	100	New	20	80	...	...	
NO_2	H	H	$MeOC_6H_4NH$	H	221	16	...	50	75	...	
NO_2	H	H	HNPh	H	208	16	...	45	70	...	
$NHCO_2Me$	NO_2	H	H	A[a]	195	10	...	40	90	...	
NO_2	Me	H	H	C	98	15	...	30	85	...	
NO_2	H	H	OMe	C + D	160	16	...	30	70	...	
NO_2	H	H	NHCOMe	C + D	210	16	5	30	70	...	
CO_2H	H	CH_3	H	A[a]	210	10	...	30	65	...	
H	NO_2	H	H	A	72	*f*	...	25	90	...	
NO_2	NHPh	H	H	H + D	208	16	...	15	20	...	
NO_2	H	NO_2	Me	C	123	*g*	...	10	70	...	
NO_2	H	H	Me	C + D	167	15	...	10	65	...	
NO_2	H	NO_2	H	C	172	3	...	5	95	...	
H	NHMe	Cl	H	A	141	New	...	0	50	95	...
NH_2	NO_2	H	H	A[a]	250	10	...	...	15	50	...
MeO	Br	H	H	B	95	8b	...	...	15	45	...
Cl	H	Cl	H	A	107	New	...	...	5	50	...
H	OCOMe	H	H	A[a]	65	9	...	...	15	...	85
H	OMe	H	H	A	118	13	...	...	...	5	35
OMe	Cl	H	H	B	81	8a	...	...	20	...	...
H	CO_2Et	H	H	A[b]		5c	...	...	...	...	60
CO_2Et	H	H	H	A[c]	108	New	...	...	...	...	45
CO_2Me	H	H	H	A[d]	147	10	...	...	...	...	40
N_2O_2		H	H	A	94	*h*	...	...	...	...	30
$CONH_2$	H	H	H	A[e]	181	10	...	...	...	...	25
N_2O		H	H	A	53	*h*	...	...	...	...	20
H	Me	H	H	A	98	18	...	...	...	...	20
H	CHO	H	H	A[a]	69	New	...	...	...	...	20
H	Cl	H	H	B	48	7	...	...	...	...	0
H	CO_2H	H	H	A[a]	130	9	...	...	...	...	0
CO_2H	H	H	H	A	208	New	...	...	...	...	0
H	H	H	H	A	72	18	...	...	...	...	0

[a] Made by P. B. G. while at the University of East Anglia, Norwich, England. [b] Kindly supplied by Professor A. R. Katritzky, Norwich, England. [c] Made by esterification of the acid. [d] Azide esterified before decomposition. [e] From the Me ester. [f] W. Noeleting and A. Kohn, *Chem. Ztg.*, **18**, 1905 (1894). [g] P. Drost and T. Zincke, *Ann. Chem.*, **313**, 309 (1900). [h] A. J. Boulton, A. C. Gripper Gray, and A. R. Katritzky, *J. Chem. Soc.*, 5958 (1965).

detected (by ir absorption at 2120 cm^{-1}). The 5- and 7-substituted benzofurazans readily nitrate in the 4 position[13] (method C). 5-Methylbenzofurazan afforded the 4-nitro isomer as shown by the nmr spectrum (in $CDCl_3$) which revealed a methyl signal at τ 7.18 and an AB quartet (τ_A = 1.75, τ_B = 2.31; J_{AB} = 9.0 cps), eliminating the possibility of nitration in the 7 position originally suggested by Drost.[14]

4-N,N-Dimethylamino-7-nitrobenzofurazan (XX) was obtained conveniently from 5-N,N-dimethylaminobenzofuroxan (XIX) by nitrosation and spontaneous rearrangement.[10]

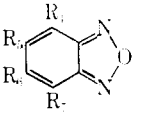
The chloro group in the 5- and 7-chloro-4-nitrobenzofurazans and nitrobenzofuroxans is extremely labile and may be replaced by a wide variety of nucleophiles[16] (method H) under fairly mild conditions. Thus, refluxing 7-chloro-4-nitrobenzofurazan with an



XIX XX

equimolar amount of thiophenol in ethanol for 1 hr in the presence of traces of pyridine or potassium acetate afforded 4-nitro-7-thiophenylbenzofurazan. When this active chloro compound was mixed with methylamine or dimethylamine, replacement occurred almost immediately. However, on treating 5-chloro-4-nitrobenzofuroxan with thiophenol or alkylamines, under these conditions, the expected 5-substituted product

Table II
Benzo-2,1,3-oxadiazoles Tested in Approximate Descending Order of Biological Activity



Substituent							% inhib for drug concn (μM) of				
R_4	R_6	R_5	R_7	Prepn	Mp, °C	Ref	5	20	100	250	500
NO_2	H	H	$PhCH_2S$	H	115	16	...	100	...	...	...
NO_2	H	H	PhS	H	157	16	65	95	...	...	...
NO_2	H	H	H	C	93	13	55	95	...	...	...
NO_2	SPh	H	H	H	141	16	55	100	...	...	...
NO_2	H	H	Cl	C	96	10	55	80	...	...	...
NO_2	Cl	H	H	C	64	*e*	...	90	...	...	...
NO_2	H	H	SCN	H	98	16	35	95	...	...	...
NO_2	H	H	H + PhS	H[a]	...	New	30	85	...	...	...
NO_2	H	H	H + OMe	H[a]	...	New	30	55	95	...	...
NO_2	H	H	*p*-$O_2NC_6H_4O$	H	144	16	...	78	...	...	...
NO_2	H	H	Tyr-O[b]	H	141	16	74	74	...	...	...
NO_2	H	H	PhNH	H	150	16	...	45	70	...	...
NO_2	H	H	N_3	H	84	10	...	35	95	...	...
NO_2	H	H	$PhCH_2NH$	H	205	16	15	35	...	...	...
H	MeO	H	H	E	99	13	...	20	85	...	...
NO_2	H	H	NMePh	H	166	16	...	20	55	...	...
NO_2	H	H	NHCOMe	H[c]	211	16	...	24	33	...	62
OMe	Cl	H	H	E	70	8a	...	10	65	...	...
H	NO_2	H	H	E	65	8a	...	10	50	...	...
NO_2	Me	H	H	C	83	New	...	10	30	...	...
Br	H	Me	H	G	81	19	...	...	40	...	...
NO_2	OMe	H	H	C	120	16	...	...	35	80	...
NO_2	H	H	NH_2	H	237	10	...	...	24	...	55
NO_2	H	H	SCH_2CO_2H	H	158	16	...	...	20	...	75
Br	H	Br	H	G	67	New	...	...	20	...	...
H	NMe_2	H	H	E	97	9	...	...	20	...	70
NO_2	H	H	NMe_2	H	250	10	...	...	15	15	...
NO_2	H	H	$NHCH_2$-CO_2H	H	190	16	...	...	10	...	25
NO_2	NHPh	H	H	H	211	16	...	...	10	...	...
NO_2	H	H	NHMe	H	252	16	...	...	5	...	...
C_4H_4		H	H	F	78	*f*	...	...	...	80	...
Cl	H	H	H	G	83	19	...	...	...	76	...
H	Cl	H	H	F	44	16	...	...	...	55	...
H	H	H	H	F	55	18	...	...	...	25	60
H	CO_2H	H	H	E	43	New	...	...	...	...	26
N	Me	H	H	E	37	13	...	...	...	...	10
MeCO	H	H	H	E[d]	Liquid	*d*	...	...	...	...	5
NO_2	NMe_2	H	H	H	286	16	...	...	...	...	0

[a] Meisenheimer complexes. [b] Tyr = N-acetyltyrosyl ethyl ester. [c] By acetylation of 7-amino-4-nitrobenzofurazan. [d] By rearrangement of 3-methyl-7-nitroanthranil; private communication from A. J. Boulton. [e] Footnote *h*, Table I. [f] A. G. Green and F. M. Rowe, *J. Chem. Soc.*, **111**, 612 (1917).

was not obtained because of the rearrangement which occurred either before or after replacement of the chloro group, yielding 4-nitro-7-substituted benzofuroxans. The reaction of 7-chloro-4-nitrobenzofuroxan with aniline gave a mixture of 7-anilino-4-nitrobenzofuroxan and 5-anilino-4-nitrobenzofuroxan.[16]

Biological Activity. Relationship of Structure to Activity.—Tables I and II show that the following substituents either much reduced or entirely abolished the drug activity of 4-nitrobenzofurazan in inhibiting the incorporation of uridine-5-^{3}H into RNA by sheep lymphocytes *in vitro:* 1-N-oxide, 5- or 7-methyl, 5- or 7-anilino, 5-methoxy, 5- or 7-dimethylamino.

A second nitro group (at C-6) diminished the drug activity of 4-nitrobenzofuroxan. The 4-carboxy isosteres and 4-acetylbenzofurazan were inactive but the 4-methoxycarbonyl-, 4-ethoxycarbonyl-, and 4-carboxyl-6-methylbenzofuroxans displayed some drug activity *in vitro*, presumably due to their greater lipophilic character.

The benzofuroxans were generally less potent *in vitro* than the corresponding benzofurazans. However, benzofuroxans with the following substituents, 5-nitro- and 4-nitro-7-alkylamino, N,N-dimethylamino, N-methylanilino, N-benzylamino, and 4-nitro-5-methyl, were more potent drugs *in vivo* than the corresponding benzofurazans. The drug activity of 4-nitro-7-dimethylamino- and 4-nitro-7-N-methylanilinobenzofuroxans was rather remarkable, by comparison with that of the related benzofurazans. Some 5- and 7-thio derivatives and 7-phenoxy derivatives of 4-nitrobenzofurazan and 4-nitrobenzofuroxan (*e.g.*, thiocyanato benzylthio, phenylthio) were as potent as the parent nitro compounds, but the corresponding amino compounds (benzylamino, anilino) were very much less potent *in vitro*. A thio analog, 4-nitrobenz-2,1,3-

thiadiazole, was almost devoid of drug activity (only 30% inhibition of uridine incorporation with 200 μM thiadiazole). The few 1,2-benzoquinone dioximes examined (*e.g.*, 3-nitro, 3-carboxy, and no substituent) were also less active than the corresponding benzofurazans (formed on dehydration), indicating that the intact oxadiazole ring is necessary for optimal drug activity. Other data in Tables I and II establish that the 4-nitro group (not hindered by a 5 substituent) is also required for benzo-2,1,3-oxadiazoles to exhibit their considerable drug activity *in vitro*.

The drug activity of these potent 4-nitro compounds and 5-methoxybenzofurazan was abolished by preincubation with a number of thiols at pH 7.4, glutathione being particularly effective in this respect.[4] During this preincubation (glutathione, 2-mercaptoethanol), characteristic colorations were formed with the nitro compounds, the 7-phenoxy and 7-thio-substituted nitro compounds with drug activity became noticeably more water soluble, and the 7 substituent (*e.g.*, thiocyanate, thiophenol, benzyl mercaptan, *p*-nitrophenol) was liberated into solutions. The 7-thioglycollic acid derivative of 4-nitrobenzofurazan was only a weak drug *in vitro*, but also gave a coloration with glutathione.

The drug activity of 7-anilino-4-nitrobenzofurazan and the 7-anilino- and 7-dimethylamino-4-nitrobenzofuroxans was not abolished by preincubation with excess glutathione.

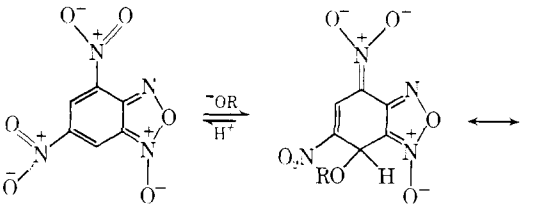
Freshly prepared solutions of the blue Meisenheimer-type adducts of 4-nitrobenzofurazan with sodium methoxide and with thiophenol were also potent drugs *in vitro*, further demonstrating that substitution at, or addition to, the reactive C-7 position in 4-nitrobenzofurazan did not necessarily abolish drug action *per se*.

There was an apparent inverse relationship between the fluorescence and drug activity *in vitro* for many of the 7-substituted 4-nitrobenzofurazans. Those compounds which strongly inhibited RNA synthesis (*i.e.*, 4-nitrobenzofurazan itself and its 7-phenoxy and 7-thio derivatives) are nonfluorescent or fluoresce only weakly in organic solvents (acetone, ethyl acetate). The 7-alkylaminonitrobenzofurazans which are highly fluorescent[21] were only weak drugs *in vitro*, whereas the corresponding 7-alkylamino-4-nitrobenzofuroxans and the 7-anilino derivatives of 4-nitrobenzofurazan, which are highly colored but do not fluoresce, were the more potent inhibitors of RNA synthesis in lymphocytes, though possibly not acting by the same mechanisms as other nitrobenzofurazans.

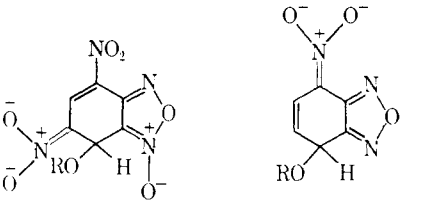
Discussion

Of the 39 benzofuroxans and 38 benzofurazans tested, those containing nitro groups were by far the most active as inhibitors of lymphocyte metabolism *in vitro*. The explanation of drug activity given below is based on experiments conducted with these potent nitro compounds. It cannot necessarily be assumed that the other compounds of this series, which are weaker inhibitors of lymphocyte nucleic acid biosynthesis, act in the same fashion.

Two independent studies[22] have shown that 4,6-dinitrobenzofuroxan (XXIV) reacts with potassium hydroxide or methoxide to give Meisenheimer-type complexes (XXV). The nmr spectrum of the complex (XXV, R = CH_3) revealed the presence of ring protons



XXIV XXV



XXVI

at τ 1.03, 3.98, and a methoxyl proton at 6.63. Neutralization of the potassium salt of XXV (R = CH_3) with D_2SO_4 regenerated unchanged 4,6-dinitrobenzofuroxan. We have found the pK_a of 4,6-dinitrobenzofuroxan to be 3.77 and so it would be completely complexed at physiological pH. We have examined the four mononitrobenzofuroxans, and nitrobenzofurazans under similar conditions, and have found that reversible addition complexes such as XXVI are formed as shown by a reversible spectral change in alkali (see Figure 1), but with the exception of the adduct formed from 4-nitrobenzofurazan (XXVI, R = H) (50% complexed at pH 10.65) these complexes were not sufficiently stable to be isolated for further examination. From observations of the rate of change of the uv spectra of these compounds at pH 13 with time we have assessed the relative stabilities of complexes to be in the order 4-nitrobenzofurazan >> 4-nitrobenzofuroxan > 5-nitrobenzofuroxan > 5-nitrobenzofurazan. This order of stability correlates exactly with the relative biological activities of these compounds (see Tables I and

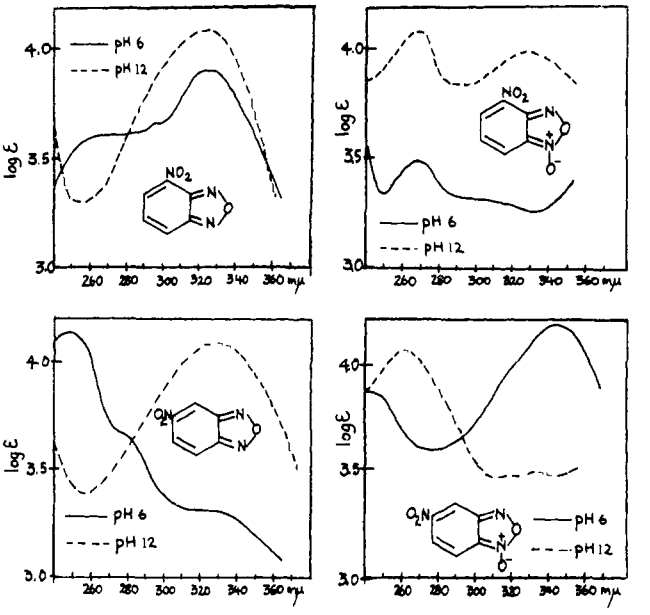


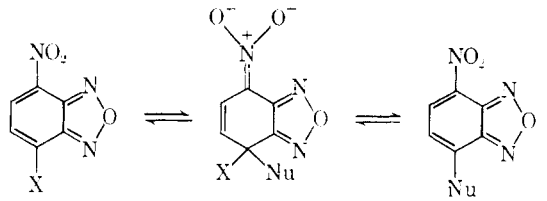
Figure 1.—Uv spectra of mononitrobenzofurazans and nitrobenzofuroxans in buffers of pH 6 and 12.

(21) P. B. Ghosh and M. W. Whitehouse, submitted for publication.

(22) (a) A. J. Boulton and D. P. Clifford, *J. Chem. Soc.*, 5414 (1965); (b) W. P. Norris and J. Osmundsen, *J. Org. Chem.*, **30**, 2407 (1965).

II) and suggested that complexes similar to those described above might be formed within the cell.

The lifetime of the Meisenheimer-type complex XXVIII depends on the nature of X and we have found that when X = H and Nu = PhS^-, OH^-, or



XXVII XXVIII XXIX

$^-OCH_3$ the complex can be isolated, whereas if X is thiocyanato and Nu = RSH, ROH, RNH_2, or N_3^- the complex is seen as an intense transient color (usually red). When X = Cl, and Nu = RSH, ROH, RNH_2, SCN^-, or N_3^- the intermediate (XXVIII) rapidly passes to the substituted product (XXIX) which has been isolated. A 5 substituuent in XXVII diminished (or entirely abolished) the drug activity and must sterically hinder formation of the postulated intermediate (XXVIII), in which the nitro group is coplanar with the benzene nucleus.

The reaction with glutathione rendering these drugs inactive (on preincubation at pH 7.4) is probably an example of this general reaction (XXVII → XXIX) and a possible model for the extracellular irreversible combination of the drug with its receptor. Present evidence, admittedly circumstantial, suggests that it is an intracellular thiol group with which these drugs combine.[4]

Experimental Section

Microanalyses were performed by Dr. Joyce Fildes and her staff, ir spectra were measured on a Unicam S. P. 200, and nmr on a Perkin-Elmer R10 60 Mc machine. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Chemical Syntheses. Method A.—The 4- or 5-substituted *o*-nitrophenyl azides (10 mmoles) were refluxed in 50 ml of AcOH until the evolution of N_2 ceased (indicated by a bubbler connected to the top of the condenser). Dilution with 100 ml of ice–water yielded the benzofuroxan which was filtered off and washed (H_2O). Generally these benzofuroxans crystallized well from EtOH or EtOH–H_2O.

The 3- and 6-substituted 2-nitrophenyl azides were more difficult to decompose, and higher boiling solvents such as xylene or sulfolane were used.

Method B.—The *o*-nitroanilines (300 mmoles) were dissolved in 250–750 ml of 95% EtOH saturated with KOH. The resulting deep red solutions were cooled to 0°, and *fresh* NaOCl (prepared from 130 mmoles of NaOH in 250 ml of H_2O and 60 mmoles of Cl_2[8c]) was added dropwise with vigorous stirring over 10 min. Crushed ice (250 g) was then added, and the yellow precipitate was collected, washed (H_2O), and crystallized from EtOH–H_2O.

4,6-Dichlorobenzofuroxan was prepared by alkaline hypochlorite oxidation of 4,6-dichloro-2-nitroaniline[23] using method B. The dichlorobenzofuroxan crystallized from H_2O–EtOH (1:2) as pale yellow plates mp 107–108°. *Anal.* ($C_6H_2Cl_2N_2O_2$) C, H, N.

2-Azido-3-nitrobenzoic Acid (VI).—To a solution of 2.47 g (10 mmoles) of 2-bromo-3-nitrobenzoic acid[24] in 15 ml of DMSO was added 0.71 g (11 mmoles) of NaN_3 in DMSO (10 ml). After 10 min of stirring at 120° the purple solution was cooled and poured into 100 ml of ice–water. Acidification with 2 *N* HCl yielded a yellow precipitate which was collected, washed (H_2O), and crystallized from EtOH to give 1.57 g (78%) of VI as pale yellow needles, mp 202° (lit.[10] mp 202°).

3-Azido-2-nitrobenzoic Acid (IV).—3-Amino-2-nitrobenzoic acid[25] (18.2 g, 100 mmoles) was dissolved in 150 ml of boiling AcOH, rapidly cooled to 25°, and added in a continuous stream to vigorously stirred ice-cooled nitrosylsulfuric acid, prepared from 7.6 g (110 mmoles) of $NaNO_2$ and 50 ml of concentrated H_2SO_4. After 15 min of stirring the solution was poured into 200 g of crushed ice and the resulting clear solution was slowly added to a vigorously stirred solution of 10 g of NaN_3 in 200 ml of H_2O. The tan precipitate which separated was collected, washed (H_2O), and crystallized from EtOH to give 18 g (87%) of IV as light tan prisms: mp 178–180° dec; ν_{max} 2150 (N_3), 1703 cm^{-1} (CO). *Anal.* ($C_7H_4N_4O_4$) C, H; N: calcd, 26.9; found, 26.3.

4-Carboxybenzofuroxan (V).—3-Azido-2-nitrobenzoic acid (IV) (1 g) in 10 ml of sulfolane was heated slowly to 180°, maintained at this temperature for 3 min, cooled, and poured into 100 ml of 1 *N* NaOH. After extracting with ether, the alkaline solution was acidified with 2 *N* HCl, the benzofuroxan acid was extracted into ether and dried ($MgSO_4$), and the solvent was removed by distillation. The residue crystallized from H_2O to give 0.4 g (41%) of the monohydrate as yellow prisms with an ill-defined melting point around 190°; ν_{max} 3550 (H_2O), 1700 (CO), 1607, 1590, 1520 cm^{-1} (benzofuroxan). *Anal.* ($C_7H_4N_2O_4 \cdot H_2O$) C, H, N. Several recrystallizations from EtOH gave the anhydrated acid which melted sharply at 204°; pK_a (water) = 2.9 ± 0.05; ν_{max} 1691 (CO), 1609, 1590, 1550 cm^{-1} (benzofuroxan). *Anal.* ($C_7H_4N_2O_4$) C, H, N.

Methyl 3-Azido-2-nitrobenzoate.—3-Azido-2-nitrobenzoic acid (1 g) was dissolved in 25 ml of MeOH and saturated with dry HCl. After 24 hr of reflux the volume of MeOH was reduced to *ca.* 10 ml and the white solid that separated was collected and recrystallized from MeOH to give 0.85 g (80%) of the azido ester as white needles: mp 118–120°; ν_{max} 2210 (N_3), 1720 (CO), 1520, 3120 cm^{-1} (NO_2). *Anal.* ($C_8H_6N_4O_4$) C, H, N.

4-Methoxycarbonylbenzofuroxan.—Methyl 3-azido-2-nitrobenzoate (0.5 g) was refluxed in 10 ml of diglyme for 1 hr. On dilution with 40 ml of H_2O a white precipitate separated which was filtered off, washed (H_2O), air-dried, and crystallized from EtOAc-petroleum ether (bp 60–80°) (1:1) to give 0.3 g (67%) of the ester as white needles, mp 144–145° (lit.[10] 145–146°).

4-Ethoxycarbonylbenzofuroxan was prepared by esterification of 4-carboxybenzofuroxan with dry HCl and EtOH and crystallized from EtOH as pale yellow plates: mp 107.5–108°; ν_{max} 1705 (CO), 1605, 1595, 1555 cm^{-1} (benzofuroxan). *Anal.* ($C_9H_8N_2O_5$) C, H, N.

1-Amino-4-chloro-5-methylamino-2-nitrobenzene (VIII) was prepared from 1-amino-4,5-dichloro-2-nitrobenzene,[26] mp 176–177° (lit.[13] 178–179°).

6-Chloro-5-methylaminobenzofuroxan (IX).—1-Azido-4-chloro-5-methylamino-2-nitrobenzene (VIII) (2 g), prepared from VII by a procedure as described for the dimethylamino analog,[9] was refluxed in 25 ml of AcOH for 1 hr. Dilution with 100 ml of ice–water afforded a brown solid which was crystallized three times from H_2O–EtOH (1:1) to give 1 g (42%) of IX as small orange prisms, mp 140–141.5°. *Anal.* ($C_7H_6ClN_3O_2$) C, N; H: calcd, 2.0; found, 3.1.

4-Azido-3-nitrobenzaldehyde (XI).—A mixture of 4 g of 4-chloro-3-nitrobenzaldehyde (X)[12] and 1 g of NaN_3 in 30 ml DMSO was heated at 75° for 30 min. The solution was cooled to 30°, poured into 100 ml of H_2O, and extracted with ether. After drying ($MgSO_4$) and evaporating the solvent a yellow oil was obtained which solidified on standing at 0° for 1 hr. It was crystallized from EtOH to give 3.0 g (72%) of XI as very pale yellow plates: mp 74–75° dec; ν_{max} 2130 (N_3), 1705 cm^{-1} (CO). *Anal.* ($C_7H_4N_4O_3$) C, H, N.

5-Formylbenzofuroxan (XII).—The azide XI (1 g) was refluxed for 30 min in 15 ml of toluene. The solvent was evaporated leaving an oil which was redissolved in 5 ml of boiling EtOAc. Petroleum ether (bp 60–80°) (20 ml) was added and the solution was cooled to give 0.7 g (82%) of XII as pale yellow needles: mp 68.5–69°; ν_{max} 1705 (CO), 1615, 1590, 1540 cm^{-1} (benzofuroxan). *Anal.* ($C_7H_4N_2O_4$) C, H, N.

(23) O. N. Witt, *Ber.*, **7**, 1603 (1874).

(24) F. C. Whitmore, P. J. Culhane, and H. T. Neher, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p 56.

(25) A. Kaiser, *Ber.*, **18**, 2946 (1885).

Method C.—The benzofuroxan (10 mmoles) was dissolved in 50 ml of ice-cold concentrated H_2SO_4 and treated with shaking with 11 mmoles of fuming HNO_3 in 10 ml of concentrated H_2SO_4. The mixture was allowed to stand 30 min at 0° and then poured into 200 ml of ice–water. The precipitated nitro compound was filtered off, washed (H_2O) and air dried. 5-Methyl-4-nitrobenzofuroxan and 5-chloro-4-nitrobenzofuroxan were crystallized from CH_2Cl_2 to avoid rearrangement. Other nitro compounds were crystallized from EtOH or EtOAc.

5,6-Dichloro-4-nitrobenzofuroxan was prepared by the above method but the temperature of the nitration mixture was raised to 60° for 1 hr before pouring into ice–water. The compound was recrystallized from ether as pale yellow needles, mp 99–100°. *Anal.* ($C_6HClN_3O_4$) C, H, N.

Method D.—The 4-nitro-5-substituted benzofuroxan was refluxed for 15 min in a solvent which boiled above the melting point of the compound. Thus, 10 mmoles of 5-chloro-4-nitrobenzofuroxan (mp 78–81°)[15] or 5-methyl-4-nitrobenzofuroxan (mp 98–100°)[15] was refluxed in AcOH for 15 min and diluted with 100 ml of ice–water, and the yellow precipitates were collected, washed (100 ml of H_2O), and recrystallized from EtOH or AcOH–H_2O (1:1).

Method E.—The benzofuroxan (1 g) and 5 ml of triethyl phosphite in 50 ml of absolute EtOH were refluxed for 30 min. The EtOH was removed at 10 mm and the residue was steam distilled to give the benzofurazan which crystallized from the distillate on cooling.

5-Carboxybenzofurazan.—5-Carboxybenzofuroxan was deoxygenated by method E to yield the corresponding benzofurazan [purified by sublimation (100° at 15 mm)] as white plates, mp 42–43°. *Anal.* ($C_7H_4N_2O_3$) C, H, N.

Method F.—To 10 moles of the benzofuroxan in 100 ml of EtOH was added 12 mmoles of $NH_2OH \cdot HCl$ in 10 ml of H_2O. The mixture was cooled to 0° and 35 mmoles of KOH in H_2O (40 ml) was added with shaking. The deep red solution was allowed to stand for 1 hr at room temperature, then the bulk of EtOH was removed at 10 mm. The residue of KOH, KCl, and the dipotassium salt of the *o*-quinone dioxime was either steam distilled to yield the benzofurazan which was collected, dried, and sublimed [100° (20 mm)] or acidified with 2 *N* HCl to liberate the *o*-quinone dioxime, which was filtered off and purified by crystallization.

3-Carboxy-1,2-benzoquinone Dioxime.—4-Carboxybenzofuroxan was treated with NH_2OH as described in method F. The quinone dioxime separated on neutralization of the dipotassium salt as an orange solid, which was recrystallized from EtOH–H_2O (1:1) as a powder, mp (darkens considerably at 150°) 154–155° dec. *Anal.* ($C_7H_6N_2O_4$) C, H, N.

3-Nitro-1,2-benzoquinone dioxime was prepared by the general method but using 30 mmoles of KOH for 10 mmoles of 4-nitrobenzofuroxan and rotary evaporating at 30°. The dioxime separated as a brown solid on neutralization of the dipotassium salt. Attempts to purify this material further caused decomposition. The analysis figure given could not therefore be improved on. *Anal.* Calcd for $C_6H_3N_3O_4$: C, 39.4; H, 2.7; N, 23.0. Found: C, 38.5; H, 2.6; N, 24.2.

Method G.—To 10 mmoles of the dihalogenonitrosobenzene (XVI) in 25 ml of DMSO at room temperature was added 10.5 mmoles of NaN_3 in 10 ml of DMSO. When N_2 evolution had ceased, the mixture was heated to 120° for 3 min, cooled to room temperature, and poured into 100 g of crushed ice. After standing for 1 hr the benzofurazan was filtered off, air dried, and sublimed at 100° (20 mm).

4,6-Dibromobenzofurazan was obtained from 2,4,6-tribromonitrosobenzene using method G but a temperature of 160° was necessary to complete the cyclization; mp 67–67.5°. *Anal.* ($C_6H_2Br_2N_2O$) C, H, N.

Method H.—To 10 mmoles of the chloronitrobenzofuroxan or benzofurazan in 20 ml of EtOH was added 11 mmoles of the nucleophile in 10 ml of EtOH. The mixture was refluxed for 5 min, and if a colorization had not developed 0.05 mmoles of KOAc in H_2O (1 ml) or 0.1 ml of pyridine was added and reflux was continued for a further 55 min. If a strong coloration did develop within 5 min no catalyst was added and reflux was continued for a further 10 min. The product either separated from the alcohol on cooling, or after fourfold concentration, and crystallized from either EtOH, Me_2CO, or aqueous DMF.

Meisenheimer Complexes.—4-Nitrobenzofurazan (0.165 g, 1 mmole) in 20 ml of dry EtOH was treated with 1 mmole of the sodium or potassium salt of the base (OH, OCH_3, SPh) under dry N_2 at room temperature. After 12 hr of standing the complex was collected and washed with 100 ml of EtOH. Attempts to crystallize these explosive salts from H_2O or other solvents were unsuccessful due to partial reversal to 4-nitrobenzofurazan and the base. Thus, on boiling the violet complex (green by reflected light) of thiophenol with H_2O the unpleasant odor of thiophenol was detected. Acidifying the intensely colored solutions of these complexes caused complete reversal to the complex's components. *Anal.* Calcd for $C_7H_6NaO_4$ ($^-OCH_3$ complex): N, 19.2. Found: N, 19.7. Calcd for $C_6H_4KN_3O_4$ (^-OH complex): N, 19.0. Found: N, 19.5. Calcd for $C_{12}H_8N_3NaO_3S$ ($^-SC_6H_5$ complex): N, 14.2. Found: N, 15.1.

Evaluation of Drug Activity.—Compounds dissolved in DMF were added to suspensions of lymphocytes (10–15 × 10^6 cells/ml) in Krebs–Ringer phosphate buffer pH 7.4 containing added glucose (7 m*M*) and heparin (2 IU/ml); 2 min later 1 μcurie of uridine-5-^{3}H was added. The final concentration of DMF was 1% (v/v). The mixture was incubated with slow shaking in air for 30 min at 37° and then acidified with 3 ml of 10% (w/v) trichloroacetic acid (TCA). The acid-insoluble material was washed twice with 5% TCA dissolved in 0.5 *N* NaOH for liquid scintillation counting to determine the amount of radioactivity incorporated into RNA by these cells (in the presence and absence of drugs).

Lymphocytes were isolated by centrifugation from sheep popliteal lymph, collected from conscious ewes over 24-hr periods through a plastic cannula[26] into sterile containers containing approximately 150 units of heparin and 2 mg of penicillin G and washed free of lymph with Hank's medium (containing added heparin, 2 IU/ml).

Data for percentage inhibition given in Tables I and II was obtained from (at least) duplicate incubations at a given drug concentration.

Acknowledgments.—We are most grateful to Dr. Bede Morris for supplying cannulated sheep and to Professor F. C. Courtice, F. A. A., and Dr. D. J. Brown for providing facilities.

(26) J. G. Hall and B. Morris, *Quart. J. Exp. Physiol.*, **46**, 199 (1961).