anecarboxylic acid[19] (1.53 g, 0.01 mole) was dissolved in $SOCl_2$ (5 ml) and the soln was gently refluxed for 1 hr and then concd to a syrup *in vacuo* at low temp (bath temp was below 80°). A soln of this syrup in dry C_6H_6 (20 ml) was added to a soln of benzyl 4-hydroxyphenylpropionate (2.6 g, 0.01 mole) and NEt_3 (2.0 g, 0.02 mole) in dry C_6H_6 (20 ml) with stirring. The reaction mixt was warmed on a water bath for 30 min with stirring. The sepd $NEt_3 \cdot HCl$ was filtered off, and the filtrate was concd to dryness *in vacuo*. The white residue (3.5 g, 89.5%), mp 58–62°, was recrystd from MeOH to give a pure sample of the cyano ester, mp 61–65°; yield, 3.1 g (80%). *Anal.* ($C_{24}H_{25}NO_4$) C, H, N.

A soln of this ester (1.5 g, 0.004 mole) in a mixt of AcOEt–EtOH–H_2O (15:40:20) (75 ml), and 28% NH_4OH (0.25 ml, *ca.* 0.002 mole) was placed in an autoclave and Raney Ni catalyst (W-5) (1.5 ml) was added. Hydrogenation was achieved at an initial pressure of 140 kg/cm² at 70°. After completion of the hydrogenation, the catalyst was filtered off and washed with MeOH and H_2O and the washings were combined with the filtrate and the combined soln was concd *in vacuo* at low temp (bath temp was 40–50°). The sepd crystals were collected by filtration and washed with hot MeOH; yield, 0.8 g. The crystals, mp 200–280° dec, were dissolved in a small amt of AcOH, an equimolar HCl–AcOH was added, and then *i*-Pr_2O was added to the soln. The ppt was collected, washed with enough *i*-Pr_2O, and recrystd from MeOH–Et_2O to give 0.7 g of 99.

4-Nitrophenyl *trans*-4-Aminomethylcyclohexanecarboxylate Hydrobromide (109). A soln of II (14.6 g, 0.05 mole) and *p*-nitrophenol (8.4 g, 0.06 mole) in AcOEt (90 ml), DCC (12.4 g, 0.06 mole) was added at room temp. Gradually cryst materials sepd from the reaction mixt which was allowed to stand at room temp overnight. White crystals were collected and washed with cold AcOEt. Recrystn from EtOH gave pale yellow crystals, mp 130–132°, yield, 17.5 g (85%). These crystals (2.1 g, 0.005 mole) were suspended in 15% HBr–AcOH (10 ml) and warmed at 50° for 10 min and then the resulting homogeneous soln was cooled. Dry Et_2O was added to the soln and the ppt was recrystd from EtOH to give pale yellow needles of 109.

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References

(1) A. Nagamatsu, T. Okuma, M. Watanabe, and Y. Yamamura, *J. Biochem. (Tokyo)*, 54, 491 (1963).
(2) M. Muramatsu, T. Onishi, and S. Fujii, *Proc. Symp. Chem. Physiol. Pathol.*, 3, 142 (1963).
(3) M. Muramatsu, T. Onishi, S. Makino, S. Fujii, and Y. Yamamura, *J. Biochem. (Tokyo)*, 57, 402 (1965).
(4) M. Muramatsu, T. Onishi, S. Makino, S. Fujii, and Y. Yamamura, *ibid.*, 57, 450 (1965).
(5) M. Muramatsu, T. Onishi, S. Makino, Y. Hayakumo, and S. Fujii, *ibid.*, 58, 214 (1965).
(6) M. Muramatsu, and S. Fujii, *ibid.*, 65, 17 (1969).
(7) F. Markwardt, P. Neuland, and H. P. Klöcking, *Pharmazie*, 21, 345 (1966).
(8) H. Landmann, and F. Markwardt, *Hoppe-Seyler's Z. Physiol. Chem.*, 348, 745 (1967).
(9) H. G. Kazmirowski, P. Neuland, H. Landmann, and F. Markwardt, *Pharmazie*, 22, 465 (1967).
(10) M. Iwamoto, Y. Abiko, and M. Shimizu, *J. Biochem. (Tokyo)*, 64, 759 (1968).
(11) K. Tanizawa, S. Ishii, and Y. Kanaoka, *Chem. Pharm. Bull.*, 18, 2346 (1970).
(12) A. Okano, T. Miki, M. Inaoka, and S. Isoda, submitted for publication in *Chem. Pharm. Bull.*
(13) M. Muramatsu and S. Fujii, *Biochim. Biophys. Acta*, 242, 222 (1971).
(14) M. Shimizu, T. Aoyagi, M. Iwamoto, Y. Abiko, T. Naito, and A. Okano, *Chem. Pharm. Bull.*, 16, 357 (1968).
(15) S. Okamoto and U. Okamoto, *Keio J. Med.*, 11, 105 (1962).
(16) Y. Abiko, M. Iwamoto, and M. Tomikawa, *Biochim. Biophys. Acta*, 185, 424 (1969).
(17) Y. Abiko and M. Iwamoto, *ibid.*, 214, 411 (1970).
(18) M. Fetizon, M. Golfier, and N. Laffont, *C. R. Acad. Sci.*, 254, 3376 (1962); *Chem. Abstr.*, 57, 7186g (1962).
(19) S. Siegel and J. M. Komarmy, *J. Amer. Chem. Soc.*, 82, 2547 (1960).

Potential Antileukemic and Immunosuppressive Drugs. 3. Effects of Homocyclic Ring Substitution on the *in Vitro* Drug Activity of 4-Nitrobenzo-2,1,3-oxadiazoles (4-Nitrobenzofurazans) and Their *N*-Oxides (4-Nitrobenzofuroxans)[1]

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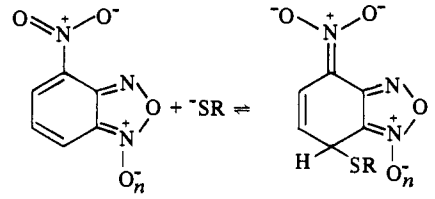
4-Nitrobenzofuroxans and benzofurazans bearing electron-withdrawing substituents in the 5 and 6 positions (relative to NO_2) have been examined for their ability to inhibit nucleic acid synthesis in rabbit thymocytes *in vitro*. None of the compds tested were more potent in this screen than the unsubstituted nitrobenzofurazan or nitrobenzofuroxan, suggesting that the formation and stability of Meisenheimer complexes with cellular thiols and amino groups is diminished by the presence of substituents in the 5 as well as 6 position. The 5-halogeno (F, Cl, Br) benzofuroxans nitrated in the 4 position, in contrast to 5-CF_3, 5-CN, 5-CONHR, and 5-COOR benzofuroxans which direct NO_2 to the 7 position. Some unique chemical and biological properties of 5-F (*vs.* 5-Cl or 5-Br) benzofuroxan are discussed.

Benzofuroxans and benzofurazans bearing NO_2 groups in the 4 and 5 positions have been shown to be potent *in vitro* inhibitors of nucleic acid synthesis in lymphocytes.[2] It was suggested[2] that a possible mode of action of these compounds at the cellular level was by forming Meisenheimer complexes with essential cellular SH and/or amino groups.



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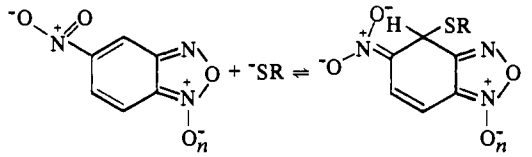

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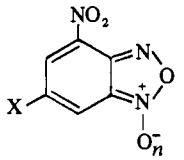

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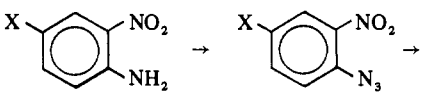
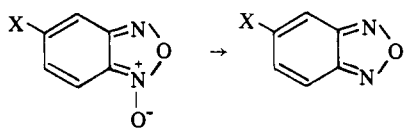
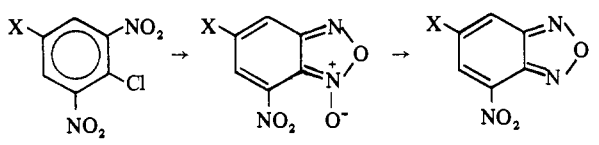


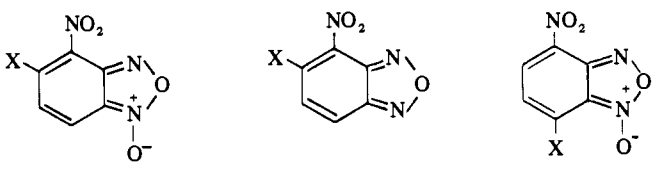
IV V



VI VII



VIII IX X



XI XII XIII

The suggested complexes formed by interactions of these compds with thiols are shown in I and II.

The potencies of some individual nitrobenzofuroxans and benzofurazans as *in vitro* drugs were related to the ease of formation, and stability of the Meisenheimer complexes formed with model thiols,[2] suggesting that the presence of electron-withdrawing substituents, which are known stabilizers of Meisenheimer complexes,[3] might considerably increase the intrinsic drug activity (SH-binding ability) of nitrobenzofuroxans and nitrobenzofurazans.

Previous studies[1,2] of these compds had established that complex stability (and consequently drug activity) was very dependent on the position of the substituent relative to the NO_2 group. Thus substituents ortho to NO_2 considerably diminished the drug activity, probably due to steric interaction between the substituent and NO_2, thereby reducing the ability of NO_2 to become coplanar with the aromatic ring which is required to form the Meisenheimer type complexes (I and II). This report describes the effect on drug activity of the introduction of electron-withdrawing substituents into the 6 position (iii) (*i.e.*, meta to 4-NO_2) of nitrobenzofurazans and benzofuroxans.

Preparation of Compounds. 4-Nitro-6-trifluoromethylbenzofuroxan (III, X = CF_3, n = 1) was synthesized by nucleophilic displacement of Cl in 4-chloro-3,5-dinitrobenzotrifluoride (VIII, X = CF_3) with N_3^- in DMSO, followed by pyrolysis. However, this synthetic route was not generally applicable to other compds in this series because (a) the appropriate starting materials (VIII) were not readily available and (b) because of the difficulty[4,5] in deoxygenating nitrobenzofuroxans to the corresponding nitrobenzofurazans (*i.e.*, IX → X).

A more general method of synthesis was therefore sought which would yield both the required benzofuroxans and corresponding benzofurazans from the readily available 4-amino-3-nitro-1-substituted benzenes (IV) *via* 5-substituted benzofuroxans and benzofurazans. Direct nitration of these 5-substituted benzofuroxans seemed promising. However, it was not clear from the literature at what position nitration would occur in benzofuroxans and benzofurazans bearing electron-withdrawing substituents. Indeed, in those instances reported, NO_2 entered at different positions. With 5-nitrobenzofuroxan (VI, X = NO_2), substitution occurred at the 7 position (IX, X = NO_2),[6] while 5-chlorobenzofuroxan[7] (VI, X = Cl) and 5-chlorobenzofurazan (VIII)[8] direct NO_2 into the 4 position, yielding XI (X = Cl) and XII (X = Cl), respectively.

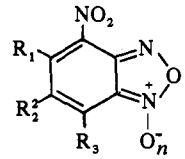
Nitration of 5-trifluoromethylbenzofuroxan (VI, X = CF_3), derived from 4-amino-3-nitrobenzotrifluoride (IV, X = CF_3), by conversion to the azide (V, X = CF_3), followed by pyrolysis, afforded the 7-NO_2 isomer (IX, X = CF_3) as shown by comparison (mp, ir, nmr) with material prepared by the previous route (VII) → (IX). The magnitude of the spin-spin coupling constant (J_{HH}) together with the chemical shift positions have been found to be reliable guides to the substitution pattern in heteroaromatic compounds,[9] as well as in this series.[10]

The nmr spectrum of 4(7)-nitro-6(5)trifluoromethylbenzofuroxan† (see Table I) exhibited a coupling of 1.28 Hz between the protons of the benzene ring. These protons were also coupled to CF_3. The lower field doublet was broad and the very small HCCCF coupling could not be resolved, while the higher field doublet was readily resolvable into a 5-line pattern, to show J^4_{HF} = 1.30 Hz. The difference between these coupling constants reflects the different bond order between C_5–C_6 and C_5–C_4.

5-Methoxycarbonyl- and 5-ethoxycarbonylbenzofuroxan (VI, X = COOMe or COOEt) and benzofurazans (VII, X = COOEt) nitrated in the 7 position, as shown by their H^+–H^+ coupling constants and chemical shifts (See Table I). A similar result was obtained by nitration of 5-carboxyaminobenzofuroxan (VI, X = $CONH_2$) or 5-carboxyisopropylaminobenzofuroxan [VI, X = CONHCH $(CH_3)_2$] derived from 5-carboxybenzofuroxan (VI, X = CO_2H). However, 5-cyanobenzofuroxan (VI, X = CN) and 5-formylbenzofuroxan (VI, X = CHO) and benzofurazan (VII, X = CHO) decomposed under nitration conditions employed. The nmr spectrum of the crude nitration product of the CN compound revealed the presence of only the 7-NO_2 deriva-

†Since benzofuroxans are tautomeric systems with the oxide apparently "flipping" between the 1 and 3 positions,[11] the numbers in parentheses should also be cited to be technically correct, but will be omitted hereafter in this text to minimize confusion.

Table I. Chemical Shifts and Coupling Constants of Substituted Nitrobenzofurazans and Benzofuroxans.

n	R_1	R_2	R_3	δ_1	δ_2	δ_3	J_{12}	J_{13}	J_{23}
									
1	H	CF_3	H	8.720		8.630		1.28	
1	F	H	H		7.954	7.266			9.80
1	Cl	H	H		7.864	7.517			9.30
1	Br	H	H		7.682	7.803			9.40
1	H	COOEt	H	8.813		8.563		1.35	
0	H	COOEt	H	9.114		8.933		1.15	
1	H	$CONH_2$	H	8.900		8.473		1.35	
1	H	$CONHCH(CH_3)_2$	H	8.950		8.510		1.35	
1	H	CN	H	8.836		8.655		0.97	
1	H	H	Br	8.383	7.860		7.80		
1	H	H	Cl	7.905	7.046		8.07		

tive. 5-Formylbenzofurazan was prepared from 5-methylbenzofurazan[12] (VII, X = Me), by dibromination of the Me group, followed by hydrolysis, since deoxygenation of 5-formylbenzofuroxan[1] with trialkyl phosphites (the usual procedure for deoxygenation[13]) proved unsatisfactory. Attempts to obtain the 5-hydroxymethyl-, and 5-cyanomethylbenzofurazans (VII, X = CH_2OH or CH_2CN) from 5-bromomethylbenzofurazan (VII, X = CH_2Br) or its pyridinium salt (VII, X = $CH_2N^+C_5H_5 \cdot Br^-$) were unsuccessful.

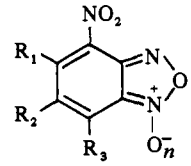
5-Fluorobenzofuroxan (VI, X = F) was initially prepared in 6 stages (IV → VI) starting from *p*-fluoroaniline. The overall low yield (6%) *via* this route prompted the alternative (V → VI) starting from *p*-difluorobenzene to be attempted. This method afforded 5-fluorobenzofuroxan in 42% overall yield. 5-Fluoro- and 5-bromobenzofuroxans[14] (VI, X = F or Br) like their Cl analog, readily nitrated in the 4 position as shown by the coupling constants and chemical shift data (see Table I).

It thus appears from these studies that nitration (and presumably other electrophilic reactions) of benzofuroxans and benzofurazans bearing electron-withdrawing 5 substituents occurs preferentially in the 7 position; with the exception of the 5-halogenobenzofuroxans where the resonance contribution of the halogen atom is sufficiently dominant to promote electrophilic substitution in the ortho position, *i.e.*, at C-4.

Heating 5-bromo-4-nitrobenzofuroxan (XI, X = Br) (J_{HH} = 9.40 Hz) to 120° in the dry state for 10 min afforded the rearranged isomer, 4-bromo-7-nitrobenzofuroxan (XIII, X = Br), as demonstrated by the decreased coupling constant, J_{HH} = 7.80 Hz (*cf.* the rearrangement of the Cl analog,[10] J_{HH} = 9.5 → 8.07 Hz). However, attempts to rearrange 5-fluoro-4-nitrobenzofuroxan (XI, X = F) to 4-fluoro-7-nitrobenzofuroxan (XIII, X = F) under the same conditions, and also in solution, failed as demonstrated by its unchanged coupling constant, J_{HH} = 9.80 Hz. This finding demonstrates again[15] that release of steric strain in the conversion of 4-nitro-5-subsituted benzofuroxans into the 4-substituted-7-nitro isomers is an important factor for the facilitation of this rearrangement. On the other hand, the greater electron-withdrawing ability of F compared to Cl or Br substituents no doubt should also be considered.

Biological Activity. Table II shows that none of the 6-substituted-4-nitrobenzofurazans or benzofuroxans that were

Table II. Inhibition of Uridine-*5-t* (UR) and Thymidine-*6-t* (TdR) into Acid-Insoluble Material (Nucleic Acids) of Washed Rabbit Thymocytes Incubated *in Vitro* with Various Compounds

Compound	*n*	R_1	R_2	R_3	Conc, m*M*	% inhibition UR	% inhibition TdR
							
1	0	H	H	H	0.10	96	98
	0	H	H	H	0.02	20	90
2	1	H	H	H	0.10	97	97
	1	H	H	H	0.02	10	90
3	0	H	COOEt	H	0.20	92	98
	0	H	COOEt	H	0.04	0	42
4	1	H	COOEt	H	0.20	90	98
	1	H	COOEt	H	0.04	0	32
5	1	H	COOMe	H	0.20	92	98
	1	H	COOMe	H	0.04	0	30
6	1	H	CONH-*i*-Pr	H	0.10	95	93
	1	H	CONH-*i*-Pr	H	0.04	56	90
	1	H	CONH-*i*-Pr	H	0.02	6	80
7	1	H	$CONH_2$	H	0.20	77	96
	1	H	$CONH_2$	H	0.04	13	62
8	1	H	CF_3	H	0.10	96	98
	1	H	CF_3	H	0.04	50	95
	1	H	CF_3	H	0.02	0	68
9	1	Br	H	H	0.10	96	94
	1	Br	H	H	0.02	5	92
10	1	Cl	H	H	0.10	95	97
	1	Cl	H	H	0.02	22	92
11	1	F	H	H	0.10	0	0
		Maleimide			0.10	80	99
					0.02	5	77

examined for their effect on nucleic acid synthesis in intact lymphocytes, was more potent *in vitro* than the parent unsubstituted NO_2 compounds. In every instance the electron-withdrawing substituent (CF_3, COX) effectively reduced the drug activity of the parent compds (Table II, $R_1 = R_2 = R_3 = H$; *n* = 0 or 1). However, both these 6-substituted derivatives and the parent compds showed the same qualitative characteristics as maleimide in preferentially inhibiting thymidine-*t* incorporation (a marker of DNA synthesis) at low drug levels and only inhibiting uridine-*5-t* (a marker of RNA synthesis) at somewhat higher levels *in vitro*. This decrease of *in vitro* drug activity upon introducing the substituent into the 6 position may be due to (i) steric hindrance to nucleophilic attack at the 5 or 7 positions by the 6 substituent or (ii) diminished lipid solubility and cell penetration by these 6 substituted compds—and may not necessarily reflect the effect of the 6 substituent upon the intrinsic ability of the parent compounds to form Meisenheimer compds with freely accessible intracellular thiols.

In further studies of the effect of these compounds upon the (glutathione-sensitive) binding of *N*-ethylmaleimide-*2,3*-^{14}C to the same intact cell preparations, we obtained evidence that all these 6-substituted compds (at 0.1 m*M* or greater) could bind to cellular thiols at pH 7.4, since they all inhibited the ^{14}C labeling of acid-insoluble proteins by this labeled maleimide when added to the cells before the *N*-ethylmaleimide-^{14}C.

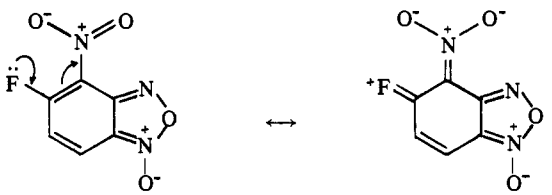
5-Fluoro-4-nitrobenzofuroxan was remarkably different from the Cl and Br analog in both chemical and biological properties. Under conditions where 5-chloro and 5-bromo-

Table III. Absorption Spectra and Thiol-Binding Properties of 5-Halogeno-4-nitrobenzofuroxans

Substituent	λ_{max}, nm	ϵ	% inhibition of NEM-^{14}C binding to cell proteins[b]
None[a]			58
5-Cl	223.5, 320.5, 336.7, 390.6, 480.8	16,100, 2550, 2660, 5750	52
5-Br	223.2, 287.3, 416.7	16,200, 4800, 8050	51
5-F	223.5, 319, 333.3, 375.9, 480.8	15,700, 6700, 5850, 6000, 5000	5

[a] *I.e.*, 4-nitrobenzofuroxan. [b] Assayed by preincubating cells (5×10^6/ml) with those compounds (0.1 m*M*) for 10 min at pH 7.4 at 37°, then adding 0.2 μCi of *N*-ethylmaleimide-^{14}C (1.7 mCi/nmole drug) (Amersham/Searle, Chicago, Ill.) and incubating for a further 5 min. ^{14}C incorporated into the acid-insoluble portions of the cells (a measure of residual thiol after drug binding) was assayed after precipitating and copious washing with 5% (w/v) Cl_3CCO_2H.

4-nitrobenzofuroxan rearrange to the 4-chloro- or 4-bromo-7-nitro isomer (*i.e.*, XI–XIII), 5-fluoro-4-nitrobenzofuroxan could not be rearranged. The uv spectrum (see Table III) of 5-fluoro-4-nitrobenzofuroxan was also different from that of the Cl and Br analog, especially in absorbing at a higher wavelength, suggesting considerable interaction between the F atom and the aromatic ring (A and B).



A B

A greater contribution from resonance structure B would undoubtedly decrease the ability of 5-fluoro-4-nitrobenzofuroxan to bind to acid-insoluble cell thiols, as shown by its failure to prevent significantly the addition of *N*-ethylmaleimide-^{14}C to protein thiol groups of intact lymphocytes (Table III), as well as possibly increasing its hydrophilic character.

Experimental Section

Microanalyses were performed by the Australian Micro-analytical Service, C.S.I.R.O., Melbourne, ir spectra recorded on a Unicam SP 1200, and nmr spectra on a Varian Associates A60D, 60-MHz spectrometer.

4-Nitro-6-trifluoromethylbenzofuroxan (III, X = CF_3; *n* = 1). 4-Chloro-3,5-dinitrobenzotrifluoride (Pierce Chem. Co.) (VIII, X = CF_3) (2.7 g, 0.01 mole), NaN_3 (1.0 g), and DMSO (50 ml) were heated on a steam bath for 30 min, than at 120° for 10 min. After cooling, the mixt was poured onto crushed ice, and the solid was collected, washed (H_2O), and crystd from EtOH to yield III (X = CF_3; *n* = 1) (2.0 g 80%) as yellow needles: mp 126–127°. *Anal.* ($C_7H_2F_3N_3O_4$) C, H, N.

5-Trifluoromethylbenzofuroxan (VI, X = CF_3). 4-Amino-3-nitrobenzotrifluoride (Eastman Kodak) (20.6 g, 0.1 mole) in glacial AcOH (200 ml) was added gradually to a stirred ice-cooled soln of nitrosylsulfuric acid made from $NaNO_2$ (7.5 g) and H_2SO_4 (200 ml, sp gr 1.84) in such a way that the temp did not exceed 25°. When addn was completed, stirring was continued for 1 hr, the soln poured into ice water (1000 ml), and the mixt then added to a vigorously stirred soln of NaN_3 (7.1 g, 0.11 mole) in H_2O (150 ml). When effervescence had ceased, the azide (V, X = CF_3) was extracted into Et_2O and dried ($MgSO_4$), and the solvent was removed under vacuum at 30°. The remaining oil was not purified because of the danger of decomposition. However, the presence of N_3 and NO_2 groups was established by their absorption. The azide (V, X = CF_3) (17.2 g) was then refluxed with glacial AcOH (100 ml) until N_2 ceased to be evolved (*ca.* 1 hr). Treatment with charcoal followed by dilution with salt water (200 ml), gave a yellow oil, which was extracted into Et_2O, dried ($MgSO_4$), and vacuum distd. 5-Trifluoromethylbenzofuroxan [9.3 g, 45% based on (IV, X = CF_3)] was obtd as a pale yellow oil: bp 118–120° (1 mm). *Anal.* ($C_7H_3F_3N_2O_2$) C, H, N.

Nitration of 5-Trifluoromethylbenzofuroxan. To stirred, ice-cooled 5-trifluoromethylbenzofuroxan (VI, X = CF_3) (2.04 g, 0.01 mole) in H_2SO_4 (50 ml, sp gr 1.84) was added HNO_3 (0.7 g, sp gr 1.5) in H_2SO_4 (5.0 ml, sp gr 1.84) over a period of 20 min. Stirring at 6° was then contd for a further hr. Addn to crushed ice (200 g) afforded a yellow ppt which was collected, washed (H_2O, 100 ml), vacuum dried, and recrystd from CH_2Cl_2 to give a NO_2 deriv (1.6 g, 64%) as yellow needles: mp 126–127°. This was identical in all respects with III (X = CF_3; *n* = 1) obtd by the direct route described above. *Anal.* ($C_7H_2F_3N_3O_4$) C, H, N.

5-Methoxycarbonylbenzofuroxan (VI, X = COOMe). 5-Carboxybenzofuroxan[16] (0.9 g, 0.005 mole) in dry MeOH (200 ml) satd with dry HCl was refluxed with exclusion of moisture for 16 hr. After cooling, MeOH was removed under vacuum, and the residue redissolved in Et_2O. The soln was then extd with NaOH (25 ml, 0.1 *M*), washed (H_2O, 50 ml), dried ($MgSO_4$), and Et_2O removed under vacuum. The yellow residue was recrystd from MeOH (charcoal) to yield the ester (VI, X = COOMe) (0.5 g, 50%) as pale yellow plates: mp 90–91°. *Anal.* ($C_8H_6N_2O_4$) C, H, N.

5-Ethoxycarbonylbenzofuroxan (VI, X = COOEt) was prepd according to the procedure of Boulton, *et al.*,[17] mp 64–65° (lit.[17] mp 66°).

5-Ethoxycarbonylbenzofurazan (VII, X = COOEt). The benzofuroxan ester (VI, X = COOEt) (2.08 g, 0.01 mole) and $P(OEt)_3$ (1.70 g) in EtOH (75 ml) were refluxed on a steam bath for 2 hr. After removal of the solvent under vacuum, the residue was shaken with H_2O (100 ml) and allowed to stand at 0-2° overnight. The tan ppt was collected, washed (H_2O), air-dried, and sublimed [100° (20 mm)] to yield VII (X = COOEt) (1.8 g, 94%) as white needles: mp 44–45°; ν_{max}: 1705 cm^{-1} (COOEt); nmr ($CDCl_3$): Et. *Anal.* ($C_9H_8N_2O_3$) C, H, N.

Nitration of 5-Alkoxycarbonylbenzofuroxans (VI, X = COOMe or COOEt). The 5-alkoxycarbonylbenzofuroxan (0.005 mole) in H_2SO_4 (10 ml, sp gr 1.84) was treated at 0–5° with stirring with HNO_3 (0.0075 mole, sp gr 1.51) in H_2SO_4 (5 ml, sp gr 1.84) at such a rate that the temp did not exceed 10°. After standing at 20° for 30 min the mixt was poured onto crushed ice, the ppt collected, washed (H_2O), and crystd from EtOH to yield the derivatives. 5-Methoxycarbonyl-7-nitrobenzofuroxan (IX, X = $COOCH_3$) was obtd as yellow needles: mp 134–135°. *Anal.* ($C_8H_5N_3O_6$) C, H, N. 5-Ethoxycarbonyl-7-nitrobenzofuroxan (IX, X = $COOC_2H_5$) crystd from EtOH as yellow needles: mp 116–117°. *Anal.* ($C_9H_7N_3O_6$) C, H, N.

5-Ethoxycarbonyl-7-nitrobenzofurazan (X, X = COOEt). 5-Ethoxycarbonylbenzofurazan (VII, X = COOEt) was nitrated using identical conditions with those described for the alkoxybenzofuroxans above. 5-Ethoxycarbonyl-7-nitro-benzofurazan separated from EtOH as pale yellow needles: mp 91–92°. *Anal.* ($C_9H_7N_3O_5$) C, H, N.

5-Aminocarbonylbenzofuroxan (VI, X = $CONH_2$). 5-Chlorocarbonylbenzofuroxan[16] (VI, X = COCl) (1.80 g, 0.01 mole) in Me_2CO (25 ml) treated dropwise with NH_3 (10 ml) (sp gr 0.88) with vigorous shaking and cooling in ice. Evapn of the solvent afforded a crystalline residue which was stirred with H_2O (100 ml) and filtered. Crystallization from EtOH yielded the amide (VI, X = $CONH_2$) (1.2 g, 75%) as pale yellow prisms: mp 168–169°. *Anal.* ($C_7H_5N_3O_3$) C, H, N.

5-Isopropylaminocarbonylbenzofuroxan [VI, X = $CONHCH(CH_3)_2$]. Treatment of 5-chlorocarbonylbenzofuroxan[16] (0.01 mole) in Me_2CO (25 ml) with *i*-$PrNH_2$ (10 ml) in a similar manner to that described above afforded VI [X = $CONHCH(CH_3)_2$] (80%) as yellow needles from EtOH: mp 172–173°. *Anal.* ($C_{10}H_{11}N_3O_3$) C, H, N.

5-Aminocarbonyl-7-nitrobenzofuroxan (IX, X = $CONH_2$). To the amide VI (X = $CONH_2$) (0.8 g, 0.005 mole) in H_2SO_4 (20 ml, sp gr 1.84) at 5° was added HNO_3 (0.5 g, sp gr 1.51) in H_2SO_4 (2 ml, sp gr 1.51) such that the temp of the ice-cooled mixt did not exceed 15°. After stirring at this temp for 15 min, the mixt was poured into ice water, and the ppt was collected and washed (H_2O) to yield IX (0.8 g, 72%) as yellow plates, mp 212–213°. *Anal.* ($C_7H_4N_4O_5$) C, H, N.

5-Isopropylaminocarbonyl-7-nitrobenzofuroxan [IX, X = $CONHCH(CH_3)_2$] was prepared as described above in 85% yield.

It crystallized from MeOH as yellow needles: mp 198–199°. *Anal.* ($C_{10}H_{20}N_4O_5$) C, H, N.

5-Cyanobenzofuroxan (VI, X = CN). An intimate mixt of 5-aminocarbonylbenzofuroxan (VI, X = $CONH_2$) (1.79 g, 0.01 mole) and P_2O_5 (1.5 g) in quinoline (25 ml) was heated at 170° with stirring for 5 min, after which more P_2O_5 (1.5 g) was added and heating at 70° was maintained for another 10 min. After cooling, the mixt was shaken with HCl (100 ml, 5 *N*) and extd (Et_2O). Removal of the solvent under vacuum after drying ($MgSO_4$), afforded a red oil which solidified on standing. Recrystallization from petr ether (50–60°)–Et_2O (1:1) yielded VI (X = CN) (0.7 g, 39%) as yellow plates: mp 75–76°. *Anal.* ($C_7H_3N_3O_2$) C, H, N.

Nitration of 5-Cyanobenzofuroxan. 5-Cyanobenzofuroxan was nitrated using identical conditions with that described for the amides (IX, X = CONHR). The oily solid which separated on work-up was collected and washed (H_2O). Attempts to purify this material further were unsuccessful.

5-Methylbenzofurazan (VII, X = CH_3). To 5-methylbenzofuroxan[12] (60 g) in refluxing EtOH (500 ml) was added dropwise $P(OEt)_3$ (100 ml). When addition was complete (*ca.* 1 hr), refluxing was continued for a further 1 hr. The solvent was removed by rotary evaporation and the residue shaken with H_2O (400 ml) and allowed to stand overnight at 0–5°. The brown solid so obtained was washed with H_2O and steam distd to yield 5-methylbenzofurazan (33 g, 62%) as white needles: mp 35° (lit.[12] mp 37°).

5-Bromomethylbenzofurazan‡ (VII, X = CH_2Br). 5-Methylbenzofurazan (26.8 g, 0.2 mole), NBS (26.3 g), and Bz_2O_2 (0.05 g) were refluxed in CCl_4 (200 ml) with stirring and exclusion of moisture for 20 hr. The cooled mixt was extd with H_2O (150 ml), the CCl_4 layer was dried ($MgSO_4$), and the solvent was removed by rotary evaporation. The resulting oil (43 g) was triturated with petr ether (200 ml, boiling point range 40–50°) and filtered, and the residue was washed with petr ether (100 ml), and then recrystd from PhH–petr ether (1:2) to give VII(X = CH_2Br) (26.5 g, 62%) as pale yellow prisms: mp 78–79°. The mother liquors afforded a further 5 g of material on evapn: *m/e* 214; ν_{max}: 664 cm^{-1} (CBr); nmr ($CDCl_3$): CH_2Br. *Anal.* ($C_7H_5BrN_2O$) C, H, N.

Reaction of 5-Bromomethylbenzofurazan with Pyridine. The bromomethyl compd VII(X = CH_2Br) (2.14 g), EtOH (50 ml), and pyridine (1 g) were refluxed for 30 min on a steam bath. After cooling, the white ppt was collected and recrystd from EtOH to yield the pyridinium salt (VII, X = $CH_2N^+C_5H_5 \cdot Br^-$) (2 g, 69%) as white needles: mp > 200°. *Anal.* ($C_{12}H_{10}BrN_3O$) C, H, N.

Attempts to Prepare 5-Cyanomethylbenzofurazan (VII, X = CH_2CN). 5-Bromomethylbenzofurazan (VII, X = CH_2Br) (2.14 g, 0.01 mole) in EtOH (25 ml) was mixed with KCN (1 g, 0.012 mole) in H_2O (10 ml). The resulting dark brown soln was refluxed on a steam bath for 4 hr. Removal of the solvent by rotary evaporation afforded a tarry material which was uncharacterized. Similar results were obtained using DMF or DMSO as solvents, or the pyridinium salt (VII, X = $CH_2N^+C_5H_5 \cdot Br^-$) in place of the CH_2Br compound.

5-Dibromomethylbenzofurazan‡ (VII, X = $CHBr_2$). 5-Methylbenzofurazan (13.4 g, 0.1 mole), NBS (35.7 g), and Bz_2O_2 (0.1 g) were refluxed with stirring in CCl_4 (500 ml) with exclusion of moisture for 30 hr. Extn with H_2O and sepn of the CCl_4 layer gave a yellow oil after removal of the solvent. This was redissolved in PhH (200 ml) and petr ether was added until the soln became turbid. After 16 hr standing at 0° the dibromo derivative VII (X = $CHBr_2$) (15 g, 51%) deposited as tan prisms: mp 75–76°; *m/e* 294; nmr (DMSO-d_6): $CHBr_2$. *Anal.* ($C_7H_4Br_2N_2O$) C, H, N.

5-Formylbenzofurazan (VII, X = CHO). To a refluxing mixt of the dibromomethyl compd (VII, X = $CHBr_2$) (2.94 g, 0.01 mole) in EtOH (150 ml) was added $AgNO_3$ (3.5 g) in H_2O (30 ml). Refluxing was contd for 10 min after addn, the mixt was cooled, and the AgBr was removed by filtn. Removal of the solvent left a mixt of Ag salts and the formyl compd as an oil. Extn with PhH, and treatment with charcoal gave, on evapn, an oil which solidified on standing. Sublimation at 100° (10 mm) gave the aldehyde (0.9 g, 60%) as white needles: mp 55–56°; ν_{max} 2900 (CHO), 1705 cm^{-1} (CHO); nmr ($CDCl_3$) (CHO). *Anal.* ($C_7H_4N_2O_2$) C, H, N.

4-Azido-3-nitrobenzaldehyde. 4-Amino-3-nitrobenzaldehyde[18] (IV, X = CHO) (3.0 g) in glacial AcOH (50 ml) was added to stirred ice-cold nitrosylsulfuric acid from $NaNO_2$ (6.5 g) and H_2SO_4 (50 ml, sp gr 1.84) at such a rate that the temp did not exceed 20°. When the addn was completed, the mixt was poured onto crushed ice (250 g), and the clear soln was added to NaN_3 (10.0 g) in H_2O (50 ml) with vigorous shaking. Addition of an ice-salt mixt (100 ml) pptd the azide as a white solid (3.0 g, 86%) which was not further purified but used directly for the next stage.

5-Formylbenzofuroxan (VI, X = CHO). The azide (3.0 g) obtained above was refluxed 30 min with PhMe (50 ml), treated with charcoal, and filtered, and petr ether (20 ml) added. After standing for 2 hr at 0° the pale yellow cryst solid was collected and recryst from EtOAc–petr ether (1:2) to yield the formyl comp (2.5 g) as pale yellow needles: mp 67–68° (lit.[1] mp 68°).

4-Fluoroacetanilide. 4-Fluoroaniline (50 g), H_2O (700 ml), and Ac_2O (275 ml) were heated at 70° for 15 min and cooled to 5° and the ppt was collected, washed with ice-water (1000 ml), and vacuum dried to yield 54.2 g (79%) of colorless plates: mp 151–152° (lit.[19] mp 150.5–151.5°).

4-Fluoro-3-nitroacetanilide. To a stirred mixt of 4-fluoroacetanilide (54.2 g) in H_2SO_4 (192 g, sp gr 1.84) was added HNO_3 (33.8 g, sp gr 1.50) in H_2SO_4 (37.3 g, sp gr 1.84) at such a rate that with ice cooling the temp did not exceed 3°. When addn was complete, stirring at 0° was contd for a further 2 hr. After pouring into ice water (1500 g) and crystn from EtOH the nitro compd (38.3 g, 55%) was obtained as pale yellow plates: mp 68–70° (lit.[19] mp 69–70.5°). Mother liquors afforded a further 7.0 g on concentration under vacuum.

4-Fluoro-2-nitroaniline (IV, X = F). 4-Fluoro-2-nitroacetanilide (38.3 g) and HCl (170 ml, 3 *N*) were refluxed together for 30 min. Cooling, then diluting with H_2O (330 ml) afforded the amine (19.7, 70%) as yellow prisms: mp 89–91° (lit.[19] mp 90–92°). The ir spectrum indicated a primary amine, and no amide carbonyl stretch. This material was used without further purification for the next stage.

4-Fluoro-2-nitrophenyl Azide (V, X = F). 4-Fluoro-2-nitroaniline (19.7 g) in glacial AcOH (200 ml) was gradually added to ice-cooled stirred nitrosyl sulfuric acid [from $NaNO_2$ (9.7 g) and H_2SO_4 (200 ml, sp gr 1.84)] such that the temp did not exceed 30°. When the addn was compl, stirring was contd for a further 1 hr at 5°, then the soln was poured onto crushed ice (1000 g). Addition of this diazonium soln to NaN_3 (8.2 g) in H_2O (250 ml) pptd the azide as a cream solid; mp 25–25.5°, which was not purified further because of the possibility of decompn: ir N_3, NO_2.

5-Fluorobenzofuroxan (VI, X = F). The crude damp azide (above) was refluxed in glacial AcOH (100 ml) for 1 hr. Diln with H_2O (400 ml) afforded a pale yellow oil which solidified on standing at 0° for 12 hr. Purification by sublimation [100° (20 mm)] afforded VI, X = F, as pale yellow needles (5.5 g, 8% overall from 4-fluoroaniline): mp 47–48°. *Anal.* ($C_6H_3FN_2O_2$) C, H, N.

2,5-Difluoronitrobenzene. To a stirred mixt of HNO_3 (7.0 g, sp gr 1.5) in H_2SO_4 (10 g, sp gr 1.84) at −40° was added dropwise *p*-difluorobenzene (Aldrich Chemical Co.) (7.0 g) at such a rate that the temp did not exceed −30°; after which stirring was contd for a further 3 hr at 0°. Addition to crushed ice (1000 g), neutralization with K_2CO_3, and extraction with Et_2O followed by distillation afforded the product (6.0 g, 62%): bp 103–104° (25 mm) [lit.[20] bp 103° (25 mm)].

5-Fluorobenzofuroxan (VI, X = F). A mixture of 2,5-difluoronitrobenzene (6.0 g) and NaN_3 (2.3 g) in DMSO (30 ml) was heated at 125–130° until the evolution of N_2 ceased (*ca.* 15 min). Dilution with H_2O (100 ml) followed by extraction with Et_2O gave after sublimation [100° (20 mm)] the fluoro compound VI (X = F) (4.0 g, 42%) as yellow needles: mp 47–48°; identical (ir, analysis, mp) with material obtained by the alternative route described above.

5-Fluoro-4-nitrobenzofuroxan (XI, X = F). To a stirred ice-cooled soln of 5-fluorobenzofuroxan (1.54 g, 0.01 mole) in H_2SO_4 (20 ml, sp gr 1.84) was added dropwise HNO_3 (0.7 g, sp gr 1.5) in H_2SO_4 (9.2 g, sp gr 1.84) such that the temp did not exceed 5°. Stirring was then continued a further 40 min at 0°, then the soln poured onto crushed ice (200 g). The resulting yellow solid was collected, washed (H_2O) (100 ml), air-dried, and then recrystallized from Et_2O to give the nitro product (XI, X = F) (1.6 g, 80%) as yellow prisms: mp 169–171° dec. *Anal.* ($C_6H_2FN_3O_4$) C, H, N.

5-Bromobenzofuroxan (VI, X = Br) was obtained by pyrolysis of 4-bromo-2-nitrophenylazide according to Forster and Barker:[14] mp 70° (lit.[14] mp 69°).

5-Bromo-4-nitrobenzofuroxan (XI, X = Br). The nitration of 5-bromobenzofuroxan was performed by a procedure identical with that of the nitration of the 5-F analog (XI, X = F). The product crystallized from CH_2Cl_2 as yellow needles: mp 129–130°. *Anal.* ($C_6H_2BrN_3O_4$) C, H, N.

4-Bromo-7-nitrobenzofuroxan (XIII, X = Br). 5-Bromo-4-nitrobenzofuroxan (XI) (0.1 g) was heated for 10 min a test tube

‡Caution. 5-Monobromomethyl and 5-dibromomethylbenzofurazans are highly lacrymatory and cause irritation of the skin.

in an oil bath maintained at 120°. After cooling the residue was crystd from AcOH to yield XIII (X = Br) (0.06 g, 60%) as orange prisms: mp 140–142°. *Anal.* ($C_6H_2BrN_3O_4$) C, H, N.

Biological Testing. Compounds were freshly dissolved in DMSO and added to 2-ml suspensions of freshly isolated and twice-washed rabbit thymocytes (0.5–1.0 × 10^6 cells/ml) in Hanks medium buffered with 0.2 vol of 0.1 *M* sodium phosphate buffer, pH 7.4. The final DMSO concns did not exceed 1% (v/v). Cells and drugs were then incubated at 37° with uridine-*5-t* or thymidine-*6-t* (0.25 μCi/ml) for 40 min. Radioactivity incorporated into the acid-insoluble fraction was determined after adding 1 vol of 10% (w/v) CCl_3COOH to each incubation, washing the pptd material twice with 5% CCl_3COOH and redissolving the ppt in 0.4 ml of 0.5 *N* NaOH. After decolorization with H_2O_2 (1 drop, 30% vol H_2O_2), 0.1-ml aliquots of the alkaline digest were assayed for ^3H by liquid scintillation counting.

Radioactivity incorporated into RNA (from uridine-*t*) or DNA (from thymidine-*t*) in control incubations containing DMSO only, in replicate incubations with a given preparation of thymus lymphocytes varied by not more than ±5%. The mean radioactivity of RNA or DNA from these replicated drug-free control incubations was assigned the value of 100%. Each compd was assayed in duplicate incubations at any one drug level. Drug activity was computed as the per cent inhibition of nucleic acid labeling with ^3H. For reference purposes a standard drug (maleimide or 4-nitrobenzofurazan) was included in each series of incubations with a new preparation of lymphocytes. The reproducibility of the drug response to these standard compds with different preparation of lymphocytes was generally within 8–10%. Data obtained with cell preparations which responded abnormally to these standard compds were not used for compiling Table II.

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References

(1) P. B. Ghosh and M. W. Whitehouse, *J. Med. Chem.*, 12, 505 (1969) (paper 2).
(2) P. B. Ghosh and M. W. Whitehouse, *ibid.*, 11, 305 (1968).
(3) J. Miller, "Aromatic Nucleophilic Substitutions," Elsevier, Amsterdam, 1967.
(4) F. B. Mallory and S. P. Varimbi, *J. Org. Chem.*, 28, 1656 (1962).
(5) G. Englert and H. Prinzbach, *Z. Naturforsch*, 17b, 4 (1962).
(6) P. Drost, *Justus Liebings Ann. Chem.*, 307, 49 (1899).
(7) A. J. Boulton and A. R. Katritzky, *Rev. Chim. Acad. Repub. Pop. Roum.*, 7, 691 (1962).
(8) A. J. Boulton, A. C. Gripper Gray, and A. R. Katritzky, *J. Chem. Soc.*, 5958 (1965).
(9) A. R. Katritzky, Y. Takeuchi, B. Ternai, and G. J. T. Tiddy, *Org. Magn. Res.*, 2, 357 (1970).
(10) P. B. Ghosh, *J. Chem. Soc. B*, 334 (1968).
(11) A. J. Boulton and P. B. Ghosh, *Advan. Heterocycl. Chem.*, 10, 8 (1969).
(12) R. J. Gaughran, J. P. Picard, and I. V. R. Kaufman, *J. Amer. Chem. Soc.*, 76, 2233 (1954).
(13) J. H. Boyer and S. E. Ellzey, *J. Org. Chem.*, 26, 4684 (1961).
(14) M. O. Forster and M. F. Barker, *J. Chem. Soc.*, 103, 1918 (1913).
(15) A. J. Boulton and R. C. Brown, *J. Org. Chem.*, 35, 1662 (1970).
(16) A. J. Boulton, P. B. Ghosh, and A. R. Katritzky, *J. Chem. Soc. C*, 971 (1966).
(17) A. J. Boulton, A. R. Katritzky, M. J. Sewell, and B. Wallis, *ibid., B*, 914 (1967).
(18) H. G. Beard and H. H. Hodgson, *J. Chem. Soc.*, 24 (1927).
(19) M. Bil, *Chem. Ind. (London)*, 198 (1969).
(20) F. Swartz, *Bull. Acad. Roy. Belg.*, 241 (1913).

β-Adrenergic Blocking Agents. 12. Heterocyclic Compounds Related to Propranolol

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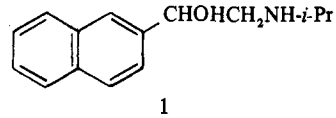
The synthesis and biological properties of some heterocyclic compounds related to propranolol (2) are described. Most compounds have the side chain attached to the benzenoid part of a heterocyclic system, and activity is highest when the position of attachment is α. Also included is a series of phenoxypropanolamines with a heterocyclic substituent. Many compounds have the same level of β-adrenergic blocking potency as propranolol.

In our previous paper[1] we described the synthesis and properties of a series of heterocyclic analogs of pronethalol (1).† We now report the synthesis of some heterocyclic compounds related to propranolol (2).‡,[2-4]

In most of these compounds (Tables I–VI) the oxypropanolamine side chain is attached to the benzenoid ring of a heterocyclic system, but a series of carbostyrils is also described (Table VII) in which the side chain is attached to the heterocyclic ring. We have also included a series of compounds (Table VIII) in which the heterocyclic nucleus is a substituent on the 1-amino-3-phenoxy-2-propanol molecule.

Chemistry. The methods used to prepare these compounds were in general analogous with those previously reported[5] (Scheme I). In routes A and B (those most frequently employed) the intermediate chlorohydrin or epoxide was not usually characterized, the crude product being treated immediately with the appropriate amine. As before,[5a,b] we confirmed that the epoxide opened in the desired manner by the use in one or two instances of the alternative synthesis (method C).



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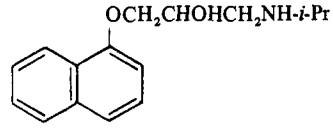

2

In one case the use of method D led also to hydrogenation of a quinoline ring to give **49**.

Reaction of **5** with AcCl gave the ester **18**, while the oxazolidines **36** and **80** were obtained from **35** and **67**, respectively, by treatment with CH_2O. Bromination of **34** gave a monobromo derivative which we consider from spectroscopic evidence to be either the 6- or 8-Br compound **44**.

When the heterocyclic nucleus carried a strongly basic N atom, side reactions (presumably quaternization) were a serious complication. Thus, the quinoline compounds in

†Alderlin.
‡Inderal.