ester, which crystallized from 50% EtOH as white needles, mp 116–117°.

3,5-Dialkyl-4-(4-aminophenoxy)phenyl-DL-alanines (Table III, 19–20).—Diethyl 4-(4-aminoaryloxy)-3,5-dialkylbenzylacetamidomalonate (**13, 14**; 0.005 mol) was heated under reflux with 50 ml of 50% H_2SO_4 for 4 hr. On cooling, white plates of the hydrated sulfate salt pptd. The salt was recrystd from H_2O containing a few drops of H_2SO_4.

3,5-Dialkyl-3'-halo-DL-thyronines (Table III, 21–24).—To the 3,5-dialkyl-DL-thyronines (**16, 17**; 0.4–0.8 mmol) dissolved in 10 ml of vigorously stirred 40% $MeNH_2$ maintained at 5–10° was quickly added 90–100% of the calcd amount of I_2 as a 1 *N* solution in aq KI.[17] Stirring was continued for 10 min after the addition was complete. The solution was adjusted to pH 5 with concd HCl. The pptd 3'-I derivatives (**23, 24**) were collected by filtration and purified by several isoelectric pptns from 10% NaOH solution by adjusting the pH to 5.0 with concd HCl.

The 3'-Br derivatives (**21, 22**) were prepared by dropwise addition of Br_2 (176 mg, 1.10 mmol) in 5 ml of AcOH to a solution (maintained at 50–60°) of 3,5-dialkyl-DL-thyronine (**16, 17**; 1.0 mmol) in 30 ml of AcOH containing a few drops of concd HCl.[18] Fifteen minutes after addition was complete, the solution was decolorized with sodium metabisulfite, diluted with H_2O, and adjusted to pH 3.7 with 20% NaOAc. The ppt was washed with H_2O, dissolved in aq EtOH containing a few drops of concd HCl, and reprecipitated at pH 5.0 with 20% NaOAc.

Acknowledgments.—We are indebted to Dr. S. B. Barker and Dr. R. E. Taylor, Jr., for biological evaluations by the rat heart-rate and tadpole metamorphosis methods. We are grateful for the assistance of Mr. A. Ishimoto and Miss S. M. Vora in the preparation of some intermediate compounds. Dr. J. R. Nuhn provided valued assistance in the conduct of rat antigoiter assays.

(17) E. C. Jorgensen and R. A. Wiley, *J. Pharm. Sci.*, **52**, 122 (1963).

(18) E. C. Jorgensen and P. A. Lehman, *J. Org. Chem.*, **26**, 805 (1961).

Synthetic Schistosomicides. XVI. 5-(Mono- and Dialkylamino)-2-nitrosophenols, 2-Amino-5-(dialkylamino)phenols, and Related Compounds[1]

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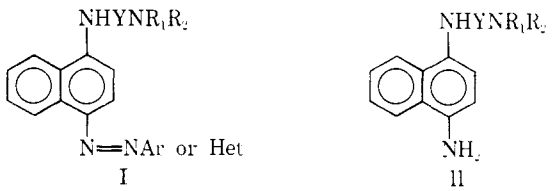
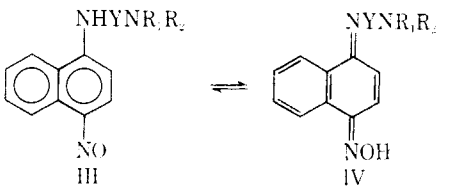
Various 5-(mono- and dialkylamino)-2-nitrosophenols VIII were prepared by nitrosation of the corresponding *m*-(mono- and dialkylamino)phenols. The latter intermediates were obtained by heating resorcinol with an excess of the appropriate amine at 200°, or by alkylation of *m*-aminophenol with an alkyl halide. 5-(Dimethylamino)-2-nitrosophenol (**6**), 5-(diethylamino)-2-nitrosophenol (**9**), 2-nitroso-5-(1-pyrrolidinyl)phenol (**7**), and 2-amino-5-(diethylamino)phenol (**17a**), a potential metabolite of **9**, displayed strong schistosomicidal activity and effected a 70–100% reduction of adult *Schistosoma mansoni* in mice at daily doses of 177–568 mg/kg for 14 days. Structure-activity relationships are summarized, and information concerning potential metabolites and the possible mode of action of the nitrosophenols is discussed.

The potent chemotherapeutic effects of various *N,N*-dialkyl-*N'*-(4-arylazo- and 4-heterocyclic azo-1-naphthyl)alkylenediamines (I)[2–9] and the corresponding *N*-(dialkylaminoalkyl)-1,4-naphthalenediamines (II)[10]



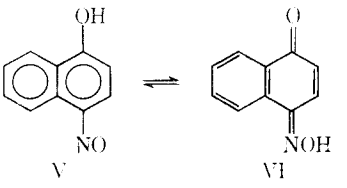
I II

against infections of *Schistosoma mansoni* and *S. japonicum* in experimental animals stimulated the synthesis of various *N,N*-dialkyl-*N'*-(4-nitroso-1-naphthyl)alkylenediamines (III) that also displayed strong anti-



III IV

schistosome properties.[11] The latter substances exist in a higher oxidation state than the naphthylamine derivatives II, and also have the potential to exist in the tautomeric quinoid structure IV, a form possibly necessary for biological activity within these series.[10,12] Moreover, 4-nitroso-1-naphthol (V), which is tautomeric with 1,4-naphthoquinone monoxime (VI), has



V VI

(1) For paper XV, see E. F. Elslager, M. P. Hutt, and L. M. Werbel, *J. Med. Chem.*, **13**, 542 (1970).

(2) E. F. Elslager, D. B. Capps, L. M. Werbel, D. F. Worth, J. E. Meisenhelder, H. Najarian, and P. E. Thompson, *ibid.*, **6**, 217 (1963).

(3) E. F. Elslager, D. B. Capps, D. H. Kurtz, L. M. Werbel, and D. F. Worth, *ibid.*, **6**, 646 (1963).

(4) S. T. Ch'en, I. F. Ch'en, P. C. Kun, Y. C. Hu, J. H. Yao, and T. H. Chou, *Yao Hsueh Hsueh Pao*, **13**, 30 (1966).

(5) E. F. Elslager, D. B. Capps, D. H. Kurtz, F. W. Short, L. M. Werbel, and D. F. Worth, *J. Med. Chem.*, **9**, 378 (1966).

(6) A. Korolkovas, *Rev. Fac. Farm. Bioquim. Univ. Sao Paulo*, **5**, 5 (1967).

(7) E. F. Elslager, D. B. Capps, D. H. Kurtz, and D. F. Worth, *J. Med. Chem.*, **11**, 1201 (1968).

(8) A. Korolkovas, *Rev. Fac. Farm. Bioquim. Univ. Sao Paulo*, **6**, 115 (1968).

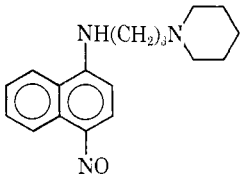
(9) E. F. Elslager and A. A. Phillips, *J. Med. Chem.*, **12**, 519 (1969).

(10) E. F. Elslager, D. B. Capps, L. M. Werbel, D. F. Worth, J. E. Meisenhelder, and P. E. Thompson, *ibid.*, **7**, 487 (1964).

(11) L. M. Werbel, E. F. Elslager, and D. F. Worth, *ibid.*, **11**, 950 (1968).

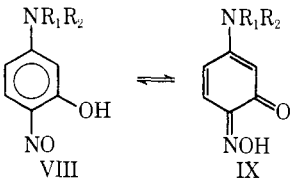
(12) E. F. Elslager, D. B. Capps, and L. M. Werbel, *ibid.*, **7**, 658 (1964).

been proposed as a likely metabolite of the *N,N*-dialkyl-*N'*-(4-nitroso-1-naphthyl)alkylenediamines (III) and exhibits significant activity against *S. mansoni in vitro* and in mice and monkeys.[1,11] The present communication describes a further extension of this work, namely the synthesis of representative 5-(mono- and dialkylamino)-2-nitrosophenols and related substances. Several of the 5-(dialkylamino)-2-nitrosophenol derivatives and 2-amino-5-(diethylamino)phenol displayed noteworthy activity against *S. mansoni* in mice, but none was more promising than 1-{3-[(4-nitroso-1-naphthyl)amino]propyl}piperidine (VII) described previously.[11]



VII

Chemistry.—The 5-(mono- and dialkylamino)-2-nitrosophenols (VIII) (**6–14**, Table I) were obtained in

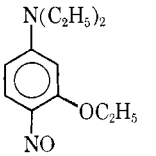


VIII IX

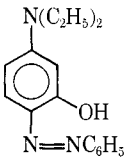
14–79% yield by nitrosation of the corresponding *m*-(mono- and dialkylamino)phenols utilizing the general procedure described by Möhlau[13] in 1892 for the preparation of 5-(dimethylamino)-2-nitrosophenol (**6**) and 5-(diethylamino)-2-nitrosophenol (**9**) (procedures IV and V). These compounds are tautomeric with the corresponding *o*-benzoquinone monooximes (IX). The uv absorption properties of **6** have been investigated in detail by Burawoy and coworkers.[14] These authors concluded that in all of the solvents used (CCl_4, Et_2O, C_6H_6, $CHCl_3$, EtOH, and H_2O) absorption bands due to each of the tautomers VIII and IX were present. Each of the compounds (**6–14**) now reported gave consistent curves in MeOH, with maxima at 402 and 330 mμ (neutral), 390 (alkaline), and 410 and 331 (acid). As expected, the ir curves indicated very strong H bonding due to the *ortho* substituents of the tautomers VIII and IX.[15] Curves from **7** and **9**, for example, showed no OH absorption ($CHCl_3$ and CCl_4) in the 3500–3600 cm^{-1} region and only a suggestion of very broad bands in the 1800–2600 cm^{-1} region.[15] In addition these curves also exhibited a strong somewhat broadened absorption peak at 1630 cm^{-1}.

The intermediate *m*-(mono- and dialkylamino)-phenols (**1–3**, **5**, Table II) were obtained in low yield (7–23%) by heating resorcinol with an excess of the appropriate amine at 200° (procedures I and III).[16]

Alternatively, *m*-{[2-(diethylamino)ethyl]amino}phenol (**4**, Table II) was prepared by alkylation of *m*-aminophenol with 2-chlorotriethylamine in C_6H_6 in the presence of K_2CO_3.[16] The nitrosation of *m*-ethoxy-*N,N*-diethylaniline afforded 3-ethoxy-*N,N*-diethyl-4-nitrosoaniline (**15**) in 66% yield, while 5-(diethylamino)-2-(phenylazo)phenol (**16**) was obtained in 67% yield

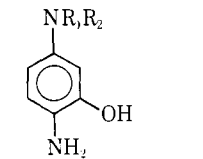


15



16

by coupling *m*-diethylaminophenol with PhN_2Cl. 2-Amino-5-(diethylamino)phenol (**17a**)[17] and 2-amino-5-morpholinophenol (**17b**), which represent potential metabolites of 5-(diethylamino)-2-nitrosophenol (**9**) and 5-morpholino-2-nitrosophenol (**8**), respectively, were



17a, $NR_1R_2 = N(C_2H_5)_2$
b, NR_1R_2 = N⟨morpholino⟩O

synthesized in 26 and 58% yield by the catalytic hydrogenation of **9** and **8** in MeOH over Raney Ni.

A variety of commercially available analogs[18] (Table III) of the 5-(mono- and dialkylamino)-2-nitrosophenols (VIII) were also procured to enable a broader delineation of structure–schistosomicidal relationships. Key compounds among them included *N,N*-dimethyl-*p*-nitrosoaniline (**18a**), *N,N*-diethyl-*p*-nitrosoaniline (**18b**), *p*-nitrosophenol (**19**), 5-methoxy-2-nitrosophenol (**20**), and 2-hydroxy-*p*-benzoquinone 1-oxime (**21**).

Biology.—The compounds described in the present communication were tested in mice against a Puerto Rican strain of *S. mansoni*[2,19] by Dr. Paul E. Thompson and coworkers of these laboratories. Drugs were given in a powdered diet for 14 days and drug amounts are expressed as free base. When indicated, expanded studies were carried out against *S. mansoni in vitro* or in Rhesus monkeys. Several of the 5-(dialkylamino)-2-nitrosophenols (**6**, **7**, **9–12**, Table I) exhibited significant antischistosome activity in mice (Table IV). Among them, 5-(dimethylamino)-2-nitrosophenol (**6**), 2-nitroso-5-(1-pyrrolidinyl)phenol (**7**), and 5-(diethylamino)-2-nitrosophenol base (**9**), hydrochloride (**10**), and 1,5-naphthalenedisulfonate (**11**) displayed strong schistosomicidal activity and effected a 97–100% reduction of live schistosomes in infected mice at doses ranging from 276 to 568 mg/kg per day when administered orally in the diet for 14 days. 2-Amino-5-(diethylamino)phenol

(13) R. Möhlau, *Ber.*, **25**, 1055 (1892).

(14) A. Burawoy, M. Cais, J. T. Chamberlain, F. Liversedge, and A. R. Thompson, *J. Chem. Soc.*, 3727 (1955).

(15) E. D. Amstutz, I. M. Hunsberger, and J. J. Chessick, *J. Amer. Chem. Soc.*, **73**, 1220 (1951); P. M. Boll, *Acta Chem. Scand.*, **12**, 1777 (1958); D. Hadzi, *J. Chem. Soc.*, 2725 (1956).

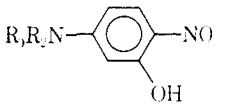
(16) I. G. Farbenindustrie Aktiengesellschaft, British Patent 274,058 (1928).

(17) R. L. Bent, J. C. Dessloch, F. C. Duennebier, D. W. Fassett, D. B. Class, T. H. James, D. B. Julian, W. R. Ruby, J. M. Snell, J. H. Sterner, J. R. Thirtle, P. W. Vittum, and A. Weissberger, *J. Amer. Chem. Soc.*, **73**, 3100 (1951).

(18) These analogs were purchased from Distillation Products Industries, Division of Eastman Kodak Company, Rochester, N. Y.

(19) For a description of test methods, see P. E. Thompson, J. E. Meisenhelder, and H. Najarian, *Amer. J. Trop. Med. Hyg.*, **11**, 31 (1962).

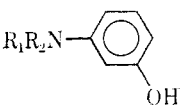
Table I
5-(Mono- and Dialkylamino)-2-nitrosophenols[a]



No.	NR_1R_2	Mp, °C[b]	Yield purified, %	Purification solvent	Procedure	Formula	Anal.[c]
6	$N(CH_3)_2$	169–170[d]	e	MeOH	e	$C_8H_{10}N_2O_2$	C, H, N
7		147–149	71	*i*-PrOH	IV	$C_{10}H_{12}N_2O_2$	C, H, N
8		172–173	75	MeOH	IV	$C_{10}H_{12}N_2O_3$	C, H, N
9	$N(C_2H_5)_2$	88–90[f]	79	*i*-PrOH	IV	$C_{10}H_{14}N_2O_2$	C, H, N
10	$N(C_2H_5)_2$	173[g]	70	EtOH–Et_2O	IV	$C_{10}H_{14}N_2O_2 \cdot HCl$	C, H, Cl[h], N
11	$N(C_2H_5)_2$	275–280	77	*h*	IV	$C_{10}H_{14}N_2O_2 \cdot 0.5C_{10}H_8O_6S_2$	C, H, N
12		106–110	14	*i*-PrOH	IV	$C_{10}H_{14}N_2O_2$	C, H; N[i]
13	$NH(CH_2)_2N(C_2H_5)_2$	128	68	*j*	V	$C_{12}H_{19}N_3O_2 \cdot 2HCl$	C, H, N
14	$N(CH_3)(CH_2)_2N(C_2H_5)_2$	157	37	*i*-PrOH	V	$C_{13}H_{21}N_3O_2 \cdot 2HCl$	C, H, N

[a] Compounds ranged from yellow-green to purple. [b] Compounds melted with decomposition. [c] See ref 39. [d] Lit.[13] mp 169°. [e] Purchased from Aldrich Chemical Co. and recrystallized. [f] Lit.[13] mp 84°. [g] Lit.[13] mp 170°. [h] Not recrystallized. [i] N: calcd, 13.58; found, 13.00. [j] Triturated with boiling Me_2CO.

Table II
m-(Mono- and Dialkylamino)phenols

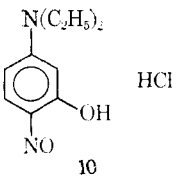


No.	NR_1R_2	Mp, °C	Yield purified, %	Purification solvent	Procedure	Formula	Anal.[a]
1		129–131	8	EtOH–H_2O	I	$C_{10}H_{13}NO$	C, H, N
2		129–131	16	*i*-PrOH	I	$C_{10}H_{13}NO_2$	C, H, N
3		114–118	7	*i*-PrOH–H_2O	I	$C_{11}H_{15}NO$	C, H, N
4	$NH(CH_2)_2N(C_2H_5)_2$	130–132 dec	50	*i*-PrOH–Et_2O	II	$C_{12}H_{20}N_2O \cdot 2HCl$	C, H, Cl, N
5	$N(CH_3)(CH_2)_2N(C_2H_5)_2$	195–196 dec	23	*i*-PrOH	III	$C_{13}H_{22}N_2O \cdot 2HCl$	C, H, Cl, N

[a] See ref 39.

dihydrochloride (**17a**) also possessed strong schistosomicidal activity and reduced the live worm burden 89% at a dose of 177 mg/kg (Table III). These compounds were, therefore, comparable with or superior to lucanthone hydrochloride,[19,20] the tris(*p*-aminophenyl)carbonium salts,[19,21] 4,4′-(heptamethylenedioxy)dianiline dihydrochloride,[22,23] *N*-[5-(*p*-aminophenoxy)pentyl]-phthalimide,[24] and 3-[4-(3-chloro-*p*-tolyl)-1-piperazinylcarbonyl]acrylic acid[25] when tested under comparable experimental conditions.[19] All other analogs, including the *m*-(mono- and dialkylamino)phenols (**1**–**5**, Table II), several of the 5-(mono- and dialkylamino)-2-nitrosophenols (**8**, **13**, **14**, Table I), 3-ethoxy-*N*,*N*-diethyl-4-nitrosoaniline (**15**), 5-(diethylamino)-2-(phenylazo)-phenol (**16**), 2-amino-5-morpholinophenol (**17b**), and **18a**, **18b**, and **19**–**21** lacked appreciable antischistosome effects when administered to mice at high dose levels ranging from 220 to 1166 mg/kg per day for 14 days (Tables III and IV).

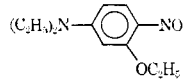
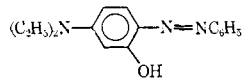
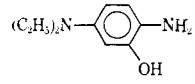
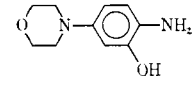
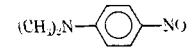
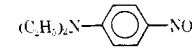
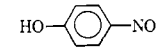
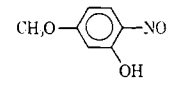
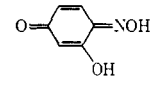
5-(Diethylamino)-2-nitrosophenol hydrochloride (**10**) was one of the most promising schistosonicides in mice and was therefore designated for expanded chemotherapeutic and toxicological evaluation. Against *S. mansoni* infections in Rhesus monkeys, the drug caused strong permanent egg suppression when administered in gavage doses of 50 or 100 mg/kg per day for 10 to 20 days, but was not curative.[19] The nitrosophenol produced only slight to moderate egg suppression at daily gavage doses of 25 mg/kg given for 5 or 10 days. 5-(Diethylamino)-2-nitrosophenol hydrochloride also killed 10-week old male and female *S. mansoni in vitro*



10

(20) W. Kikuth and R. Gönnert, *Ann. Trop. Med. Parasitol.*, **42**, 256 (1948).
(21) E. F. Elslager, F. W. Short, D. F. Worth, J. E. Meisenhelder, H. Najarian, and P. E. Thompson, *Nature*, **190**, 628 (1961).
(22) C. G. Raison and O. D. Standen, *Brit. J. Pharmacol.*, **10**, 191 (1955).
(23) R. F. Collins, M. Davis, N. D. Edge, and J. Hill, *ibid.*, **13**, 238 (1958).
(24) R. F. Collins, M. David, N. D. Edge, J. Hill, H. W. Reading, and E. R. Turnbull, *ibid.*, **14**, 467 (1959).
(25) G. Lämmler, *Z. Tropenmed. Parasitol.*, **9**, 294 (1958).

TABLE III
EFFECTS OF 2-AMINO-5-(DIALKYLAMINO)PHENOLS AND OTHER RELATIVES OF THE 5-(MONO- AND DIALKYLAMINO)-2-NITROSOPHENOLS AGAINST *S. mansoni* IN MICE

No.	Structure	Drug: Route X days[a]	Drug: mg/kg per day	Live schistosomes: % mice pos	Live schistosomes: % redn
15		D × 14	274	100	2
16		D × 14	295	100	0
17a		D × 14	177	60	90
		G × 10	100	67	45
		G × 5	100	90	47
17b		D × 14	334	100	0
18a		D × 14	215	100	0
		D × 14	220	100	11
18b		D × 14	323	100	0
19		D × 14	470	100	5
		G × 10	200	100	0
20		D × 14	307	100	0
21		D × 14	377	100	0

[a] D represents drug-diet, G represents gavage.

within 3–18 hr at a concentration of 50 μg/ml and within 18–50 hr at 12.5 μg/ml.[19]

Potential Metabolites.—1,2-Naphthoquinones inhibit the glycolysis of adult *S. mansoni in vitro* at low concentrations.[26] A similar mode of action can be reasonably postulated for 5-(diethylamino)-2-nitrosophenol and related substances which are tautomeric with the corresponding *o*-benzoquinone oximes IX. Alternatively, the schistosomicidal effects of 5-(diethylamino)-2-nitrosophenol can be accounted for by assuming metabolic conversion into the reduction product 2-amino-5-(diethylamino)phenol (**17a**) which similarly shows strong activity against *S. mansoni* in mice (*vide supra*). Further studies have demonstrated that 2-amino-5-(diethylamino)phenol also kills 10-week old male and female *S. mansoni in vitro* within 2–70 hr at drug concentrations of 12.5–50 μg/ml. Conversely, the antischistosome effects of 2-amino-5-(diethylamino)-phenol may be due to metabolic oxidation to *o*-benzoquinone derivatives.

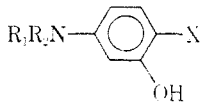
The effects of other simple aminophenol and phenylenediamine derivatives on *S. mansoni* were also investigated. *p*-Aminophenol hydrochloride, which can presumably be converted into quinoid products *in vivo*, was inactive against *S. mansoni* in mice when administered in the diet for 14 days at a high dose (377 mg/kg), but killed schistosomes *in vitro* within 60–84 hr at concentrations of 2.5–10 μg/ml. Although *p*-aminophenol or substituted *p*-aminophenols have not been demonstrated to be metabolites of the orally active *p*-aminophenoxyalkane schistosomicides,[22-24,27-29] it is known that the phenoxyalkanes possess only low schistosomicidal activity *in vitro*[30] and exert their action through a combined effect on the parasite and on the host.[27,30] Therefore, it is not unreasonable to postulate that *p*-aminophenol metabolites may be implicated in the mode of action of these drugs.

Among the phenylenediamines, Nabih and Helmy[31] recently reported that *p*-phenylenediamine when administered by gavage at 80 mg/kg b.i.d. for 15 days killed adult *S. mansoni* and rendered eggs nonhatchable. In the course of earlier work in these laboratories relating to the antischistosome properties of *N*-(dialkylaminoalkyl)-*p*-phenylenediamines[32] and *N*-(dialkylaminoalkyl)-1,4-naphthalenediamines,[10] we had occasion to test both *p*-phenylenediamine and 2-methoxy-*p*-phenylenediamine by drug-diet against *S. mansoni* infections in mice.[19] Neither compound exhibited appreciable antischistosome effects when administered at doses ranging from 125 to 630 mg/kg per day for 14 days.

Several highly colored metabolites of 5-(diethylamino)-2-nitrosophenol hydrochloride (**10**) were found in the urine of rats and Rhesus monkeys that had been dosed with the drug.[33] Unfortunately, none of these materials was more active than 5-(diethylamino)-2-nitrosophenol against *S. mansoni in vitro* and therefore their structures were not elucidated. However, based upon known chemical reactions of *o*-nitrosophenols and

(26) E. Bueding and L. Peters, *J. Pharmacol. Exp. Ther.*, **101**, 210 (1951).
(27) O. D. Standen, *Trans. Roy. Soc. Trop. Med. Hyg.*, **49**, 416 (1955).
(28) See R. F. Collins, V. A. Cox, M. Davis, N. D. Edge, J. Hill, K. F. Rivett, and M. A. Rust, *Brit. J. Pharmacol. Chemother.*, **29**, 248 (1967), and previous papers.
(29) See R. F. Collins and M. Davis, *J. Chem. Soc., C*, 61 (1968), and previous papers.
(30) E. Bueding and C. Swartzwelder, *Pharmacol. Rev.*, **9**, 329 (1957).
(31) I. Nabih and E. Helmy, *J. Pharm. Sci.*, **54**, 1698 (1965).
(32) L. M. Werbel, E. F. Elslager, M. W. Fisher, Z. B. Gavrilis, and A. A. Phillips, *J. Med. Chem.*, **11**, 411 (1968).
(33) W. A. Dill and A. J. Glazko, Parke Davis and Co., Ann Arbor, Mich., unpublished results.

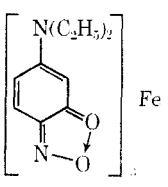
TABLE IV
EFFECTS OF *m*-(MONO- AND DIALKYLAMINO)PHENOLS AND 5-(MONO- AND DIALKYLAMINO)-2-NITROSOPHENOLS AGAINST *S. marinum* IN MICE



No.	NR_1R_2	X	Drug: Route × days[a]	Drug: mg/kg per day	Live schistosomes: % mice pos	Live schistosomes: % reduc
1		H	D × 14	327	100	0
2		H	D × 14	322	100	0
4	$NH(CH_2)_2N(C_2H_5)_2$	H	D × 14	397	100	0
5	$N(CH_3)(CH_2)_2N(C_2H_5)_2$	H	D × 14	271	100	0
6	$N(CH_3)_2$	NO	D × 14	557	17	99
			D × 14	289	0	100
			D × 14	139	100	46
			D × 14	74	100	0
7		NO	D × 14	568	0	100
			D × 14	315	50	69
			D × 14	61	100	0
			G × 10	400	78	84
			G × 10	200	89	40
8		NO	D × 14	1166	100	0
			D × 14	321	100	0
9	$N(C_2H_5)_2$	NO	D × 14	276	22	97
			D × 14	75	100	7
10	$N(C_2H_5)_2 \cdot HCl$	NO	D × 14	409	0	100
			D × 14	283	33	97
			D × 14	172	45	70
11	$N(C_2H_5)_2 \cdot 0.5C_{10}H_8O_6S_2$	NO	D × 14	322	11	99
			D × 14	72	100	13
			G × 4	200	100	61
12		NO	D × 14	376	83	48
13	$NH(CH_2)_2N(C_2H_5)_2$	NO	D × 14	269	100	1
14	$N(CH_3)(CH_2)_2N(C_2H_5)_2$ Lucanthone·HCl	NO	D × 14	318	100	0
			D × 14	140[b]	90	71
			D × 14	70	100	48
			G × 10	100[b]	100	59

[a] See footnote *a*, Table III. [b] Maximum tolerated dose.

o-aminophenols, it is interesting to speculate that 5-(diethylamino)-2-nitrosophenol and/or the reduction product 2-amino-5-(diethylamino)phenol (**17a**) might undergo the following metabolic transformations: (1) formation of metal complexes (**22**); (2) condensation of **17a** with **10** to give a phenoxazonium salt (**23** or **24**);[34] (3) alkaline oxidation of **17a** to the phenoxazinone **25**;[13,34] (4) acidic oxidation of **17a** to the phenoxazonium salt



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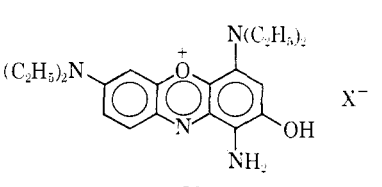

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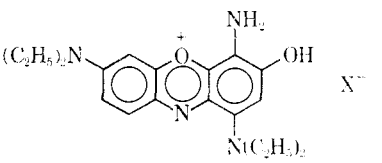
26;[34] (5) dimerization of **17a** to the phenazine **27**;[34] and (6) reaction of **17a** with **10** to form *p*-quinone imine dyes such as **28** or **29**.[35] With regard to postulations 3 through 5, it is interesting to note that a series of 5-amino-9-alkylaminobenzo[*a*]phenoxonium salts have



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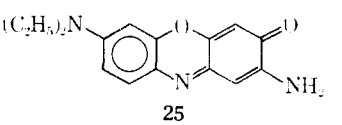

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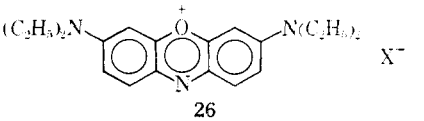

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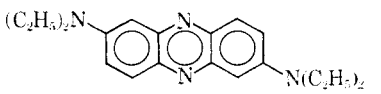
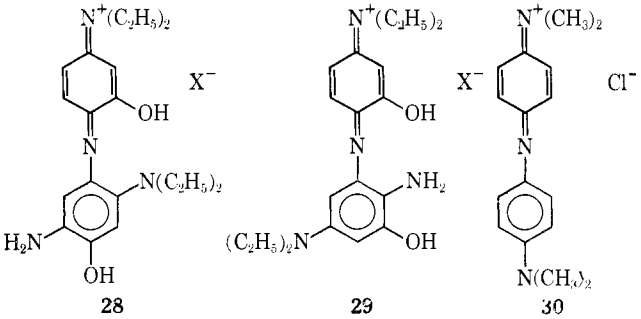


27

been prepared[36] from certain 5-amino-2-nitrosophenols and were reported to possess significant activity against a bovine strain of tuberculosis.[36]

(34) D. E. Pearson, *Heterocyc. Compounds*, **6**, 624 (1957).
(35) "Colour Index," 2nd ed, Vol. 3, Oberley and Pickersgill Ltd., Leeds, England, 1957, pp 3407–3432.
(36) M. L. Crossley, P. F. Dreisbach, C. M. Hofmann, and R. P. Parker, *J. Amer. Chem. Soc.*, **74**, 573 (1952).

An array of commercial phenoxazonium dyes,[35] including Gallo Violet D, C. I. Mordant Violet 54,



Brilliant Cresyl Blue, C. I. Mordant Blue 14, and C. I. Mordant Blue 45, together with representative phenazine dyes[35] such as C. I. Basic Blue 5, Rhoduline Violet, and C. I. Basic Violet 5, has been tested in these laboratories against *S. mansoni* in mice. None exhibited significant activity. By contrast, Binschedler's Green (**30**)[35] showed substantial antischistosome activity in mice at high doses (1129 mg/kg) and effected a 40% reduction of live worms.

Toxicology.—In view of the overall promise of 5-(diethylamino)-2-nitrosophenol hydrochloride (**10**) against *S. mansoni in vitro* and in mice and Rhesus monkeys, the drug was subjected to preliminary toxicological studies.[37] Acute toxicity studies were done in male mice and rats, while subacute oral tolerance work was carried out in male rats. The acute single dose LD_{50} values for the nitrosophenol in mice were 122 ± 3.6 mg/kg orally, 36.8 ± 1.1 mg/kg intraperitoneally, and 34.5 ± 1 mg/kg intravenously.[37] In rats, the acute single dose LD_{50} values were 315 ± 3 mg/kg orally, 84 ± 2.8 mg/kg subcutaneously, and 52 ± 1.3 mg/kg intravenously.[37] In both species the clinical signs following acute oral, intraperitoneal, and intravenous dosing included immediate slight depression followed variably by salivation, tonic–clonic convulsions, dyspnea, trembling, and incoordination. The first symptom listed above appeared with the lowest dose level, and the balance of the symptoms with gradually increasing dose levels. These symptoms appeared in mice after oral administration of 75 to 100 mg/kg, after intraperitoneal administration of 25 mg/kg, and after intravenous administration of 20 mg/kg. In rats these signs appeared after oral administration of 125 mg/kg, after subcutaneous administration of 75 to 125 mg/kg, and after intravenous administration of 10 mg/kg. The most frequent cause of death in the acute experiments was respiratory failure. In rats no animal survived 150 mg/kg subcutaneous doses of **10**, and the intravenous administration of 80 mg/kg of the drug was always fatal.[37]

In subacute oral toxicity studies in rats 5-(diethylamino)-2-nitrosophenol hydrochloride (**10**) was administered in powdered ration for 5 weeks at drug concentrations ranging from 0.0625 to 0.5% (63–538 mg/kg).[37] There was an overall weight gain depression which appeared to be out of proportion to the food intake depression. This suggested the possibility that the drug had produced a heightened metabolism. In the three higher dose levels of 123, 254, and 538 mg/kg there was a food intake depression throughout, while at the lowest dose level of 63 mg/kg there was only an initial depression of food intake. There was evidence of hemoconcentration in 13 of the 40 animals manifest by high hematocrit, and in 6 instances by high hemoglobin values. These elevated values were not dose-related. There were scattered instances of leukocytosis and leukopenia in the treated animals, but leukopenia was also observed in the control animals.[37] Grossly at all dose levels there was evidence of atrophy of the liver with depletion of peritoneal fat reserves. In the groups of animals at the three highest dose levels there was atrophy of the seminal vesicles and prostate, and in the two highest dose level groups, evidence of staining of the esophageal mucosa by the drug. At necropsy there was evidence of some suppression of hematopoiesis since the nucleated cell counts were depressed at all dose levels.[37] At the lowest dose level there were microscopic evidences of excess hemosiderin deposition in the spleen and depletion of the PAS stainable material in the kidney. At the next higher dose level there was added depletion of the hepatic stainable fat, and at the two highest dose levels evidence of bone marrow suppresssion.[37]

(37) D. H. Kaump, R. A. Fisken, and J. Scharlein, unpublished data in the files of Parke Davis and Co., Ann Arbor, Mich.

Experimental Section[38,39]

***m*-(Mono- and Dialkylamino)phenols (1–5, Table II). Procedure I.**—A mixture of 220 g (2.0 mol) of resorcinol, 434 ml (5.0 mol) of morpholine, and 120 g (1.0 mol) of 85% H_3PO_4 was heated in a steel bomb at 200° for 12 hr. The bomb was opened, the yellow-brown solution was filtered, and the filtrate was treated with 190 g (2.0 mol) of 50% aq NaOH. The mixture was steam distilled to remove excess morpholine and the residue was cooled and neutralized with concentrated HCl. Upon standing at room temperature for 18 hr, a tan crystalline precipitate separated (58.0 g), mp 126–129°. For analysis the product was recrystallized from *i*-PrOH to give 16.0 g of *m*-morpholinophenol (**2**), mp 129–131°.

Procedure II.—A mixture of 109 g (1.0 mol) of *m*-aminophenol, 173 g (1.1 mol) of 2-chlorotriethylamine·HCl, and 345 g (2.5 mol) of K_2CO_3 was heated under reflux in C_6H_6 for 20 hr. H_2O (1 l.) was added and the mixture was shaken vigorously. The C_6H_6 layer was separated, washed thoroughly (H_2O), and dried (Na_2SO_4). C_6H_6 was removed on a rotary evaporator and the dark brown residual oil (172 g, 83%) was distilled *in vacuo* through a 15-cm Vigreaux column. Five fractions, bp 136–140° (0.15–0.25 mm), were combined (142 g, 68%), dissolved in *i*-PrOH, and treated with excess HCl in *i*-PrOH. The solution was poured into Et_2O, the supernatant was decanted, and the sticky residue was crystallized from *i*-PrOH to give 140 g (50%) of *m*-{[2-(diethylamino)ethyl]amino}phenol dihydrochloride (**4**) as colorless crystals, mp 130–132° dec.

Procedure III.—A solution of 110 g (1.0 mol) of resorcinol and 130 g (1.0 mol) of *N*,*N*-diethyl-*N'*-methylethylenediamine was heated under reflux (195–200°) for 18 hr. A Dean–Stark water trap was then placed in the system, heating was continued, and 30 ml of distillate was collected over a period of 9 hr. An additional 20 g of *N*,*N*-diethyl-*N'*-methylethylenediamine was added during this period to replace diamine that was lost through the trap. The mixture was cooled to room temperature and dissolved in 2 l. of 1 *N* HCl. The solution was washed twice with Et_2O, and the aqueous layer was separated and made strongly alkaline with 200 ml of 50% aqueous NaOH. The mixture was washed with Et_2O, adjusted to pH 8, and extracted with Et_2O and $CHCl_3$. The combined Et_2O and $CHCl_3$ extracts were dried (Na_2SO_4), and volatile materials were removed *in vacuo*. The residue was distilled *in vacuo* through a 15-cm Vigreaux column

(38) Melting points (corrected) were taken on a Thomas–Hoover capillary melting point apparatus.

(39) Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

to give 69.0 g (31%) of a viscous oil, bp 124–130° (0.12 mm). The base was dissolved in *i*-PrOH and the solution was treated with an excess of an *i*-PrOH–HCl mixture. The crystalline precipitate was collected by filtration, washed with *i*-PrOH and Et_2O, and dried. The *m*-{[2-(diethylamino)ethyl]methylamino}-phenol·2HCl (67.0 g, 23%) was obtained as colorless crystals, mp 195–196° dec.

5-(Mono- and Dialkylamino)-4-nitrosophenols (VIII) (6–14, Table I). Procedure IV.—*m*-Diethylaminophenol (165 g, 1.0 mol, Eastman) was dissolved in a mixture of 350 ml of concentrated HCl and 200 ml of H_2O and the solution was cooled to 0° with stirring. To this cold solution was added dropwise over a period of 2.5 hr a solution of 69 g (1.0 mol) of $NaNO_2$ in 500 ml of H_2O while maintaining the temperature at 0–5°. A vigorous exothermic reaction occurred. When the addition was complete, the reaction mixture was stirred at 0–5° for 0.5 hr and the crude HCl salt that separated was collected by filtration and dried *in vacuo* at 50° for 18 hr. The product was dissolved in 2 l. of boiling EtOH, the solution was cooled to 40°, and 1 l. of Et_2O was added slowly until crystallization began. The mixture was cooled to 5° and the product was collected by filtration, dried, and crystallized once again from EtOH–Et_2O. The 5-(diethylamino)-2-nitrosophenol·HCl (**10**) was thus obtained as green crystals, mp 173° dec (lit.[13] mp 170°), yield, 161 g (70%).

A solution of 15.0 g (0.065 mol) of **10** in 300 ml of H_2O was treated with 5.5 g (0.065 mol) of $NaHCO_3$. The precipitate was collected by filtration, washed (H_2O), dried, and crystallized from *i*-PrOH. The purified product was dried *in vacuo* at 45° for 18 hr to give 10.0 g (79%) of 5-(diethylamino)-2-nitrosophenol base (**9**) as shiny maroon needles, mp 88–90° dec (lit.[13] mp 84°).

To a warm aqueous solution of 11.5 g (0.05 mol) of **10** was added a warm aqueous solution of 9.2 g (0.025 mol) of 1,5-naphthalenedisulfonic acid disodium salt dihydrate (Eastman). The mixture was allowed to stand at room temperature for 18 hr, and the salt was collected by filtration and dried at 50° *in vacuo* for 18 hr. The 5-(diethylamino)-2-nitrosophenol salt with 0.5 f wt 1,5-naphthalenedisulfonic acid (**11**) (13.0 g, 77%) was obtained as a yellow-brown solid, mp 275–280° dec.

Procedure V.—A stirred solution of 29.5 g (0.1 mol) of *m*-{[2-(diethylamino)ethyl]methylamino}phenol·2HCl (**5**) in 150 ml of H_2O and 10 ml of concentrated HCl was cooled to 0° and a solution of 6.9 g (0.1 mol) of $NaNO_2$ in 50 ml of H_2O was added dropwise over a period of 0.5 hr while maintaining the temperature at 0–5°. The reaction was exothermic and the solution turned deep red. The mixture was allowed to stand at room temperature for 18 hr and volatile materials were removed *in vacuo*. The residue was crystallized from *i*-PrOH and the product was dried *in vacuo* at 45° for 72 hr to give 12.0 g (37%) of 5-{[2-(diethylamino)ethyl]methylamino}-2-nitrosophenol·2HCl (**14**) as a green solid, mp 157° dec.

3-Ethoxy-*N*,*N*-diethyl-4-nitrosoaniline·HCl (15).—A stirred solution of 38.6 g (0.2 mol) of *m*-ethoxy-*N*,*N*-diethylaniline (Eastman) in 70 ml (0.82 mol) of concentrated HCl and 40 ml of H_2O was cooled to 0° and to it was added dropwise over 1 hr a solution of 13.8 g (0.2 mol) of $NaNO_2$ in 100 ml of H_2O. The temperature was maintained at 0–5° during the addition. The reaction mixture was stirred for 1 hr, diluted with ice and H_2O, and made alkaline with concentrated NaOH, keeping the temperature below 20°. The product was extracted with $CHCl_3$, and the combined $CHCl_3$ extracts were washed (H_2O) and dried (K_2CO_3). Volatile materials were removed *in vacuo* and the viscous green-red residue was dissolved in Et_2O. Excess dry HCl was bubbled into the Et_2O solution and the yellow solid that precipitated was collected by filtration and crystallized from EtOH–Et_2O to give 34.0 g (66%) of product as chartreuse crystals, mp 153–155°. *Anal.* ($C_{12}H_{18}N_2O_2$·HCl) C, H, Cl⁻, N.

5-(Diethylamino)-2-(phenylazo)phenol (16).—A solution of 9.3 g (0.1 mol) of aniline in 250 ml of H_2O and 25 ml of concentrated HCl was treated with 6.9 g (0.1 mol) of $NaNO_2$ in 100 ml of H_2O at 0–5°. When diazotization was complete, the cold solution of the diazonium salt was added slowly with stirring to a solution of 16.5 g (0.1 mol) of *m*-diethylaminophenol (Eastman) in 500 ml of H_2O and 17 ml of concentrated HCl while maintaining the temperature at 0–5°. The solution was then stirred for 0.5 hr at 0° and allowed to warm to 17° over a 2-hr period. The mixture was neutralized by the slow, cautious addition of 25 g (0.3 mol) of NaOAc in 150 ml of H_2O and was allowed to stir at room temperature for 18 hr. The product was collected by filtration, dried, and crystallized from *i*-PrOH to give 18.0 g (67%) of maroon crystals, mp 114–115°. *Anal.* ($C_{16}H_{19}N_3O$) C, H, N.

2-Amino-5-(diethylamino)phenol·2HCl (17a).—To a solution of 23.1 g (0.1 mol) of hydrochloride **10** in MeOH was added 4.0 g (0.1 mol) of NaOH and the mixture was hydrogenated over Raney Ni at an initial H_2 pressure of 3.5 kg/cm.² When the theoretical amount of H_2 had been absorbed, the catalyst was removed by filtration and the filtrate was treated immediately with excess concentrated HCl to arrest air oxidation. Volatile materials were removed *in vacuo* and the residue was dissolved in *i*-PrOH and the NaCl was removed by filtration. The *i*-PrOH solution was poured into Et_2O, and the deliquescent off-white solid that formed was collected and triturated with hot *i*-PrOH. The product was thus obtained as the dihydrochloride (5.0 g, 26%), mp 224–226° dec (lit.[9] mp 201–203° for the monohydrochloride obtained by reduction of the corresponding phenylazo compound). *Anal.* ($C_{10}H_{16}N_2O$·2HCl) C, H, Cl, N.

2-Amino-5-morpholinophenol (17b).—5-Morpholino-2-nitrosophenol (**8**) (19.5 g, 0.094 mol) was hydrogenated in MeOH over Raney Ni according to the procedure used for the preparation of 2-amino-5-(diethylamino)phenol (**17a**). The base of the product was relatively insoluble in MeOH, and was collected and recrystallized rapidly from 400 ml of boiling MeOH. The product was obtained (10.5 g, 58%) as lavender crystals, mp 160° dec. *Anal.* ($C_{10}H_{14}N_2O_2$) C, H, N.

Acknowledgments.—The authors express their appreciation to Dr. Paul E. Thompson and coworkers for the antischistosome testing, Mr. William Pearlman for carrying out the hydrogenations, Mr. C. E. Childs and associates for the microanalyses, and Dr. J. M. Vandenbelt and coworkers for the spectral data.