3-Hydroxymethyl-5-methoxy-1-(β-methylmercaptoethyl)-2,6-dimethylindole-4,7-dione (**XVe**) was obtained from ether–petroleum ether as red crystals: mp 91–93°; λ_{max} 230, 286, 345, 460 mμ (ε 18,200, 14,500, 3160, 1300); λ 2.90, 6.03, 6.12, 6.21, 6.65 μ: pmr,[14] 116 (3s, 6-CH_3), 131 (3s, SCH_3), 137 (3s, 2-CH_3), 166 (2t, J = 7 cps, NCH_2CH_2S), 239 (3s, OCH_3), 265 (2t, J = 9 cps, NCH_2CH_2S), 270 cps (2s, CH_2O).

Anal. Calcd for $C_{15}H_{19}NO_4S$: C, 58.24; H, 6.19; N, 4.53; S, 10.36. Found: C, 58.55; H, 6.89; N, 4.70; S, 10.11.

Reduction of 1-(β-azidoethyl)-5-methoxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxaldehyde (XVId), 1-(β-chloroethyl)-5-methoxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxaldehyde (XVIc), 5-methoxy-2,6-dimethyl-4,7-dioxo-1-[β-(2-tetrahydropyranyloxy)-ethyl]-3-indolecarboxaldehyde (XVIf), and 5-methoxy-2,6-dimethyl-4,7-dioxo-1-(β-thiocyanoethyl)-3-indolecarboxaldehyde (XVIg) gave oils which were converted into the carbamate esters without purification.

General Procedure for Conversion of the Indoloquinone Alcohols into the Carbamate Esters.—The following preparation illustrates this procedure. 1-(β-Fluoroethyl)-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione (XVa) (387 mg, 1.38 mmoles) and 15 ml of methyl isocyanate were heated at gentle reflux for 20 hr. The excess isocyanate was removed and the residue was recrystallized from CH_2Cl_2–petroleum ether to give 290 mg (62%) of the methylcarbamate XIVa as orange needles, mp 162–163°. Complete characterization of this substance and the other compounds (XIVb–d and g) prepared analogously is given in Table II.

1-(β-Hydroxyethyl)-3-hydroxymethyl-5-methoxy-2,6-dimethylindole-4,7-dione 3-Methylcarbamate (**XIVe**).—A solution of 300 mg (0.72 mmole) of 3-hydroxymethyl-5-methoxy-2,6-dimethyl-1-[β-(2-tetrahydropyranyloxy)ethyl]indole-4,7-dione methylcarbamate (XIVd) in 60 ml of methanol and 15 ml of 0.1 *N* HCl was stirred at room temperature for 23 hr. Thin layer chromatography showed two spots, each being more polar than starting quinone. The crude material was isolated with CH_2Cl_2 and adsorbed from benzene onto a column prepared from silica gel and benzene. The column was washed with ether; upon eluting the first orange band, 125-ml fractions were collected. After collection of 12 fractions, the more polar band was eluted with acetone to furnish the product, the characterization of which is given in Table II.

3-Hydroxymethyl-5-methoxy-2,6-dimethyl-1-(β-dimethylsulfoniumethyl)indole-4,7-dione Methylcarbamate Iodide (**XIVg**).—A solution of 50 mg (0.14 mmole) of XIVf in 5 ml of CH_3I was allowed to stand at ambient temperature in the dark for 6 days, after which time the solvent was removed. Characterization of the product is given in Table II.

Acknowledgment.—Microanalyses were furnished by Mr. L. Brancone and his staff, and spectral data were determined by Mr. W. Fulmor and his associates. Separations by partition chromatography were carried out by Mr. C. Pidacks and his group. The *in vitro* antibacterial data were furnished by Mr. A. C. Dornbush and his associates.

Antimicrobial Properties of Pyrrole Derivatives[1]

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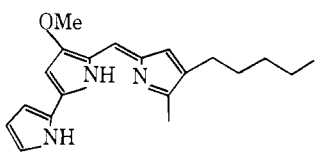
The activity of pyrroles, 2,2′-dipyrrylmethenes, 2,2′,2″-tripyrrylmethenes, 2,2′-bipyrrole, and congeners against representative bacteria, fungi, and yeast, and of prodigiosin against pathogenic fungi is described. The activities of the pyrroles and methenes vary with substitution. Reversal studies indicate that 2-pyrrol-2-yl-1-pyrroline interferes with glycine metabolism.

There have been a number of studies[2–10] devoted to an investigation of the bipyrrylpyrrylmethene prodigiosin (**1**), which occurs in the pigment produced by the bacterium *Serratia marcescens*. Notable among these is the claimed[5,6] activity of this compound against the pathogenic fungus *Coccidiodes immitis*, the causative agent for coccidioidomycosis (San Joaquin Valley fever). The potential application of the bacterial



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metabolite has been hampered apparently by its toxicity. In view of the activity that has been described for this compound and the generally important role of pyrrole derivatives in biological systems, it was of interest to investigate the antibiotic properties of simpler and more readily attainable pyrroles, dipyrrylmethenes, and certain congeners.

Among a number of pyrroles investigated (alkyl derivatives, aldehydes, ketones, esters), 2,4-dimethyl-3-ethylpyrrole was found to be qualitatively active (agar diffusion–filter paper disk method) against *Bacillus subtilis, Staphylococcus aureus, Mycobacterium smegmatis, Candida albicans, Trichophyton mentagrophytes, Penicillium* sp., *Aspergillus niger*, and *Saccharomyces cerevisiae*. An isomeric mixture of 2- and 3-heptylpyrrole (5.4:1) showed activity against *B. subtilis, S. aureus, Pseudomonas aeruginosa, C. albicans, A. niger*, and *S. cerevisiae*. In contrast 2-methylpyrrole, 2,4-dimethylpyrrole, and an isomeric mixture of 2- and 3-ethylpyrrole (2- chiefly) were shown to be inactive against the same microorganisms. However, all of the alkyl-

(1) (a) This investigation was supported by Public Health Research Grants E-1335 (National Institute of Allergy and Infections Diseases), CA 06255 (National Cancer Institute), and GM-09389 (National Institute of General Medical Sciences). (b) Presented largely before the Division of Medicinal Chemistry at the 151st National Meeting of the American Chemical Society, Pittsburgh, Pa., March 1966.

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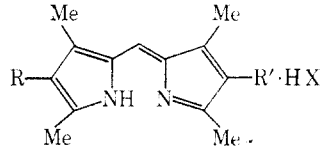
TABLE I
ANTIMICROBIAL ACTIVITY OF PYRRYLMETHANE HYDROHALIDES[a]



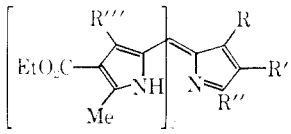
Compd no.	R	R′	HX	*B. subtilis*	*S. aureus*	*A. aerogenes*	*E. coli*	*Ps. aeruginosa*	*M. phlei*	*M. smegmatis*	*A. niger*	*C. albicans*	*M. corymbifera*	*Penicillium* sp.	*R. oryzae*	*S. cerevisiae*
2	H	H	HI	+	±	+	−	−		+						
3	H	Et	HBr	65%[b]	+	+	+	88%[b]	+	−	−	−	+	+		+
4	Et	Et	HBr		100%[c]	−	−	~100%[b]			−	~100%[b]				100%[c]
5	Et	Et	HCl	5	7	−	−	7	13	12	8	10	7	10	2	10
6	H	CO_2Et	HBr	−	2	−	−	−	6	11	8	10	10	−	7	10
7	Et	CO_2Et	HBr	−	−	−	−	−	−	−	−	5	7	−	−	5

[a] Values given show either qualitative activity (+ or −), inhibition zone on agar (in millimeters), or per cent inhibition in broth at concentration shown. See the Experimental Section. [b] At 10 μg/ml. [c] At 1 μg/ml.

pyrroles suffer from ease of oxidation, which alone could militate against their possible application as antibiotics.

In view of these findings our attention was directed next to the more stable and more complex 2,2′-dipyrrylmethenes. Results with these derivatives and analogs proved quite interesting. Thus, initially, it was found that although 2,2′-(3,3′,5,5′-tetramethyl-4,4′-diethyl)-dipyrrylmethene hydrobromide (**4**) was active against several organisms (Table I), 2,2′-(3,3′,5,5′-tetramethyl-4,4′-diethoxycarbonyl)dipyrrylmethene (**8**) and its hydrobromide (**9**) were completely inactive against these cells. It will be observed that the ring ethyl groups of the first methene have been replaced by ethoxycarbonyl groups in the second. Proceeding from this the series **2**–**11** (see Experimental Section) was synthesized and tested. The activities observed for the dipyrrylmethenes are described in Table I. *Mycobacterium phlei* and *Mucor corymbifera* were used as test organisms for **3** and **5**–**11**, but not for the others; *Rhizopus oryzae* was employed with **5**–**11** only. It is seen that the antibiotic activity of these compounds vary in a remarkable way with substitution. The activity of **4** against the pathogens *S. aureus* (especially) and *Ps. aeruginosa* is worthy of note. The results shown for different compounds against the mycobacteria and *C. albicans* likewise merit mention, and the effect of several of the methene derivatives against *S. cerevisiae* is of interest. Considering the germicidal properties of phenol, it is also of interest that of the two *p*-hydroxybenzylidene-2H-pyrrolenine (*p*-hydroxyphenylpyrrylmethene) derivatives tested (**10** and **11**), compound **11** showed qualitative activity against *S. aureus*, *M. phlei*, and *M. smegmatis* only.

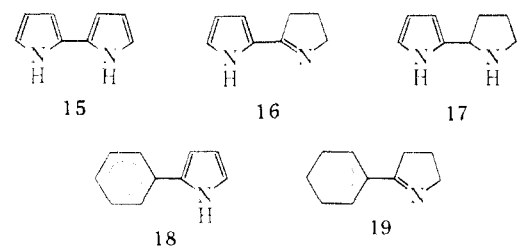
The sensitivity of pyrroles and 2,2′-dipyrrylmethene derivatives to substitution is also reflected in 2,2′,2″-tripyrrylmethenes. In a comparison of **12**–**14** against different microorganisms only **13** showed any activity



	12	**13**	**14**
R	H	Me	CO_2Et
R′	CO_2Et	CO_2Et	Me
R″	Me	Me	CO_2Et
R‴	H	Me	Me

exhibiting inhibition with no zone, or only a very small zone, at 2000 μg/ml against *Proteus vulgaris*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *Microsporum audouini*, *Histoplasma capsulatum*, and *Cryptococcus neoformans*. An inhibition zone of 0.6 mm was shown against *S. aureus* and one of 3.1 mm against *Diplococcus pneumoniae*, Type I. It showed no activity at the same concentration against *Pseudomonas* sp., *Escherichia coli*, *Klebsiella pneumoniae*, *Blastomyces dermatitidis*, *and C. albicans;* at 400 μg/ml, **13** was but weakly active (0.6-mm inhibition zone) against the *Diplococcus* organism only.

Because of the 2,2′-bipyrrole moiety in prodigiosin, compounds **15**–**19** were examined. Of these, 2,2′-bi-



15 **16** **17**

18 **19**

pyrrole (**15**) showed qualitative activity against *S. aureus*, *Aerobacter aerogenes*, and *Ps. aeruginosa* but was inactive against *B. subtilis*, *E. coli*, *M. smegmatis*, *A. niger*, *C. albicans*, *Penicillium* sp., *T. mentagrophytes*, and *S. cerevisiae*. 2-Phenylpyrrole (**18**), tested against the same microorganisms, was found to inhibit the growth of *B. subtilis* and *Ps. aeruginosa* 100% at a concentration of 100 μg/ml, and to be qualitatively active against *A. aerogenes*, *M. smegmatis*, *A. niger*, *C. albicans*, *Penicillium* sp., and *S. cerevisiae*. 2-Pyrrol-2-yl-1-pyrroline (**16**) inhibited *S. cerevisiae* specifically and the other compounds were inactive. Interestingly it was found using the "reversal method"[11] that the inhibitory action of **16** can be overcome by glycine, pyridoxal phosphate, and pyridoxine phosphate, but not by any other of 100 compounds tested. Considering the role of the latter two in transamination reactions, it appears that **16** may be blocking a metabolic sequence of glycine.

The purity of samples of prodigiosin used in previous antibiotic studies is questionable. Using the purified compound, the antibiotic activity against several pathogenic fungi was determined (Table II). It should be

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TABLE II
ANTIBIOTIC ACTIVITY OF PRODIGIOSIN AGAINST PATHOGENIC FUNGI IN TISSUE CULTURE[12]

Fungus	Concn (μg/ml) for 50% inhib
Blastomyces dermatitidis	2.2
Candida albicans	22
Cryptococcus neoformans	3.2
Histoplasma capsulatum	3.2
Sporotrichum schenkii	1

noted that these results were obtained by a tissue culture method.[12] Activity against *Bl. dermatitidis* and *H. capsulatum* were confirmed by agar diffusion studies, but the same method shows prodigiosin to be inactive at 80 μg/ml against *C. albicans* as well as *C. neoformans*. In a study in which only the latter method was used, prodigiosin also appeared to be only slightly active at the same concentration against the pathogens *E. floccosum*, *M. audouini*, and *T. mentagrophytes*.

Experimental Section[13]

Pyrroles.—Samples of 2-methylpyrrole,[14] 2,4-dimethylpyrrole,[15] and the isomeric mixture of 2- and 3-heptylpyrrole[16] were obtained as described in the literature references. The isomeric mixture of ethylpyrroles, bp 164–166°, was obtained from alkylation of the pyrrole Grignard reagent essentially as described for the heptyl isomers. 2,4-Dimethyl-3-ethylpyrrole was obtained from the Aldrich Chemical Co.

2,2′-Dipyrrylmethenes. A. 2,2′-(3,3′,5,5′-Tetramethyl)dipyrrylmethene hydroiodide (2) was synthesized by the general method[17a] for the synthesis of symmetrical dipyrrylmethenes from the condensation of 1.7 g of 2,4-dimethylpyrrole and 5.0 g of 98–100% formic acid in the presence of 6.0 g 47% HI. The red needles of the methene hydroiodide that separated from the reaction mixture were recrystallized from a mixture of chloroform and ligroin yielding 1.4 g (48%) of product, mp 283° dec (lit.[18] 283°).

B. 2,2′-(3,3′,5,5′-Tetramethyl-4-ethyl)dipyrrylmethene Hydrobromide (3).—Hydrobromic acid (48%, 1 ml) was added to a solution of 0.475 g of 2,4-dimethylpyrrole and 0.750 g of 2-formyl-3,5-dimethyl-4-ethylpyrrole in 10 ml of absolute ethanol in the general procedure for unsymmetrical dipyrrylmethenes.[17b] The red crystals that deposited from the reaction mixture were washed with a little ethanol and after drying in a vacuum desiccator weighed 1.25 g (86%), mp 218–222° dec (lit.[17c] 215°).

C. 2,2′-(3,3′,5,5′-Tetramethyl-4,4′-diethyl)dipyrrylmethene Hydrohalides (4 and 5).—Compound **4** was obtained from 2,4-dimethyl-3-ethylpyrrole and formic acid following the procedure for **2** and the literature description for the perchlorate.[19] After crystallization from a mixture of chloroform and petroleum ether (bp 30–60°) the red-brown methene salt (**4**) darkened at 210° when heated and melted at 249–250° dec. The methene hydrobromide was also synthesized from the HBr-catalyzed condensation of 2-formyl-3,5-dimethyl-4-ethylpyrrole with 2,4-dimethyl-3-ethylpyrrole as in the preparation of **3**.

Anal. Calcd for $C_{17}H_{25}BrN_2$: C, 60.53; H, 7.47; N, 8.31. Found: C, 60.62; H, 7.02; N, 8.15.

The hydrochloride **5** was synthesized by the procedure for **3**. After crystallizing from acetic acid, it was obtained as dark red needles, mp 215° dec.

Anal. Calcd for $C_{17}H_{25}ClN_2$: Cl, 12.11. Found: Cl, 12.42.

D. 2,2′-(3,3′,5,5′-Tetramethyl-4-ethoxycarbonyl)dipyrrylmethene Hydrobromide (6).—Following the procedure for **3** there was employed 0.450 g of 2-formyl-3,5-dimethyl-4-ethoxycarbonylpyrrole, 0.250 g of 2,4-dimethylpyrrole, 6 ml of absolute ethanol, and 0.5 g of 48% HBr. The orange needles of **6** weighed 0.750 g (92%) and after recrystallizing from glacial acetic acid melted at 197–198° dec.

Anal. Calcd for $C_{18}H_{21}BrN_2O_2$: Br, 22.62. Found: Br, 22.55.

E. 2,2′-(3,3′,5,5′-Tetramethyl-4-ethyl-4′-ethoxycarbonyl)dipyrrylmethene Hydrobromide (7).—The procedure described for **3** was used. Employing 0.450 g of 2-formyl-3,5-dimethyl-4-ethoxycarbonylpyrrole, 0.320 g of 2,4-dimethyl-3-ethylpyrrole, 0.5 g of 48% HBr, and 7.0 ml of absolute ethanol, there was obtained 0.890 g (99%) of **7**[20] as orange crystals which darkened at 204° when heated and melted at 210–212° dec. An analytical sample recrystallized from glacial acetic acid showed the same behavior.

Anal. Calcd for $C_{18}H_{25}BrN_2O_2$: Br, 20.96. Found: Br, 20.72.

F. 2,2′-(3,3′,5,5′-Tetramethyl-4,4′-diethoxycarbonyl)dipyrrylmethene (8) and Its Hydrobromide (9).—The free base **8** was derived by treatment of the hydrobromide **9** with NH_3 in chloroform. Samples obtained in this way melted in the range 187.7–191.1° (lit.[20] 186.8–188.1°) after crystallization from a mixture of chloroform and cyclohexane.

The synthesis of the hydrobromide **9** has been reported.[20]

***p*-Hydroxybenzylidene-2H-pyrrolenine Hydrobromides.**—The synthesis of 2-*p*-hydroxybenzylidene-3,5-dimethyl-4-ethyl-2H-pyrrolenine hydrobromide (**10**) and of 2-(3-methoxy-4-hydroxybenzylidene)-3,5-dimethyl-4-ethyl-2H-pyrrolenine hydrobromide (**11**) from the hydrobromic acid catalyzed condensation of *p*-hydroxybenzaldehyde and vanillin, respectively, with 2,4-dimethyl-3-ethylpyrrole and the characterization of these compounds is described in detail in another report.[21]

2,2′,2″-Tripyrrylmethenes.—Compounds **12–14** were obtained from the $KMnO_4$ oxidation of the corresponding tripyrrylmethanes as we have described in the literature.[22]

2,2′-Bipyrrole (15), 2-Pyrrol-2-yl-1-pyrroline (16), and 2-Pyrrol-2-ylpyrrolidine (17).—2,2′-Bipyrrole (**15**) was synthesized by the dehydrogenation of 2-pyrrol-2-yl-1-pyrroline[23] (**16**) essentially according to the published description.[23]

2-Pyrrol-2-ylpyrrolidine (**17**) was derived from the reaction of pyrrol and 1-pyrroline following the reported procedure.[24] The same compound was also obtained by the low-pressure hydrogenation (PtO_2, Burgess–Parr apparatus) of 2-pyrrol-2-yl-1-pyrroline in 95% ethyl alcohol.

2-Phenylpyrrole (18) and 2-Phenyl-1-pyrroline (19).—2-Phenylpyrrole (**18**) was synthesized by the nickel on nickel chromite catalyzed dehydrogenation 2-phenyl-1-pyrroline[25] (**19**) as described in the literature.[26]

Prodigiosin (1).—An authentic sample isolated and purified as reported[15] was employed.

Antimicrobial Tests. The antimicrobial spectra for pyrroles, 2,2′-dipyrrylmethenes, 2,2′-bipyrrole, and congeners were determined by impregnating 1-cm filter paper disks with a 1% solution or suspension of the compound and placing these with controls on the surface of agar plates seeded with the test organism. Trypticase soy agar was used for gram-positive and gram-negative bacteria, Sauton agar for mycobacteria, Sabouraud agar for yeast and fungi. Inhibition zones were measured as the radial segment of no growth surrounding the impregnated disks. Quantitative estimations of per cent inhibition were carried out in the appropriate liquid media without agar (*vide supra*) and calculated from turbidity measurements compared to those of controls at 640 mμ, measured with a Coleman spectrophotometer.

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(13) Melting points (Fisher-Johns) and boiling points are uncorrected. Analyses are by Dr. F. Pascher, Bonn, Germany, and Dr. G. Weiler and Dr. F. B. Strauss, Oxford, England.

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(17) H. Fischer and H. Orth, "Die Chemie des Pyrrols," Band II, 1 Hälfte, Akademische Verlagsgesellschaft, M. B. H. Leipzig, 1937: (a) p 3; (b) p 2; (c) p 13.

(18) H. Fischer, *Ber.*, **47**, 3266 (1914).

(19) H. Fischer, P. Halbig, and B. Walach, *Ann.*, **452**, 280 (1927).

(20) A. J. Castro, J. P. Marsh, Jr., and B. T. Nakata, *J. Org. Chem.*, **28**, 1943 (1963).

(21) A. J. Castro, G. Tertzakian, B. T. Nakata, and D. A. Brose, submitted for publication.

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(25) L. C. Craig, H. Bulbrook, and R. M. Hixon, *ibid.*, **53**, 1831 (1931).

(26) H. Adkins and L. G. Lunsted, *ibid.*, **71**, 2964 (1949).

Growth periods of 18–24 hr at 37° were used in all types of tests except that 50–100 hr was used for mycobacteria.

The testing of 2,2′,2″-tripyrrylmethene and of prodigiosin on Sabouraud agar was pefrormed in a similar manner by Smith Kline and French Laboratories. Inhibition was measured at the time of full growth on control plates (37°, 18–24 hr for bacteria; 30°, 40–120 hr for fungi) using 12.8-mm filter paper disks.

Acknowledgment. We are most grateful to Dr. James F. Kerwin and Smith Kline and French Laboratories for testing the tripyrrylmethenes and prodigiosin on agar, and to Dr. Michael L. Furcolow and the Communicable Disease Center, Public Health Service, Kansas City, Kansas, for testing prodigiosin by the tissue culture method. We are also indebted to Professor Aldo Ermili[27] for the synthesis of one of the methenes (**2**) used.

(27) Visiting Fulbright Hays Research Scholar from Italy: Instituto Di Chimica Farmaceutica e Tossicologica, Università di Roma, Italy.

Organophosphorus Compounds as Schistosomicides

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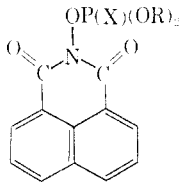
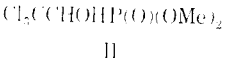
Several types of organophosphorus compounds have been shown to be effective against *Schistosoma mansoni* infections in mice. The most active compounds are the phosphate and thiophosphate derivatives of N-hydroxynaphthalimide. None of the materials, however, has shown high activity in monkeys at well-tolerated dose levels.

Some organophosphorus derivatives have proved useful as insecticides and others as drugs for the removal of intestinal helminths in animals. Apparently, these substances act primarily through cholinesterase inhibition. A relationship between the anthelmintic effect of di(2-chloroethyl)-3-chloro-4-methyl-7-coumarinyl phosphate (Haloxon) and cholinesterase inhibition has been found in several nematode parasites.[1] The antischistosomal drugs, tartar emetic[2] and tris(*p*-aminophenyl)carbonium salts,[3] inhibit cholinesterase activity in schistosomes. There is thus some rationale for the antischistosomal testing of potential cholinesterase inhibitors.

We have examined over 300 organophosphorus compounds of a wide variety of types against *Schistosoma mansoni* in mice and have tested selected compounds in monkeys. This report summarizes our results. A series of phosphate and thiophosphate derivatives of N-hydroxynaphthalimide (I) had particularly interesting activity in mice.



Ia, X = S; R = Et
b, X = O; R = Et
c, X = S; R = Me
d, X = O; R = Bu

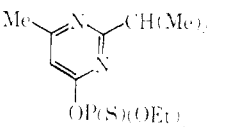


II

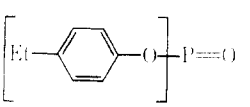
Recently, the organophosphorus compound II (Dipterex) has been reported to have activity against *S. japonicum* in mice,[4] dogs,[4] and man[5] and against *S. haematobium* in man.[6–8] However, this material did not show more than a trace of activity against a Puerto Rican strain of *S. mansoni* in albino mice by our test prodedures.[9] The therapeutic effects of this and other organophosphorus compounds against *S. mansoni* in mice are compiled in Table I.

Compound II was tested for therapeutic effect against *S. mansoni* in a rhesus monkey. It was given orally twice daily in amounts of 25.0 mg/kg/day for 5 days and 12.5 mg/kg/day for 5 days. Incoordination, sluggishness, and weight loss indicated that higher doses would not have been tolerated. Egg excretion was reduced, but numerous live worms and no dead worms were found at autopsy 2 weeks after treatment.

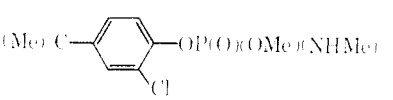
O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate (III)[10] (Diazonon) showed early in our testing considerable activity in mice. Com-



III



IV



V

pound III was tested orally against *S. mansoni* in six rhesus monkeys. It was given twice daily 5 days/week for 2 weeks. The following results were obtained. One monkey given 80 mg/kg/day died on the 9th day of medication. One monkey given 40 mg/kg/day was cured but exhibited 14% weight loss and diarrhea dur-

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(10) Supplied by Geigy Co., Inc.