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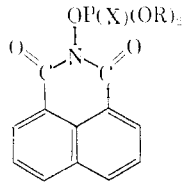
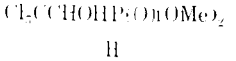
Several types of organophosphorus compounds have been shown to be effective against *Schistosoma mansoni* infections in mice. The most active compounds are the phosphate and thiophosphate derivatives of N-hydroxynaphthalimide. None of the materials, however, has shown high activity in monkeys at well-tolerated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

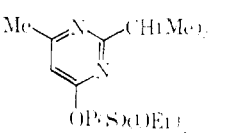


II

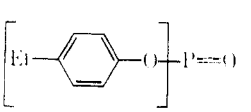
Recently, the organophosphorus compound II (Dipterex) has been reported to have activity against *S. japonicum* in mice,[4] dogs,[4] and man[5] and against *S. haematobium* in man.[6–8] However, this material did not show more than a trace of activity against a Puerto Rican strain of *S. mansoni* in 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.

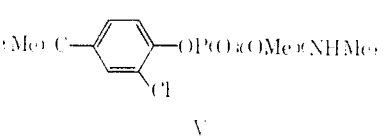
O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate (III)[10] (Diazonon) showed early in our testing considerable activity in mice. Com-



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-

(1) R. J. Hart, and R. M. Lee, *Exptl. Parasitol.*, **18**, 332 (1966).

(2) M.-L. Shen, Y.-I. Liang, and K.-S. Ting, *Acta Biochim. Sinica*, **2**, 79 (1960).

(3) E. L. Schiller and E. Buesding, *J. Parasitol.*, **51**, 43 (1965).

(4) B. F. Lu, Y. I. Liang, J.-M. Shi, and K.-S. Ting, *Acta Pharm. Sinica*, **9**, 602 (1962).

(5) B. F. Lu, Y. I. Liang, and J.-M. Shi, *ibid.*, **9**, 599 (1962).

(6) S. M. Talaat, N. Amin, and B. ElMasry, *J. Egypt. Med. Assoc.*, **46**, 827 (1963).

(7) S. M. Talaat, *ibid.*, **47**, 312 (1964).

(8) J. Cerf, A. LeBrun, and J. Hiericks, *Am. J. Trop. Med. Hyg.*, **11**, 514 (1962).

(9) For a description of test methods, see P. E. Thompson, J. E. Meisenhelder, and H. Najarian, *ibid.*, **11**, 31 (1962).

(10) Supplied by Geigy Co., Inc.

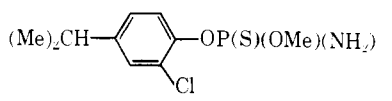
TABLE I
THERAPEUTIC EFFECTS OF ORGANOPHOSPHORUS COMPOUNDS AGAINST *S. mansoni* IN MICE

				Treated			Controls		
No.	Dose, mg/kg/day	No. of days	Regimen[a]	No. of mice	% of worms dead	Mean no. of live worms	No. of mice	% of worms dead	Mean no. of live worms
Ia	129	14	0.125% diet	9	100	0	19	0.4	16.1
	85	14	0.06% diet	11	98	0.4	23	0.4	15.8
	18	14	0.016% diet	15	37	9.6	37	0.3	18.8
	10	14	0.008% diet	5	56	8.3	11	1.0	24.9
	20	10	Gavage × 2	7	45	9.4	11	1.0	24.9
	20	10	Ip × 1	8	13	13.8	10	0	10.9
Ib	72	14	0.06% diet	5	100	0	13	0	19.5
	17	14	0.016% diet	6	100	0	7	1	12.7
	20	10	Gavage × 2	7	77	3.7	7	1	12.7
	20	10	Ip × 1	7	79	2.5	10	0	10.9
Ic	284	14	0.25% diet	6	29	8.4	13	0	14.9
	70	14	0.06% diet	5	12	9.0	11	0	13.8
Id	72	14	0.06% diet	6	98	0.2	13	0	13.0
	20	14	0.016% diet	5	56	6.6	10	0	16.0
II	872	14	0.8% diet	6	7	11.2	9	0	11.2
	287	14	0.2% diet	6	5	13.9	11	0	13.4
	71	14	0.05% diet	5	0	8.5	8	0	11.8
III	63	14	0.06% diet T[b]	7	89	0.8	10	0	15.6
	60	14	0.125% diet T	3	57	2.0	10	1	18.3
	54	14	0.06% diet T	6	58	2.3	14	0	16.1
	18	14	0.016% diet	8	69	2.1	10	0	11.3
	9	14	0.008% diet	7	18	6.4	10	0	17.5
	4	14	0.004% diet	8	0	11.7	10	0	17.5
	12	10	Gavage × 2	10	30	10.5	9	0	18.8
	6	9	Ip × 1	8	11	8.7	9	0	14.0
IV	68	14	0.06% diet T	1	74	5.0	7	0	28.6
	50	5	Gavage × 2	7	2	15.3	12	0	16.4
V	338	14	0.25% diet	6	85	0.9	11	1	15.0
	400	5	Gavage × 2	10	76	4.6	10	1	18.1
	100	5	Sc × 2	8	2	14.7	10	1	18.1
VI	205	14	0.25% diet	4	98	0.2	13	0	13.0
	74	14	0.06% diet	5	6	13.3	13	0	13.0
VII	324	14	0.25% diet	4	48	8.3	11	0	13.8
IX	554	14	0.5% diet	9	93	0.8	9	0	12.8
	212	14	0.125% diet	18	30	7.0	17	2	14.8
	800	4	Gavage × 2 T	3	95	0.6	10	0	11.1
	400	5	Gavage × 2	8	89	1.6	10	1	18.1
	200	10	Gavage × 2	9	11	9.4	10	0	15.8
	200	5	Gavage × 2	10	13	10.5	10	0	15.8
	200	5	Sc × 2	9	1	9.9	10	0	11.1
X	276	14	0.25% diet	5	99	0.1	13	1	15.2
	200	5	Gavage × 2	5	3	7.6	15	2	12.7
XII	82	14	0.06% diet	6	95	0.6	10	0	16.0
	22	14	0.016% diet	5	2	10.3	10	0	16.0

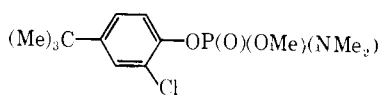
[a] When the compound is given in the diet, it is mixed with the food and comprises the stated percentage of the mouse daily diet. When the compound is administered by gavage or parenterally, the stated daily dose is given either all at once (× 1) or is divided in half and administered twice daily (× 2). [b] T, toxic (caused mortality or weight loss).

ing treatment. Two monkeys given 20 mg/kg/day showed a suppression of egg excretion but were not cured; one of these lost weight (16%) and had diarrhea but the other did not. One monkey given 10 mg/kg/day showed only a transient suppression of egg excretion and another given 5 mg/kg/day showed no evidence of therapeutic effect; both of these monkeys tolerated their doses well.

With the exception of tris(*p*-ethylphenyl) phosphate (IV),[11] which showed slight activity at toxic doses in mice, the only other active structural type was 4-*t*-butyl-2-chlorophenyl methylphosphoramidate (V, Ruelene),[12] which was quite effective in mice both in the diet at 0.25% and by gavage. Activity was fairly widespread among this structural type. Compounds VI and VII, for example, exhibited fair activity when administered at high levels in the diet. Certain minor



VI

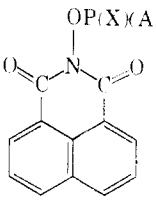


VII

structural variations did, however, abolish the biological activity. Compound VIII, for example, showed no

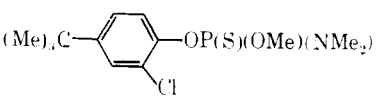
(11) Supplied by Dr. H. F. Bondy of Coalite and Chemical Products.
(12) Compounds V–X were supplied by Dr. E. Monroe of the Dow Chemical Co.

TABLE II

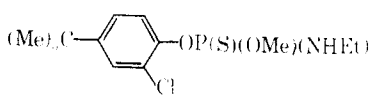


X	AR_1	AR_2	Mp, °C	Yield, %	Cryst solvent	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found
S	OMe	OMe	170–172	37	EtOH	$C_{14}H_{12}NO_5PS$	49.85	50.03	3.59	3.58	4.15	3.96
S	OPr	OPr	168–170	35	EtOH	$C_{18}H_{20}NO_5PS$	54.95	54.67	5.13	4.89	3.56	3.88
O	OBu	OBu	115–117.5	74	Dil EtOH	$C_{20}H_{24}NO_6P$	59.25	59.34	5.97	6.15	3.46	3.65
O	OC_6H_5	OC_6H_5	162–163 (prior softening)	63	EtOH	$C_{24}H_{16}NO_6P$	64.72	65.11	3.62	3.56	3.15	3.13
O	OC_6H_4-*o*-Me	OC_6H_4-*o*-Me	205–206.5	27	CH_3CN	$C_{28}H_{26}NO_6P$	65.96	65.56	4.26	4.28	2.91	2.78
O	OEt	NMe_2	196.5–199.5	41	EtOH	$C_{16}H_{17}N_2O_6P$	55.17	55.15	1.92	1.90	8.05	7.93
O	OC_6H_3-3,5-Me_2	OC_6H_3-3,5-Me_2	202–204	8	DMF–H_2O	$C_{28}H_{24}NO_6P$	67.06	66.60	4.82	5.07	2.79	3.10
O	OC_6H_4-*p*-Me	OC_6H_4-*p*-Me	173–175	6	C_6H_6	$C_{28}H_{20}NO_6P$	65.96	66.33	4.26	4.75	2.91	2.88
O	NMe_2	NMe_2	240–242.5	16	EtOH	$C_{28}H_{31}N_3O_4P$	55.33	55.23	5.22	5.37	12.40	11.84

activity at 0.25% in the diet. The best compound in this series on the basis of diet and gavage data in mice was IX.



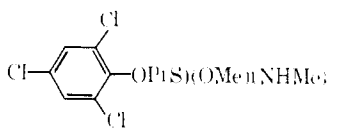
VIII



IX

Compound IX was tested orally twice daily for therapeutic action against *S. mansoni* in six rhesus monkeys. The following results were obtained. Treatment with 800 mg/kg/day for 5 days was essentially ineffective in one monkey. Doses of 400 mg/kg/day for 5 days cured one light and old infection. Doses of 200 mg/kg/day for 5 days caused in one monkey slight egg suppression but failed to cure. Doses of 100, 50, or 25 mg/kg/day for 10 days (one monkey each) were essentially ineffective. All doses were well tolerated by gross examination.

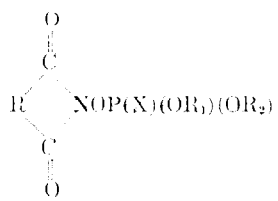
One further active type perhaps related to those above was O-methyl O-(2,4,6-trichlorophenyl)methylphosphoramidate (X)[12] which exhibited good activity



X

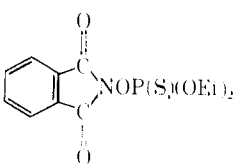
at 0.25% in the diet but showed no improvement over IX and so was not examined further.

Recently the O,O-diethyl O-phosphorothioate ester (Ia) of N-hydroxynaphthalimide (I, X = S; R = Et) was reported to be a potent insecticide[13–15] and to have anthelmintic effect in calves at toxic levels.[16] Whole blood cholinesterase activity was shown to be markedly reduced by this treatment.[16] We prepared this material and examined its activity in albino mice against *S. mansoni* and found it to be extremely potent. It killed 97–100% of all worms down to 0.06% in the diet and showed considerable reduction in live worm burdens down to 0.008% in the diet, and in addition showed fair activity when administered by gavage at 20 mg/kg for 10 days. This excellent activity prompted us to investigate this series further. Since a German patent[17] claims the equivalence of the series



X = O, S
R = an organic radical
R_1, R_2 = alkyl C_1–C_4

we studied first the closely related phthalimide derivative XI. This material, prepared according to the patent procedure by acylation of N-hydroxyphthal-



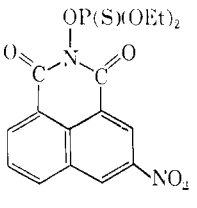
XI

imide in dimethylformamide with O,O-diethylthiophosphoryl chloride in the presence of potassium carbonate, was completely inactive against *S. mansoni* in mice at 0.25% in the diet. We returned to the naphthalimide series and prepared a series of analogs of the active lead. We were not able to prepare these materials using the patent procedure above but finally succeeded in obtaining them by forming the sodium salt of N-hydroxynaphthalimide with sodium hydride in dimethylformamide, isolating it by filtration, suspending it in benzene, and acylating by refluxing with the desired phosphoryl or thiophosphoryl chloride.

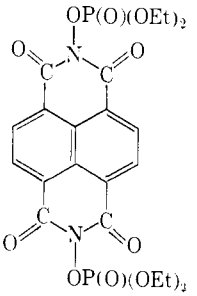
The compounds prepared are described in Table II. The oxygen analog Ib which had not been described in the literature except as a metabolite of Ia[18,19] has recently acquired a generic name (maretin) and has been reported to be effective against intestinal nematodes in

(13) H. W. Dorough and B. W. Arthur, *J. Econ. Entomol.*, **54**, 1117 (1961).
(14) S. C. Hoyt, *ibid.*, **54**, 1127 (1961).
(15) R. O. Brommond, B. Moore, and J. Warren, *ibid.*, **52**, 1220 (1959).
(16) T. S. Galvin, R. R. Bell, and R. H. Turk, *Am. J. Vet. Res.*, **23**, 191 (1962).
(17) W. Lorenz and R. Wegler (Bayer), German Patent 962,608 (April 25, 1957).
(18) N. R. Boyd, Jr., and B. W. Arthur, *J. Econ. Entomol.*, **53**, 848 (1960).
(19) J. R. Bateman and B. W. Arthur, *ibid.*, **54**, 446 (1961).

sheep and cattle.[20,21] This material also proved to be an effective schistosomicidal agent in mice; it was curative in amounts as low as 0.016% in the diet and had significant effect by gavage when administered at 20 mg/kg for 10 days. The other alkyl esters showed variable degrees of activity. The methyl and propyl analogs Ic and I (X = S; R = Pr) showed only very weak activity even at 0.25% while Id showed quite good activity even at 0.06% but was not superior to the ethyl ester. The *m*-nitro analog XII also exhibited



XII



XIII

good activity at 0.06% but was not superior to the parent compound while the bis compound XIII was inactive at 0.06% in the diet. The aryl esters and the two amine derivatives described in Table II showed essentially no activity when administered in the diet.

Further studies were then initiated with the parent compounds Ia and Ib. Ia has been shown to be converted by mammals to the oxygen analog Ib.[18,19] In certain organophosphorus insecticides thiophosphates are metabolized to the more active phosphates. The *in vitro* activity of Ia and Ib against *S. mansoni* was studied to determine whether a similar relationship was required for schistosome activity. The test preparations consisted of three pairs of worms in 2 ml of medium [75% horse serum, 20% physiologic saline solution, and 5% aqueous solutions of penicillin G (100 units/ml) and streptomycin (100 μg/ml)] and were incubated at 37°. Preparations containing 0, 2, 4, 8, or 16 μg of drug/ml were inspected at frequent intervals during 5 days. The death rate of worms in the presence of Ia was not significantly higher than the nonmedicated controls. Compound Ib also was tested *in vitro* under the same conditions as compound Ia. It also failed to kill the worms in concentrations of 2–16 μg/ml during 5 days of exposure. The lack of *in vitro* activity suggests either an insufficient concentration of drug in the medium or metabolism to an active component by the intact animal.

Compound Ia was tested orally twice daily for therapeutic action against *S. mansoni* in six rhesus monkeys. The following results were obtained. Two monkeys were given 200 mg/kg/day for 10 days. One of these had a light infection of several months duration; following treatment it stopped passing eggs and was judged cured on the basis of inability to find live worms at autopsy. The other animal had a heavy, recently induced infection; after treatment it showed a moderate suppression of egg excretion and at autopsy it had 50 live worms and about an equal number of dead worms. Two monkeys given 200 mg/kg/day for 5 days showed no discernible therapeutic response; both continued to excrete eggs in large numbers. Two others that had old, light infections were given 100 mg/kg/day for 10 days; they showed moderate suppression of egg excretion and at autopsy 18 and 19 live worms, respectively, which was close to the normal number for untreated infections of comparable duration. All doses were well tolerated by gross examination.

Compound Ib was tested for therapeutic effect against *S. mansoni* in three rhesus monkeys. One animal was given the drug orally in doses of 50 mg/kg/day (25 mg/kg twice daily) for 10 days (5 days/week for 2 weeks). Its weight declined 23% during treatment. Egg excretion was discernibly suppressed but 23 live worms were found at autopsy. Two others were treated intraperitoneally once daily 5 days/week. One received 1-mg/kg doses for the first week and 2.5-mg/kg doses during the second week. This animal died the day after the last dose and had 24 live worms; it did not survive long enough to assess an effect on egg excretion. The other monkey was given 1-mg/kg doses during the first two weeks and 2.5-mg/kg doses during the third week. This course of treatment was tolerated well but did not suppress egg excretion and left 65 live worms at autopsy.

In view of the prominence of cholinesterase inhibition in the general biological activity of organophosphorus compounds, it is logical to suspect that their antischistosomal action might be mediated *via* this mechanism. Early in the present studies, we tested three classical cholinesterase inhibitors against *S. mansoni* in mice for general guidance. The substances and test conditions were as follows: tetraethyl pyrophosphate (TEPP) in doses of 0.031 mg/kg by gavage twice daily for 10 days (near the maximum tolerated amount); 3-methyl-1-phenylpyrazol-5-yl dimethylcarbamate (Pyrolan) as a 0.5% diet for 14 days; 5,5-dimethyl-3-oxo-1-cyclohexen-1-yl dimethylcarbamate (Dimetan) as a 0.5% diet for 14 days. All were ineffective. Such results discourage the expectations of a direct relationship between cholinesterase inhibition and antischistosomal activity. Moreover, review of the results from the large number and diverse types of organophosphorus compounds tested by us has failed to suggest relationships between structure and antischistosomal activity or host toxicity. To the extent that host toxicity is due to cholinesterase inhibition, we also have been unable to detect useful structure–cholinesterase inhibition relationships. Understandably, such relationships may be obscured by physiological factors: metabolic disposition, membrane permeability, etc. The problem of selecting a promising organophosphorus drug for trial against schistosomiasis has been further complicated by dissimilar therapeutic indices among experimental hosts. Whereas some of the compounds had a relatively promising margin of safety in mice, none so far has shown high activity in monkeys at well-tolerated dose levels.

Experimental Section[22]

N-Hydroxynaphthalimide O,O-Diethyl O-Phosphorothioate Ester (Ia).—To a solution of 40.8 g (0.192 mole) of N-hydroxynaphthalimide in 1 l. of dimethylformamide (DMF) was added

(20) M. Federmann, *Deut. Tieraerztl. Wochschr.*, **71**, 62 (1964).

(21) M. Stuber and H. Ende, *Berliner Münchener Tieraerztl. Wochschr.*, 100 (1964).

(22) Melting points were taken on a Thomas–Hoover melting point apparatus and are corrected.

8.8 g of NaH (50% dispersion in oil). The mixture was heated for 3 hr at 70–80°, the solid sodium salt was removed by filtration, washed with benzene, and dried briefly in air. The solid was transferred to a 2-l. flask, suspended in about 1 l. of toluene, and heated under reflux for 24 hr with 36.1 g of diethylthiophosphoryl chloride. The mixture was cooled to room temperature and filtered, and the solvent was removed from the filtrate *in vacuo*. The residue was recrystallized three times from 95% ethanol to give 28.9 g (41%) of the product, mp 162–164°.

Anal. Calcd for $C_{16}H_{16}NO_5PS$: C, 52.60; H, 4.42; N, 3.83. Found: C, 52.49; H, 4.34; N, 4.01.

N-Hydroxynaphthalimide Diethyl Phosphate (Ib).—To a solution of 16.6 g (0.05 mole) of N-hydroxynaphthalimide in 250 ml of DMF was added 2.4 g of NaH (50% dispersion in oil). The mixture was heated for 3 hr at 70–80°, and the solid sodium salt was removed by filtration, washed with benzene, and dried briefly in air. The solid was suspended in about 250 ml of benzene and heated under reflux for 7 hr with 8.6 g (0.05 mole) of diethylphosphoryl chloride. The mixture was filtered and the filtrate was concentrated to dryness *in vacuo*. The residue was recrystallized twice from 95% ethanol to give the product as a white solid, mp 180–182°, 7.7 g (44%).

Anal. Calcd for $C_{16}H_{16}NO_6P$: C, 55.02; H, 4.62; N, 4.01. Found: C, 54.97; H, 4.96; N, 4.01.

3-Nitro-N-hydroxynaphthalimide Sodium Salt.—A mixture of 12 g (0.05 mole) of 3-nitronaphthalic anhydride, 8.8 g of hydroxylamine hydrochloride, and 11 g of Na_2CO_3 in 250 ml of water was heated under reflux for 4 hr. The warm mixture was filtered and the red solid was dried *in vacuo*, powdered, and boiled in benzene with a water take-off to remove occluded water. The solid was collected and dried to give 11 g (74%) of the product as its monohydrate.

Anal. Calcd for $C_{12}H_5N_2NaO_5 \cdot H_2O$: C, 48.33; H, 2.37; N, 9.40; H_2O, 6.04. Found: C, 48.03; H, 2.35; N, 9.46; H_2O, 5.86.

N-Hydroxy-3-nitronaphthalimide O,O-Diethyl O-Phosphorothioate (XII).—A suspension of 10.1 g (0.0339 mole) of the above sodium salt and 6.4 g of diethylthiophosphoryl chloride in benzene was heated under reflux for 4 days. The mixture was filtered and the filtrate was concentrated to dryness *in vacuo*. The residue recrystallized from ethanol gave the product as brown plates, 4.5 g (32%), mp 173–178°.

Anal. Calcd for $C_{16}H_{15}N_2O_7PS$: C, 46.83; H, 3.69; N, 6.83. Found: C, 47.03; H, 3.55; N, 6.95.

N,N′-Dihydroxynaphthalenetetracarboxylic Diimide.—A mixture of 13.4 g (0.5 mole) of 1,4,5,8-naphthalenetetracarboxylic dianhydride, 17.6 g of hydroxylamine hydrochloride, and 22 g of Na_2CO_3 in 300 ml of water was warmed gradually. Vigorous foaming occurred, heating was discontinued, and the warm suspension was stirred for about 1.5 hr. Heating was then resumed, and the mixture was heated to boiling for 1.5 hr, cooled, and filtered. The crude product was stirred with warm water, filtered, and dried *in vacuo* to give 7.1 g (37%) of the product as a reddish solid which did not melt to 300°.

Anal. Calcd for $C_{14}H_4N_2Na_2O_8 \cdot 2H_2O$: C, 44.45; H, 2.13; N, 7.41; H_2O, 9.53. Found: C, 43.75; H, 2.21; N, 7.06; H_2O, 9.98.

N,N′-Dihydroxynaphthalenetetracarboxylic Diimide Bis(diethyl Phosphate) Diester (XIII).—N,N′-Dihydroxynaphthalenetetracarboxylic diimide disodium salt (7.1 g, 0.0188 mole), suspended in about 1 l. of benzene, was heated under reflux with 6.5 g of diethylphosphoryl chloride for 24 hr. The mixture was cooled to room temperature and filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was recrystallized three times from ethanol to give 1.9 g (19.3%) of desired product, mp 248–252° (earlier softening).

Anal. Calcd for $C_{22}H_{24}N_2O_{12}P_2$: C, 46.32; H, 4.24; N, 4.91. Found: C, 46.83; H, 4.36; N, 5.16.

N-Hydroxynaphthalimide Di-*o*-tolyl Phosphate Ester. The following modification was used for those analogs that were less soluble in the reaction mixture and tended to precipitate along with the inorganic salts when the reaction was complete. A solution of 10.6 g (0.05 mole) of N-hydroxynaphthalimide in 250 ml of DMF was treated with 2.4 g of NaH (50% dispersion in oil) for 3 hr at 60–70°. The sodium salt was removed by filtration, washed with benzene, and heated under reflux with 14.8 g (0.05 mole) of di-*o*-tolyl chlorophosphate in 500 ml of benzene for 18 hr. The reaction mixture was filtered and the filtrate was concentrated to dryness *in vacuo*. The residue was recrystallized twice from acetonitrile to give 1.4 g of the product, mp 205–206.5°. The solid obtained from the reaction mixture was stirred with warm water and filtered, and the solid was recrystallized twice from acetonitrile to give an additional 5.1 g of product, mp 205–206.5°.

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