COMMUNICATIONS

Insect Chemosterilants. XII. Phosphorus Amides

Twenty-four phosphoramides not previously reported in the literature were synthesized and tested for sterilizing activity against the housefly, *Musca domestica* L., the boll weevil, *Anthonomous grandis* Boheman, and the screwworm, *Cochliomyia hominivorax* Coquerel. Based on results with these insects, 15 compounds are classed as active chemosterilants.

In 1964 we reported the sterilizing effect of hexamethylphosphoric triamide (hempa) (Chang *et al.*, 1964) on the housefly, *Musca domestica* L. Following our later description of the chemosterilant activity of several phosphoramides (Terry and Bořkovec, 1967), we now report the synthesis and biological activity of 24 more new compounds of this class.

EXPERIMENTAL SECTION

Biological Evaluation. All compounds were tested as chemosterilants in the housefly; some were also tested in the boll weevil, *Anthonomus grandis* Boheman, and in the screwworm, *Cochliomyia hominivorax* Coquerel. In all tests, young adult insects were fed a diet medicated with 1% or less of the candidate compound and their fertility was compared with that of untreated insects. Three criteria served as indicators of sterilizing effects: reduced production of eggs; reduced hatch of eggs; and reduced pupation of larvae or emergence of adult progeny. Detailed screening procedures with the housefly (Fye *et al.*, 1966), screwworm (Crystal, 1970), and boll weevil (Bořkovec *et al.*, 1972), and the relative significance of the three indicators of sterility have been described previously.

Based on results with three test insects, 15 of the compounds are classed as active (Table I).

Syntheses. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. Complete analytical data on all compounds described in this communication will appear following these pages in the microfilm edition of this volume of the journal. Boiling points and melting points (Thomas Hoover apparatus) are uncorrected.

Most of the amides were synthesized from corresponding acid chlorides and amines in a solvent with cooling (low-boiling amines) or in excess amine with heating (high-boiling amines). Evolved HCl was neutralized with amine or Et_3N. Tetramethylphosphorodiamidic chloride, tetramethylphosphorodiamidothioic chloride, and dimethylphosphoramidic dichloride, all prepared by literature methods, were used in 16 of the reactions. Isopropylphosphoramidothioic dichloride, diethylphosphoramidothioic dichloride, ethylphosphonothioic dichloride, and phosphorodichloridothioic acid, *o*-phenyl ester, obtained commerically, were used to prepare **18**, **19**, **23**, and **24**. The less obvious preparations are outlined below.

***N*-Methoxy-*N*′,*N*′,*N*″,*N*″-Pentamethylphosphoric Triamide (8).** Attempted synthesis from tetramethylphosphorodiamidic chloride and *N*-methoxy-*N*-methylamine led to a mixture of products. A pure product was obtained from *N*-methoxy-*N*-methylphosphoramidic dichloride and dimethylamine. Phosphoric trichloride (0.2 mol) in absolute diethyl ether was treated with *N*-methoxy-*N*-methylamine (0.2 mol) and triethylamine (0.2 mol) at −20°. Upon completion of the reaction, the mixture was allowed to stand at room temperature overnight, and then the salt and solvent were removed. The distilled *N*-methoxy-*N*-methylphosphoramidic dichloride, bp 71° (10 mm), was obtained in a 50% yield. *Anal.* Calcd for $C_2H_6Cl_2NO_2P$: C, 13.50; H, 3.40; N, 7.87; P, 17.41. Found: C, 13.59; H, 3.54; N, 7.50; P, 17.13. One mole of dimethylamine was condensed into a flask at −20° and 0.1 mol of *N*-methoxy-*N*-methylphosphoramidic dichloride was added dropwise. After addition of the acid chloride was completed, the reaction mixture was allowed to reflux for a few hours and then the dimethylamine was allowed to escape. Diethyl ether was added, the salt was filtered off, and the solvent was removed. On distillation a 66% yield of product, bp 117° (12 mm), was obtained. *Anal.* Calcd for $C_6H_{18}N_3O_2P$: C, 36.92; H, 9.29; N, 21.53; P, 15.87. Found: C, 36.69; H, 9.47; N, 21.75; P, 16.05.

[Bis(dimethylamino)phosphinyl]methylcarbamic Acid, Ethyl Ester (9). When a stirred suspension of sodium hydride (0.04 mol) in absolute diethyl ether was treated with an ether solution of pentamethylphosphoric triamide (0.025 mol), hydrogen was evolved gently. The mixture was allowed to stand overnight, and then after cooling (ice bath), a solution of ethyl chloroformate (0.04 mol) in ether was added. Four hours later the crude product was isolated by removing the salt and solvent and freed from impurity (pentamethylphosphoric triamide, 11% by glpc) by three successive distillations. The pure compound, bp 67° (0.01 mm), was obtained in a 55% yield. *Anal.* Calcd for $C_8H_{20}N_3O_3P$: C, 40.50; H, 8.50; N, 17.71; P, 13.06. Found: C, 40.48; H, 8.65; N, 17.91; P, 13.24.

***N*,*N*,*N*′,*N*′-Tetramethylphosphorothioic Triamide (17).** Several attempts to prepare **17** by use of a solvent and a large excess of ammonia, or by use of ammonia and the acid chloride alone at atmospheric pressure, failed. The synthesis was accomplished by putting tetramethylphosphorodiamidothioic chloride and liquid ammonia (1:4) in a sealed tube and letting the tube stand for 2 days. The reaction mixture was transferred to a beaker where the product was extracted from the salt with boiling benzene. The crude product melted at 58–63° and the yield was quantitative. Recrystallization of the crude product from a benzene–petroleum ether (1:2) mixture gave fluffy, white needles, mp 64–66°. *Anal.* Calcd for $C_4H_{14}N_3PS$: C, 28.73; H, 8.44; N, 25.13; P, 18.52. Found: C, 28.65; H, 8.53; N, 25.06; P, 18.29.

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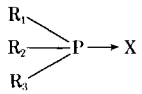
Table I. Chemical and Physical Data and Chemosterilant Activity of Phosphorus Amides



No.	X	R_1	R_2	R_3	ENT-No	Yield, % pure material	Bp (mm) or mp, °C	Formula	Analyses[a]	Sterilizing[b] activity HF	BW	SW[c]
1	O				61407	66	82–87[d]	$C_9H_{18}N_3OP$	C,H,N,P	0	0	0
2	O	$-NHCH_2CH_2OCH_3$	$-NHCH_2CH_2OCH_3$	$-NHCH_2CH_2OCH_3$	60423	50	166 (0.10)	$C_9H_{24}N_3O_4P$	C,H,N,P	0	0	0
3	O	$-N(CH_3)_2$	$-NHCH_2CH_2OCH_3$	$-NHCH_2CH_2OCH_3$	60333	31	136 (0.20)	$C_8H_{22}N_3O_3P$	C,H,N,P	0	0	0
4	O	$-N(CH_3)_2$			61406	66	63–66[e]	$C_{12}H_{26}N_3OP$	C,H,N,P	0	0	0
5	O	$-N(CH_3)_2$	$-NHCH_3$	$-NHCH_3$	62439	93[f]	145 (2.0)[g]	$C_4H_{14}N_3OP$	C,H,N,P	—	—	—
6	O	$-N(CH_3)_2$	$-N(CH_3)CH_2CH_2(CH_3)N-$		61019	52	118 (6.0)	$C_6H_{16}N_3OP$	C,H,N,P	0	++	0
7	O	$-N(CH_3)_2$	$-NHCH_2CH_2CH_2O-$		61615	81	150 (0.03)	$C_{15}H_{13}N_2O_2P$	C,H,N,P	0	0	0
8	O	$-N(CH_3)_2$	$-N(CH_3)_2$	$-N(CH_3)OCH_3$	61078	66	117 (12)	$C_6H_{18}N_3O_2P$	C,H,N,P	0	0	0
9	O	$-N(CH_3)_2$	$-N(CH_3)_2$	$-N(CH_3)COOC_2H_5$	61176	55	67 (0.01)	$C_8H_{20}N_3O_3P$	C,H,N,P	0	0	0
10	O	$-N(CH_3)_2$	$-N(CH_3)_2$	$-OCH_3$	61100[h]	83	95 (15)	$C_5H_{15}N_2O_2P$	C,H,N,P	0	0	0
11	O	$-N(CH_3)_2$	$-N(CH_3)_2$		61344	70	64 (0.01)	$C_7H_{18}N_3OP$	C,H,N,P	++	0	0
12	O	$-N(CH_3)_2$	$-N(CH_3)_2$		61175	65	77 (0.01)	$C_8H_{20}N_3OP$	C,H,[i]N,P	++	+	+
13	O	$-N(CH_3)_2$	$-N(CH_3)_2$		61958	61	100 (0.01)	$C_7H_{18}N_3OPS$	C,H,N	0	+	0
14	O	$-N(CH_3)_2$	$-N(CH_3)_2$		61182	75	95 (0.01)	$C_{10}H_{24}N_3OP$	C,H,N,P	0	++	+
15	O	$-N(CH_3)_2$	$-N(CH_3)_2$		61177	80	82 (0.01)	$C_{10}H_{24}N_3OP$	C,H,N,P	++	++	+
16	O	$-N(CH_3)_2$	$-N(CH_3)_2$		61117	69	79 (0.01)	$C_9H_{23}N_4OP$	C,H,N,P	0	0	0
17	S	$-N(CH_3)_2$	$-N(CH_3)_2$	$-NH_2$	62223	80	64–66[j]	$C_4H_{14}N_3PS$	C,H,N,P	++	++	—
18	S	$-N(CH_3)_2$	$-N(CH_3)_2$	$-NHCH(CH_3)_2$	61956	60	37–39[j]	$C_7H_{20}N_3PS$	C,[k]H,N	+	++	0
19	S	$-N(CH_3)_2$	$-N(CH_3)_2$	$-N(C_2H_5)_2$	61918	84	77 (0.01)	$C_8H_{22}N_3PS$	C,H,N	0	++	+
20	S	$-N(CH_3)_2$	$-N(CH_3)_2$		61566	82	85 (0.01)	$C_7H_{18}N_3PS$	C,H,N,P	++	+	++
21	S	$-N(CH_3)_2$	$-N(CH_3)_2$		61408	79	93 (0.01)	$C_9H_{22}N_3PS$	C,H,N,P	++	+	—
22	S	$-N(CH_3)_2$	$-N(CH_3)_2$		62468	80	104 (0.02)	$C_8H_{20}N_3OPS$	C,H,N,P	++	—	—
23	S	$-N(CH_3)_2$	$-N(CH_3)_2$	$-C_2H_5$	61916	91	53 (0.01)	$C_6H_{17}N_2PS$	C,H,N,P	0	+	—
24	S	$-N(CH_3)_2$	$-N(CH_3)_2$		61917	79	98 (0.01)	$C_{10}H_{17}N_2OPS$	C,H,N	0	+	0

[a] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. [b] Three criteria served as indicators of sterilizing effects: reduced production of eggs; reduced hatch of eggs; and reduced pupation of larvae or emergence of adult progeny. The sterilizing activity is designated with ++ when 1% or lower concentration of the compound reduced one of the indicators to zero, with + when there was a marked reduction in one or more of the indicators, and 0 when insects treated with 1% of the compound reproduced normally. [c] HF, housefly; BW, boll weevil; SW, screwworm; —, not tested. [d] Recrystallized from ligroin (bp 100–115°). [e] Recrystallized from ligroin (bp 60–90°). [f] Yield of crude product. Purification consisted of one distillation through a rotofilm molecular still followed by ordinary distillation. [g] Liquid solidifies to a solid, mp 38°. [h] The directions of Gardiner and Kilby (1950) for the ethyl ester were modified by the use of sodium methoxide. [i] H: calcd, 9.82; found, 10.36. [j] Recrystallized from petroleum ether, bp 30–60°. [k] C: calcd, 40.17; found, 39.59.

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Microbial Hydroxylation of the Herbicide *N*-(3,4-Dichlorophenyl)methacrylamide (Dicryl)

The fungus *Rhizopus japonicus* converts the herbicide *N*-(3,4-dichlorophenyl)methacrylamide (dicryl) to *N*-(3,4-dichlorophenyl)-2-methyl-2,3-dihydroxypropionamide. This metabolite was formed by the addition of two hydroxyl groups to the ethylenic double bond of dicryl. Its structure was elucidated by nuclear magnetic resonance and mass spectrometric analysis.

Fungi of the family *Mucoraceae* are known to hydroxylate fungicidal acylanilides and related compounds. The enzyme system responsible for this reaction is nonspecific. Hydroxyl groups are introduced on aromatic rings (Wallnöfer *et al.*, 1971), methyl groups attached to the furan ring system (Wallnöfer *et al.*, 1972a), and on aliphatic side chains (Wallnöfer *et al.*, 1972b).

Since fungi of the type responsible for these reactions are commonly found in soil (Domsch, 1960), it is not unreasonable to expect hydroxylations of acylanilides to be a mode of detoxication under natural soil conditions. Because of the difficulties of isolating metabolites from a complicated matrix such as soil, we are continuing to use the fungus *R. japonicus* as a model hydroxylating organism. A number of acylanilides and related compounds are being investigated with regard to finding the "biologically labile site" towards hydroxylation.

MATERIAL AND METHODS

Chemical and Instrumentation. A sample of the herbicide *N*-(3,4-dichlorophenyl)methacrylamide (dicryl), analytical grade, was obtained from Niagara Chemical Co., Naugatuck, Conn., USA. Instruments and conditions for their operation were those described earlier (Wallnöfer *et al.*, 1971, 1972a). The 220 MHz nmr spectrum, recorded on a Varian Instrument, was obtained through the Ontario Research Foundation, Sheridan Park, Ont., Canada.

Culture Methods and Analytical Procedures. *Rhizopus japonicus* was cultured in a synthetic glucose medium (500 ml) in 1-l. Fernbach vessels on a shaker at 27° in the presence of 1.5% calcium carbonate for 1 week (Wegener *et al.*, 1967).

A standard solution of dicryl (10 mg in 1 ml of acetone–propylene glycol 1:1) was added to the culture medium to a final concentration of 20 mg/l. To observe conversion of dicryl, 10-ml portions of the culture medium were extracted with chloroform (30 ml) daily and the disappearance of the starting material was followed by uv analysis after purification by tlc (solvent A, Table I; Wallnöfer *et al.*, 1971, 1972a,b).

Isolation of the Dicryl Metabolite. The culture medium was extracted with chloroform and the extract was purified by tlc (Wallnöfer *et al.*, 1972a). The solvent system for this purification was benzene–acetic acid, 9:1. The crude material after elution from the silica was recrystallized from chloroform–carbon tetrachloride, 1:1.

RESULTS AND DISCUSSION

As observed previously with other compounds, maximum conversion of dicryl was found when the fungal growth ended. Within 1 week, 87 μmol/l. of dicryl was transformed to 19 μmol/l. of metabolite M-1; 30 μmol/l. remained unchanged in the culture medium and the rest was found unchanged in the mycelium.

Some physical data (mp, uv maxima, and R_f values) for dicryl and its metabolite M-1 are given in Table I.

The mass spectrum of the metabolite M-1 showed a

Table I. Physical Data for Dicryl and Metabolite M-1

Compound	mp, °C	uv max, nm $CHCl_3$	tlc R_f A[a]	B[b]	C[c]	D[d]
Dicryl *N*-(3,4-Dichlorophenyl)methacrylamide	105.5	264	0.76	0.76	0.99	0.83
Metabolite M-1 *N*-(3,4-Dichlorophenyl) 2-methyl-2,3-dihydroxypropionamide	101–102	252	0.03	0.31	0.72	0.25

[a] A = chloroform–benzene, 9:1. [b] B = benzene–acetic acid, 9:1. [c] C = chloroform–acetone–acetic acid, 15:2:3. [d] D = ethyl acetate–benzene, 6:4.