Preparation and Fungitoxicity of Some Trialkyl Phosphorotetrathioates

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Homologous series of both symmetrical and unsymmetrical trialkyl phosphorotetrathioates were prepared and tested in agar plates as fungitoxicants for eight species of phytopathogenic fungi. Certain of the shorter homologs, such as trimethyl phosphorotetrathioate, proved to be more fungitoxic than standard commercial fungicides. These studies showed that the fungitoxicity of trialkyl phosphorotetrathioates generally decreased as alkyl groups were lengthened.

During a recent screening program on the biological properties of several types of compounds, it was found that trialkyl phosphorotetrathioates containing methyl groups had significant fungitoxicities. To obtain more information on the toxicity-structure relationships of these little known materials, 16 compounds were synthesized and evaluated. This paper is a brief account of that work, and the unique toxicity found for the methyl substituent.

Preparation of Compounds

Two methods of preparing some of the trialkyl phosphorotetrathioates were described recently (*2*). As the third method (Reaction 5) is somewhat similar, only the pertinent data are recorded (Table I). The reactions involved are:

$$6RSH + P_2S_5 \rightarrow 2(RS)_3PS + 3H_2S \quad (1)$$

$$2CH_3SH + P_2S_5 \rightarrow 2CH_3SPS_2 + H_2S \quad (2)$$

$$CH_3SPS_2 + RSH \rightarrow CH_3S(RS)P(=S)-SH \quad (3)$$

$$CH_3S(RS)P(=S)-SH + \text{olefin} \rightarrow CH_3S(RS)P(=S)-SR' \quad (4)$$

$$CH_3S(RS)P-SH + R'SH \rightarrow CH_3S(RS)P(=S)-SR' + H_2S \quad (5)$$

The symmetrical triakyl phosphorotetrathioates were made easily by allowing phosphorus pentasulfide to react with an excess of mercaptan, as shown in Reaction 1. The unsymmetrical trialkyl phosphorotetrathioates were less easily prepared and, in this work, depended upon the great reactivity of methyl phosphenotrithioate, the preparation of which is shown in Reaction 2. When methyl phosphenotrithioate reacts with one mole of a mercaptan, the product is a methyl alkyl hydrogen phosphorotetrathioate (Reaction 3). With two or more moles of a mercaptan, methyl phosphenotrithioate gives methyl dialkyl phosphorotetrathioate (Reaction 5). When a methyl alkyl hydrogen phosphorotetrathioate reacts with an olefin, the product is a methyl dialkyl phosphorotetrathioate (Reaction 4). The addition here is according to Markownikoff's rule—i.e., 1-olefins add in the 2- position.

Most of these reactions are nearly quantitative. Many of the poor yields in Table I were caused by handling losses. Because these compounds have moderately poor thermal stabilities, considerable decomposition occurred during some of the distillations. Residual solvent was removed from many of the compounds by vacuum stripping rather than by distillation. Although some of the products were contaminated with small amounts of solvent, the structures were confirmed by infrared analysis (*1*).

Fungitoxicity Evaluation

The fungitoxicity of these compounds was tested in agar at 1000 and 100 p.p.m. To prepare the 1000-p.p.m. dosage, 2.5 grams of the test compound was weighed into a 200-ml. Waring Blendor, with 2 drops of a nonionic dispersing agent (Triton X-171, Rohm and Haas Co.) and sufficient distilled water to bring the total weight to 50 grams. The mixture was homogenized at high speed and then, with the blender still operating, 1 gram of the homogenate was transferred to 50 grams of sterile liquid potato dextrose agar at 45° C. The treated medium, containing 1000 p.p.m. of test chemical, was then poured into three Petri plates and allowed to solidify. To obtain 100 p.p.m. of test chemical in the agar medium, 10.0 grams of the homogenate was weighed into another blender and the weight brought up to 100 grams with distilled water. This mixture was homogenized and 1 gram transferred to 50 grams of sterile agar medium, which was then poured into three Petri plates and allowed to solidify. In all cases, the homogenate was readily dispersed in the liquid agar medium by swirling.

The solid medium in each plate was inoculated with the mycelia of eight species of fungi: *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora cinnamomi*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, *Monolinia fructicola*, *Botrytis cinerea*, and *Alternaria solani*. Disks of agar with mycelia on the surface were cut from Petri-plate cultures with a sterile, No. 2 cork borer and were transferred to treated agar with the mycelia against the treated surface.

The disks were arranged in a circle on each plate about 1 inch apart and 0.7 inch in from the plate rim. The cultures of fast-growing species were 2 days old and the more slowly growing species 4 or 5 days old at the time of transfer. All cultures were originally isolated from plant hosts.

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Table I. Synthesis of Trialkyl Phosphorotetrathioates

Phosphorotetrathioate	Mercaptan, Moles	CH_3SPS_2, Mole	P_2S_5, Moles	Olefin, Moles	Reaction Pressure, P.S.I.G.	Reaction Temp., ° C.	Reaction Time, Hours	Boiling Point of Product, ° C./Mm. Hg	Yield, Mole %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Phosphorus, % Calcd.	Phosphorus, % Found	Sulfur, % Calcd.	Sulfur, % Found
$(CH_3S)_3PS$	42	...	3.5	...	195	100	6	126–130/0.2	86	17.7	17.1	4.4	4.3	15.2	15.1	62.7	62.8
$(C_2H_5S)_3PS$	15	...	3	...	0	100	14	110/0.15	46	29.3	28.8	6.1	6.3	12.6	12.6	52.0	51.8
$(CH_3)_2CH-S)_3PS$	3	...	0.5	...	0	70	40	123–125/0.3	79	37.5	36.6	7.3	7.4	10.8	10.7	44.4	44.7
$(CH_3CH_2CH_2S)_3PS$	8.2	...	2	...	0	100	5	136–137/0.15	38	37.5	37.1	7.3	7.4	10.8	10.9	44.4	44.6
$(CH_3S)_2P(S)SCH(CH_3)_2$	CH_3SH 0.15	0.5	...	$CH_2=CHCH_3$ 1.0	[a]	40[b] 80–100	21.5[c] 2.5	125–130/0.8	22	25.8	26.0	5.6	5.7	13.4	13.7	55.2	55.2
$(CH_3S)_2P(S)S-CH_2CH_2CH_3$	n-C_3H_7SH, CH_3SH 0.5 1.0	0.5	...	...	65	50 80	0.5 3.0	140–150/0.5–0.7	57	25.8	24.6	5.6	5.6	13.4	14.1	55.2	56.9
$(CH_3S)_2P(S)S$-n-C_4H_9	CH_3SH, n-C_4H_9SH 0.5 1.0	0.5	...	...	33	40 100	2 17	[d]	28	...	...	...	...	12.6	13.2	51.9	49.2
$(CH_3S)_2P(S)S-CH(CH_3)-C_4H_9$	CH_3SH 0.5	0.5	...	$CH_2=CHC_4H_9$ 1.0	50	5 100	2 16	[d]	80	...	...	...	...	11.3	12.1	46.8	47.8
$(CH_3S)_2P(S)S$-n-C_6H_{13}	CH_3SH, n-$C_6H_{13}SH$ 1.0 0.4	0.4	...	...	0 65	40 100	0.5 4	[d]	75	...	...	...	...	11.3	12.2	46.6	46.3
$(CH_3S)_2P(S)S$-n-C_8H_{17}	CH_3SH, n-$C_8H_{17}SH$ 0.5 0.3	0.3	...	...	0 60	40 100	0.5 1.0	[d] [d]	75	39.8	41.3	7.6	7.7	10.3	10.3	42.3	39.6
$(CH_3S)_2P(S)S$-n-$C_{10}H_{21}$	CH_3SH, n-$C_{10}H_{21}SH$ 1.0 0.2	0.2	...	...	0 60	30 100	1.2 16	[d]	77	43.6	46.3	8.2	8.8	9.4	8.8	38.8	35.7
$(CH_3S)_2P(S)S$-n-$C_{11}H_{23}$	CH_3SH, n-$C_{11}H_{23}SH$ 1.0 0.2	0.2	...	...	0 40	40 65	0.5 5.5	[d]	76	45.3	45.3	8.4	8.1	9.1	9.8	37.2	35.1
$(CH_3S)_2P(S)S$-n-$C_{12}H_{25}$	CH_3SH, n-$C_{12}H_{25}SH$ 0.2 0.2	0.2	...	...	5 0	40 50	2 17	[d]	74	...	...	...	...	...	...	...	
$CH_3S-P(S)(SC_2H_5)_2$	C_2H_5SH 0.4	0.4	...	$CH_2=CH_2$ (excess)[f]	[f]	25 100	66 18	109–129/0.5	34	25.8	24.4	5.6	5.7	13.4	14.2	55.2	56.3
$CH_3S-P(S)(SC_2H_5)(S-CH(CH_3)_2)$	C_2H_5SH 0.4	0.4	...	$CH_2=CHCH_3$ 1.0	[a]	25 100	66 16	105–115/0.2	15	...	...	...	...	12.6	12.8	52.1	53.2
$CH_3S-P(S)[S-CH(CH_3)_2]_2$	i-C_3H_7SH 0.7 0.7	0.7	...	$CH_2=CHCH_3$ 1.0	[a]	34 100	2 1.5	130–135/0.6	45	32.3	31.6	6.5	6.8	11.9	11.7	49.3	49.0

[a] Autogenous pressure.
[b] First number is temperature of reaction of CH_3SPS_2 and RSH. Second number is temperature of addition of olefin.
[c] First number is time of reaction of CH_3SPS_2 and RSH. Second number is time and addition of olefin.
[d] Product vacuum stripped rather than distilled because of instability at high temperatures.
[e] Structure confirmed by infrared analysis.
[f] 300-ml. bomb at 500 p.s.i.g./25° C., 3000 p.s.i.g./95° C.

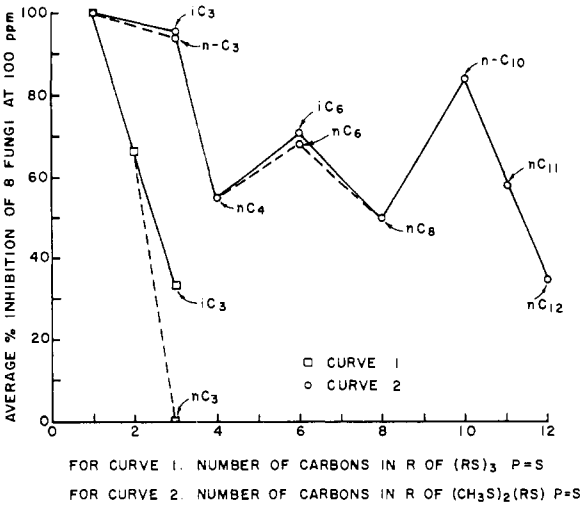

Figure 1. Effect of side chain length on agar-plate fungitoxicities of dimethyl alkyl and trialkyl phosphorotetrathioates

Inoculated plates were incubated for 2 days at 25° C. and the millimeters of mycelial growth from the edge of the inoculum disk to the margin of radial development recorded. The per cent inhibition of mycelial growth was calculated from the average of three replicates as follows:

$$100 - \left(\frac{\text{average growth on chemical-treated agar}}{\text{average growth on untreated agar}}\right) \times 100 = \% \text{ inhibition}$$

Discussion

The compounds in Tables I and II are separated into three groups: sym-

Table II. Agar-Plate Fungitoxicities of Trialkyl Phosphorotetrathioates

(Per cent inhibition of radial growth)

Phosphorotetrathioates	*Rhizoctonia Solani*		*Sclerotium Rolfsii*		*Phytophthora Cinnamomi*		*Pythium Ultimum*		*Sclerotinia Sclerotiorum*		*Sclerotinia Fructicola*		*Botrytis Cinerea*		*Alternaria Solani*		*Average*	
	100	*1000*	*100*	*1000*	*100*	*1000*	*100*	*1000*	*100*	*1000*	*100*	*1000*	*100*	*1000*	*100*	*1000*	*100*	*1000*
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
$(CH_3S)_3PS$	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
$(C_2H_5S)_3PS$	79	89	95	100	24	100	43	90	90	94	89	89	70	91	40	70	66	90
$(CH_3)_2CHS)_3PS$	63	68	61	89	10	40	16	70	48	77	58	77	0	52	14	36	34	64
$(CH_3CH_2CH_2S)_3PS$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$(CH_3S)_2P(S)-SCH(CH_3)_2$	100	100	100	100	100	100	94	100	98	100	100	100	95	95	70	100	95	99
$(CH_3S)_2P(S)-S-CH_2CH_2CH_3$	100	100	100	100	100	100	82	100	97	100	97	100	95	100	83	83	94	98
$(CH_3S)_2P(S)-S-n-C_4H_9$	60	68	76	93	89	100	48	100	39	90	60	87	55	90	12	35	55	83
$(CH_3S)_2P(S)-S-CH(CH_3)-C_4H_9$	62	82	91	99	100	100	38	100	63	100	83	100	91	96	29	59	70	92
$(CH_3S)_2P(S)-S-n-C_6H_{13}$	42	90	83	100	91	100	80	100	70	98	62	94	67	100	50	89	68	96
$(CH_3S)_2P(S)-S-n-C_8H_{17}$	40	72	48	96	100	100	62	100	41	100	60	90	49	90	0	47	50	82
$(CH_3S)_2P(S)-S-n-C_{10}H_{21}$	86	100	95	100	100	100	90	100	77	100	87	100	91	100	44	67	84	96
$(CH_3S)_2P(S)-S-n-C_{11}H_{23}$	32	87	73	94	91	100	81	100	44	98	66	100	75	94	0	77	58	94
$(CH_3S)_2P(S)-S-n-C_{12}H_{25}$	19	76	29	96	68	100	72	100	11	95	31	91	30	91	28	61	35	89
$CH_3S-P(S)(SC_2H_5)(SC_2H_5)$	92	95	100	100	81	90	76	92	87	97	100	100	93	93	54	62	86	91
$CH_3S-P(S)(SC_2H_5)(S-CH(CH_3)_2)$	80	100	100	80	80	80	80	100	100	100	...	...	80	100	...	...	87	93
$CH_3S-P(S)(SCH(CH_3)_2)_2$	58	76	73	96	12	82	21	83	72	87	53	75	42	78	0	38	41	77
Phygon[a]	72	83	15	60	90	100	65	87	56	88	74	89	52	80	50	71	59	82
Captan[b]	75	85	58	85	90	90	74	88	72	95	83	91	91	92	100	100	80	91
Terrachlor[c]	70	76	94	100	43	100	66	100	70	87	91	100	91	92	57	79	73	92
Ferbam[d]	68	92	53	100	90	97	31	100	59	95	26	91	56	95	79	100	58	96

[a] 2,3-Dichloro-1,4-naphthoquinone from U. S. Rubber Co.
[b] *N*-Trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide from Stauffer Chemical Co.
[c] Pentachloronitrobenzene from Olin Mathieson Chemical Corp.
[d] Ferric dimethyldithiocarbamate from E. I. du Pont de Nemours and Co., Inc.

metrical triakyl phosphorotetrathioates, $(RS)_3PS$; unsymmetrical dimethyl alkyl phosphorotetrathioates, $(CH_3S)_2P(=S)-SR$; and unsymmetrical methyl dialkyl phosphorotetrathioates, $CH_3S-P(=S)(SR)(SR')$. To compare the fungitoxicities of these compounds, the average inhibitions of eight fungi at 100 p.p.m. were used. Although there were variations between fungi, examination of the data in Table II shows that the variations generally were small and that the structure-toxicity relationships are consistent.

Figure 1 shows these structure-toxicity relationships graphically for the first two groups of compounds in Tables I and II. Curve I represents the symmetrical compounds and shows the linear relationship among CH_3, C_2H_5, and i-C_3H_7. The same superiority of branched chains over straight chains is shown again in curve 2 for the dimethyl propyl phosphorotetrathioates and the dimethyl hexyl phosphorotetrathioates. The differences here are not so exaggerated, however, because of the overwhelming effect of the two methyl groups. The increase in toxicity with the *n*-decyl side chain is not surprising, as this phenomenon occurs in many other toxicity-structure relationships. It is interesting to note, however, that there is a linear relationship with the *n*-decyl, *n*-undecyl, and *n*-dodecyl groups.

In view of the many reactions and modes of action in which these compounds could be involved it would be unwise to infer too much from these preliminary screening results. It is clear, however, that these compounds have significant biological activity, particularly those with small alkyl groups, and are worthy of further investigation.

Acknowledgment

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Literature Cited

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FUNGICIDE RESIDUES

Rapid Determination of Mercury in Apples by Modified Schöniger Combustion

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A modified Schöniger combustion of dried apple tissue replaces wet ashing prior to the determination of mercury. Loss of mercury by volatilization is eliminated in the closed combustion flask. Apple tissue is dried on cellophane overnight under vacuum, then burned in an oxygen-filled flask with a balloon attached for pressure control. Mercury is determined spectrophotometrically after extraction of the absorbing solution with dithizone. About 12 samples can be burned and analyzed in one day. Recovery of mercury from apples in the 0.3- to 0.6-p.p.m. range averaged 83.6%. Up to 0.18 p.p.m. of mercury was found in apples treated with mercurial fungicides for scab control.

MERCURIAL FUNGICIDES have been used in New York during the past three seasons for control of scab in apples. During analysis of these apples for mercury residues this laboratory was unable to recover small amounts of mercury consistently by wet-ashing procedures. Although oxidizing conditions were always maintained, mercury was lost by volatilization during acid digestion of apple tissues. Attempts to reduce this loss by the use of a low temperature initial digestion, hydrogen peroxide, selenium, and various condensing systems proved unsuccessful.

Southworth, Hodecker, and Fleischer (*4*) recently determined mercury in organic compounds by combustion in a Schöniger flask (*2, 3*). In the work reported a modified Schöniger flask is used to determine mercury in apples. Apple tissue containing organic mercury fungicides is dried on cellophane and burned in an oxygen-filled flask. The combustion products are absorbed in 0.1*N* hydrochloric acid and mercury is determined spectrophotometrically after extraction of the absorbing solution with dithizone (*1*). Loss of mercury by volatilization cannot occur in the closed system and recovery of mercury is consistently good.

Combustion Flask

The combustion flask and platinum holder are shown in Figure 1.

The flask is round-bottomed, of 5-liter capacity, and made of borosilicate glass. A 40/35 standard-taper, female ground joint is sealed on the neck. A side arm (1 cm. in inside diameter, 4 cm. long) is

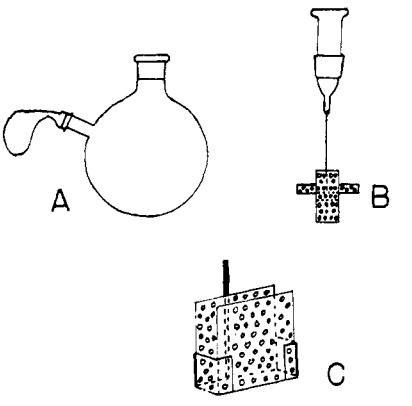

Figure 1. Combustion equipment

A. Flask
B. Platinum holder open
C. Platinum holder closed