Fungitoxicity of Organoantimony and Organobismuth Compounds

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The influence of acetone on fungal toxicity assays of organoantimony and organobismuth was determined. Significant antagonistic and synergistic solvent–compound interactions occurred when the acetone concentration exceeded 0.4% (v/v). At concentrations below 0.4% only additive responses were observed. The fungitoxicity of test compounds was therefore determined by using acetone as the carrier solvent at a final concentration of 0.1% (v/v). Trivalent organobismuth compounds were found to be the most fungitoxic. When the lipid solubility of the test compounds was increased through molecular modification, the fungitoxicities of the compounds were increased. The organoantimony compounds with the exception of diphenylantimony(III) oxine were not considered fungitoxic. The compounds were chemically stable in the test medium as shown by the comparison of substituent group fungitoxicity with the mathematically determined fungitoxicity of the substituent group. The antifungal activity of the most active compounds was compared with those of the reference fungicides benomyl and captan and found to be inferior to the former but superior to the latter.

The first organometallic fungicide, Upsulun, an organomercurial, was introducted by Bayer in 1915 (Ulfvarson, 1969). While different organomercuric compounds and formulations have been used as fungicides, many are now considered environmentally unacceptable. The antimicrobial activities of organic compounds of group IVA elements were studied extensively in the 1960s. Organic derivatives of tin, germanium, and lead were shown to be active against many fungal species, and derivatives of tetravalent metals proved to be the most toxic (Kaars Sijpesteijn et al., 1962, 1969). Studies on the antimicrobial activity of organic compounds containing VA elements, such as bismuth and antimony, are limited in number, while those involving arsenic are more numerous. This is probably related to the greater chemical stability of the latter compounds.

Beiter and Leebrick (1963) examined the activity of a series of tri- and pentavalent organoantimony and organobismuth compounds. The compounds were moderately fungitoxic with organoantimonies being more effective than organobismuths and the organobismuths being superior as antibacterial agents. Patents were issued for the use of organoantimony and organobismuth compounds as antimicrobial agents. The claims made generally related to the protection of inanimate surfaces (Gross, 1965; Leebrick, 1966a,b, 1970, 1971). One claim involved the incorporation of organobismuth compounds into products destined for use in personal hygenic formulations (Curry, 1972).

Recently it has been shown that organic solvents used as carriers for water-insoluble fungicides in bioassays play an important role in the evaluation of a compound's toxicity. Solvents and fungicides may interact with the test organism to produce additive synergistic and antagonistic responses (Burrell and Corke, 1980). Since the organometallic compounds used in this study were water insoluble, a part of this presentation deals with the influence of 2-propanone (acetone) on the fungitoxicity of one of the metallic compounds. In addition, the antifungal activities of a number of organic derivatives of trivalent as well as pentavalent antimony and bismuth are discussed.

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MATERIALS AND METHODS

Acetone–Organometallic Interactions. Because of the complex interactions that may occur when an organic solvent is used as a carrier in a toxicity bioassay, the interaction between acetone and tetraethylammonium diphenyldiazidobismuthate(III) [$Net_4BiPh_2(N_3)_2$] was examined.

The fungal isolates *Fusarium oxysporum* f. sp. *lycopersici*, and *Polyporus hirsutus* were tested with 2 μg/mL while *Pestalotia* sp. and *Sclerotinia homeocarpa* were tested with 8 μg/mL $NEt_4BiPh_2(N_3)_2$ in potato dextrose agar medium (Difco) containing levels of acetone varying from 0 to 1.0% (v/v). All control and experimental plates were in replicates of five, and colony diameters were measured after 48- or 72-h incubation at 30 °C. The percent inhibition of fungal growth on medium supplemented with the range of acetone concentrations was calculated with respect to growth on control agar plates (no acetone or test compound). The percent inhibition of growth due to the compound with the various levels of acetone was calculated also from growth on control plates. The net fungicide effect was derived from the data of inhibition of growth of the test compound plus acetone with respect to the fungal growth in the corresponding solvent control plates. All data were plotted as the logarithm of the solvent concentration vs. the percent inhibition. The detailed procedures for this type of interaction study have been described previously (Burrell and Corke, 1980; Burrell et al., 1980).

Fungitoxicity of Experimental Compounds. The test compounds listed in the tables were dissolved in acetone, forming stock solutions which were added as 0.1-mL aliquots to 100-mL volumes of potato dextrose agar to yield final concentrations of 0.1, 1.0, 10, 15, and 30 μg/mL. The final acetone level in the medium was 0.1% (v/v). Plates were incubated at 30 °C, a temperature at which good growth was obtained for all test cultures. Percent inhibition was determined after 48- or 72-h incubation with respect to growth on medium containing only 0.1% acetone (v/v). All data were plotted as the logarithms of compound concentration vs. percent inhibition, and EC_{50} values were calculated.

Two fungicides, benomyl [methyl 1-(butylcarbamoyl)-benzimidazol-2-ylcarbamate] and captan [*N*-[(trichloromethyl)thio]cyclohex-4-ene-1,2-dicarboximide] were assayed by using the technique described above.

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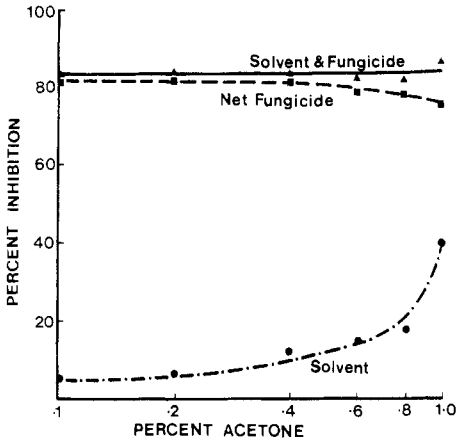

Figure 1. Additive interaction between *P. hirsutus*, acetone (0.1–1.0% v/v), and 2 μg/mL $NEt_4BiPh_2(N_3)_2$ grown on potato dextrose agar at 30 °C for 72 h.

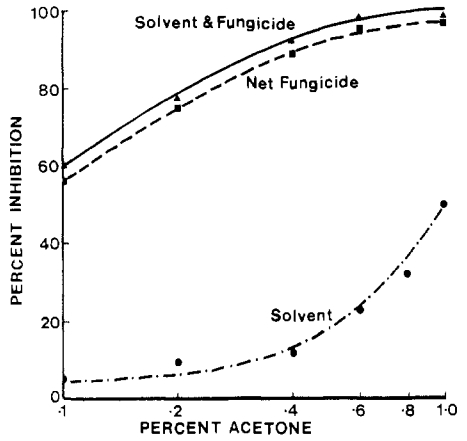

Figure 2. Synergistic interaction between *S. homeocarpa*, acetone (0.1–1.0% v/v), and 8 μg/mL $NEt_4BiPh_2(N_3)_2$ grown on potato dextrose at 30 °C for 48 h.

Test Compounds. The organoantimony and organobismuth compounds were synthesized by methods described by Goel and co-workers (Goel and Prasad, 1970, 1979; Goel and Ridley, 1972). The compounds had a chemical purity greater than 95% and were in crystalline form. The chemical compounds were stored at 4 °C.

RESULTS AND DISCUSSION

The interaction data from experiments with acetone and $NEt_4BiPh_2(N_3)_2$ are summarized in Figures 1, 2, and 3 for *P. hirsutus*, *S. homeocarpa*, and *F. oxysporum*, respectively. It is to be noted that acetone was toxic to all three fungi. Many researchers employ this solvent at 1% (v/v) in bioassays (Edgington et al., 1971; Manten et al., 1950), and in spite of its significant effect on growth at this concentration, the solvent effect is not taken into consideration in the interpretation of toxicity data.

All possible interactions between the solvent and the test compound were observed with the four test fungi. An additive response occurred with *P. hirsutus* (Figure 1). This was concluded since there was no statistically significant difference ($P = 0.02$) in the net fungicide percent inhibition (about 80%) values for 2 μg/mL of the test substance over the range of acetone concentrations tested (0.1–1.0%). A synergistic response occurred with *S. homeocarpa* as shown by the significant increase ($P = 0.02$) in inhibition from 60% with 0.1% acetone to about 98% with 1% acetone, with 8 μg/mL of the test compound (Figure 2). An antagonistic interaction occurred with *F. oxysporum* (Figure 3), and this was defined by the significant ($P = 0.02$) reduction in the net fungicide effect from 30% inhibition with 0.1% acetone to 18% inhibition with 1% acetone. *Pestalotia* sp. also yielded an antagonistic interaction in this bioassay.

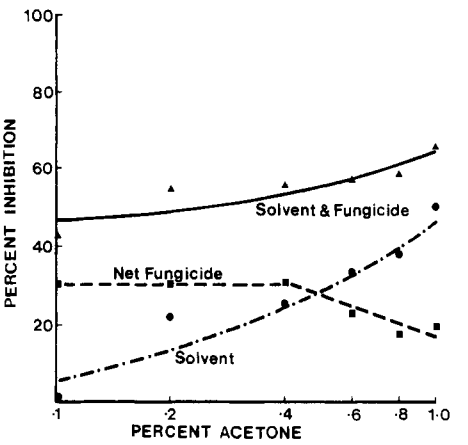

Figure 3. Antagonistic interaction between *F. oxysporum*, acetone (0.1–1.0% v/v), and 2 μg/mL $NEt_4BiPh_2(N_3)_2$ grown on potato dextrose agar at 30 °C for 72 h.

These interaction responses were observed in the study of solvent–fungicide combinations reported by Burrell and Corke (1980). In all assays performed, additive responses between the test compound and 0.1% acetone occurred, and therefore this level of solvent was considered appropriate for studies of toxicity of both the bismuth and antimony compounds considered below.

Organoantimony Compounds. The EC_{50} values obtained with the organoantimony compounds for the test fungi are summarized in Table I. Only 3 of the 15 compounds listed had EC_{50} values less than 30 μg/mL. Diphenylantimony(III) chloride (Ph_2SbCl) and diphenylantimony(III) acetylacetonate [$Ph_2Sb(acac)$] had EC_{50} values of 20 ± 1.8 and 24 ± 2.0 μg/mL against *F. oxysporum*. The compound diphenylantimony(III) oxinate [$Ph_2Sb(oxine)$] was the most effective organoantimony derivative tested.

The toxicity of $Ph_2Sb(oxine)$ and the free oxine (8-hydroxyquinoline) was compared. In addition, the EC_{50} value of $Ph_2Sb(oxine)$ was recalculated based upon its oxine content and is indicated as $Ph_2Sb(oxine)$-oxine (Table II). The activity of $Ph_2Sb(oxine)$-oxine approximated closely that of oxine with three of the test fungi but not with *P. hirsutus*. This differential toxicity with *P. hirsutus* provided indirect evidence that the $Ph_2Sb(oxine)$ molecule was not dissociated, or hydrolyzed, and the observed toxicity probably was due to the entire molecule.

Organobismuth Compounds. The toxicity data for selected organobismuth compounds are presented in Table III and IV. Triphenylbismuth(III) and the four triphenylbismuth(V) derivatives showed limited toxicity, although Ph_3Bi, Ph_3BiCl_2, and $Ph_3Bi(CH_3,cOO)_2$ yielded EC_{50} values of less than 30 μg/mL with *S. homeocarpa*. The compound $NEt_4BiPh_2Cl_2$ [tetraethylammonium diphenyldichlorobismuthate(III)] gave EC_{50} values less than 30 μg/mL with all fungi tested, while the cationic group NEt_4^+, supplied as the chloride salt (NEt_4Cl) was not as inhibitory. Diphenylbismuth(III) cyanide (Ph_2BiCN) and the phenylbismuth dichloride–dipyridine complex ($PhBiCl_2Py_2$) were also inhibitory to all fungi.

The effects of the azide-substituted compounds on fungal growth are summarized in Table IV. The reference compound sodium azide caused complete inhibition at less than 0.57 μg/mL. Diphenylbismuth(III) azide (Ph_2BiN_3) exhibited low antifungal activity with EC_{50} values of 30

Table I. Fungitoxicity of Organoantimony Compounds Determined for Four Fungi Grown on Potato Dextrose Agar Incubated at 30 °C for 72 Hours

compounds	EC_{50} values, μg/mL, for fungi			
	F. oxysporum	*Pestalotia* sp.	*P. hirsutus*	*S.*[b] *homeocarpa*
Ph_2SbCl	20 ± 1.8	>30	>30	>30
$Ph_2Sb(CH_3COO)$	>30	>30	>30	>30
$Ph_2Sb(acac)$[a]	24 ± 2.0	>30	>30	>30
Ph_2Sb(oxine)	12 ± 0.8	1.8 ± 0.2	15 ± 0.6	1.8 ± 0.2
Ph_3Sb	>30	>30	>30	>30
Ph_3SbCl_2	>30	>30	>30	>30
$Ph_3Sb(CH_3COO)_2$	>30	>30	>30	>30
$Ph_3Sb(CH_2ClCOO)_2$	>30	>30	>30	>30
$Ph_3Sb(CHCl_2COO)_2$	>30	>30	>30	>30
$Ph_3Sb(CCl_3COO)_2$	>30	>30	>30	>30
$(CH_3)_3SbCl_2$	>30	>30	>30	>30
$(CH_3)_3Sb(CH_3COO)_2$	>30	>30	>30	>30
$(CH_3)_3Sb(CH_2ClCOO)_2$	>30	>30	>30	>30
$(CH_3)_3Sb(CHCl_2COO)_2$	>30	>30	>30	>30
$(CH_3)_3Sb(CCl_3COO)_2$	>30	>30	>30	>30

[a] Diphenylantimony(III) acetylacetonate. [b] 48-h incubation period.

Table II. EC_{50} Values for Ph_2Sb(oxine) and Oxine Determined for Four Fungi Grown on Potato Dextrose Agar Incubated at 30 °C for 72 Hours

compounds	EC_{50} values, μg/mL, for fungi			
	F. oxysporum	*Pestalotia* sp.	*P. hirsutus*	*S.*[b] *homeocarpa*
Ph_2Sb(oxine)	12 ± 0.8	1.8 ± 0.2	15 ± 0.6	1.8 ± 0.2
oxine	3.6 ± 0.1	0.48 ± 0.006	>30	0.45 ± 0.007
Ph_2Sb(oxine)-oxine[a]	4.13	0.62	5.1	0.62

[a] EC_{50} values calculated as micrograms of oxine per milliliter from the molecule Ph_2Sb(oxine). [b] 48-h incubation period.

Table III. EC_{50} Values of Organobismuth Compounds Determined for Four Fungi Grown on Potato Dextrose Agar Incubated at 30 °C for 72 Hours

compounds	EC_{50} values, μg/mL, for fungi			
	F. oxysporum	*Pestalotia* sp.	*P. hirsutus*	*S.*[a] *homeocarpa*
Ph_3Bi	>30	>30	>30	20 ± 1.0
Ph_3BiCl_2	>30	>30	>30	22 ± 0.9
$Ph_3Bi(CH_3COO)_2$	>30	>30	>30	28 ± 1.2
$Ph_3Bi(CH_2ClCOO)_2$	>30	>30	>30	>30
$Ph_3Bi(CHCl_2COO)_2$	>30	>30	>30	>30
$PhBiCl_2Py_2$	7.5 ± 0.4	7.0 ± 0.3	28 ± 1.6	12.0 ± 0.9
Ph_2BiCN	15 ± 0.8	5.5 ± 0.07	17 ± 0.9	15 ± 0.7
$NEt_4BiPh_2Cl_2$	17.0 ± 1.2	10.0 ± 0.5	22.0 ± 2.3	16.0 ± 1.8
NEt_4Cl	>30	>30	>30	>30

[a] 48-h incubation period.

Table IV. Toxicity of Ph_2BiN_3, $NEt_4BiPh_2(N_3)_2$, and NaN_3 Determined for Four Fungi Grown on Potato Dextrose Agar Incubated at 30 °C for 72 Hours

compounds	EC_{50} values, μg/mL, for fungi			
	F. oxysporum	*Pestalotia* sp.	*P. hirsutus*	*S.*[c] *homeocarpa*
Ph_2BiN_3	>30	>30	>30	>30
$NEt_4BiPh_2(N_3)_2$	1.8 ± 0.04	7.5 ± 0.05	10 ± 0.04	2.6 ± 0.02
$NEt_4BiPh_2(N_3)_2$-N_3[a]	0.26	1.09	1.45	0.38
NaN_3[b]	<0.57	<0.57	<0.57	<0.57

[a] EC_{50} values calculated as micrograms of N_3^- ion per milliliter from the complex $NEt_4BiPh_2(N_3)_2$. [b] Calculated as the maximum concentration of NaN_3 possible if total ionization and volatilization occurred. This amount caused 100% kill. [c] 48-h incubation period.

Table V. EC_{50} Values for Organoantimony Compounds and Organobismuth Compounds Compared to Those of the Reference Fungicide Benomyl and Captan for Four Fungi Grown on Potato Dextrose Agar Incubated at 30 °C for 72 Hours

compounds	EC_{50} values, μg/mL, for fungi			
	F. oxysporum	*Pestalotia* sp.	*P. hirsutus*	*S.*[a] *homeocarpa*
Ph_2Sb(oxine)	12 ± 0.8	1.8 ± 0.2	15 ± 0.6	1.8 ± 0.2
$NEt_4BiPh_2(N_3)_2$	1.8 ± 0.4	7.5 ± 0.05	10 ± 0.04	2.6 ± 0.02
$NEt_4BiPh_2Cl_2$	17 ± 1.2	10 ± 0.5	22 ± 2.3	16 ± 1.8
Ph_2BiCN	15 ± 0.8	5.5 ± 0.07	17 ± 0.9	15 ± 0.7
$PhBiCl_2Py_2$	7.5 ± 0.4	7.0 ± 0.3	28 ± 1.6	12 ± 0.9
benomyl	1.05 ± 0.02	0.09 ± 0.003	3.0 ± 0.14	0.22 ± 0.02
captan	>30	7.6 ± 0.4	10.6 ± 0.8	17.8 ± 1

[a] 48-h incubation period.

μg/mL. When this molecule was modified by the addition of a tetraethylammonium moiety and a second N_3^- ion to increase lipid solubility, antifungal activity was greatly increased. The EC_{50} values for tetraethylammonium diphenyldiazidobismuthate(III) calculated on the basis of N_3^- ion content are shown in Table IV. These data were further evidence of the stability of the organometallic compounds in the test system.

GENERAL DISCUSSION

The organometallic compounds with the greatest fungitoxicity are listed in Table V with the EC_{50} values for the reference fungicides benomyl and captan. A fungicidal ranking of the compounds based upon the spectrum of fungal genera inhibited and individual toxicity is benomyl > $NEt_4BiPh_2(N_3)_2$ > Ph_2Sb(oxine) > $PhBiCl_2Py_2$ > captan > $NEt_4BiPh_2Cl_2$. Venugopal and Luckey (1978) reported that compounds of trivalent antimony were much more toxic to mammals than the pentavalent derivatives. Kaars Sijpesteijn et al. (1969) indicated that compounds of trivalent antimony and bismuth were more toxic to microbes than those of pentavalent elements. In this study the trivalent organometallic compounds also were more inhibitory to fungi than the pentavalent compounds (Table V). Very few pentavalent organobismuths have been patented while several trivalent organobismuth compounds were patented for use as antifungal agents (Gross, 1965; Leebrick, 1966a,b; Curry, 1972).

Four of the five tested organometallics that were considered fungitoxic were bismuth preparations. This suggests that fungal toxicity increased with increasing molecular weight of the organic compounds containing group VA elements. This is in contrast to Beiter and Leebrick's (1963) results which indicated that organoantimony derivatives were slightly more fungitoxic than organobismuth compounds. Kaars Sijpesteijn et al. (1969) found that Ph_2BiCl and $PhBiCl_2$ were far more active against test fungi than reported by Beiter and Leebrick (1963). This result, in conjunction with the data presented here, indicates that the organobismuth derivatives are more fungitoxic than the corresponding organoantimony compounds. The increased fungitoxicity with increased molecular weight of organic compounds containing group VA elements was the inverse of mammalian oral toxicity (Venugopal and Luckey, 1978), suggesting that organobismuth preparations may be safer to use than organoantimonies with respect to nontarget mammals, and future research on these compounds should be directed accordingly.

Registry No. $NEt_4BiPh_2(N_3)_2$, 69786-40-9; Ph_2SbCl, 2629-47-2; Ph_2Sb(acac), 83364-11-8; Ph_2Sb(oxine), 28854-28-6; oxine, 148-24-3; Ph_3Bi, 603-33-8; Ph_3BiCl_2, 594-30-9; $Ph_3Bi(CH_3COO)_2$, 7239-60-3; $NEt_4BiPh_2Cl_2$, 69786-33-0; Ph_2BiCN, 41083-18-5; $PhBiCl_2Py_2$, 83364-12-9; Ph_2BiN_3, 20157-68-0; Ph_3Sb, 603-36-1; Ph_3SbCl_2, 594-31-0; $Ph_3Sb(CH_3COO)_2$, 1538-62-1; $Ph_3Sb(CH_2ClCOO)_2$, 36971-71-8; $Ph_3Sb(CHCl_2COO)_2$, 36971-70-7; $Ph_3Sb(CCl_3COO)_2$, 83434-46-2; $(CH_3)_3SbCl_2$, 13059-67-1; $(CH_3)_3Sb(CH_3COO)_2$, 10446-33-0; $(CH_3)_3Sb(CH_2ClCOO)_2$, 83434-47-3; $(CH_3)_3Sb(CHCl_2COO)_2$, 83434-48-4; $(CH_3)_3Sb(CCl_3COO)_2$, 38172-36-0; $Ph_3Bi(CH_2ClCOO)_2$, 61217-41-2; $Ph_3Bi(CHCl_2COO)_2$, 83434-49-5; NEt_4Cl, 56-34-8; NaN_3, 26628-22-8; $Ph_2Sb(CH_3COO)$, 5613-52-5; acetone, 67-64-1; benomyl, 17804-35-2; captan, 133-06-2.

LITERATURE CITED

Beiter, C. B.; Leebrick, J. R. *Proc. Annu. Meet., Chem. Spec. Manuf. Assoc.* **1963**, *49*, 132.
Burrell, R. E.; Corke, C. T. *Bull. Environ. Contam. Toxicol.* **1980**, *25*, 554.
Burrell, R. E.; Stratton, G. W.; Corke, C. T. *Can. Tech. Rep. Fish. Aquat. Sci* **1980**, *975*, 123.
Curry, J. D. British Patent 1 341 331, 1972.
Edgington, L. V.; Khew, K. L.; Barron, G. L. *Phytopathology* **1971**, *61*, 42.
Goel, R. G.; Prasad, H. S. *Can. J. Chem.* **1970**, *48*, 2488.
Goel, R. G.; Prasad, H. S. *Spectrochim. Acta, Part A* **1979**, *35A*, 339.
Goel, R. G.; Ridley, D. R. *J. Organomet. Chem.* **1972**, *38*, 83.
Gross, F. J. U.S. Patent 3 197 314, 1965.
Kaars Sijpesteijn, A.; Luijten, J. G. A.; Van der Kerk, G. J. M. In "Fungicides"; Torgeson, D. C., Ed; Academic Press: New York, 1969; Vol. II, Chapter 7.
Kaars Sijesteijn, A.; Rijkens, F.; Loijten, J. G. A.; Willemsens, L. C. *Antonie van Leeuwenhoek* **1962**, *28*, 346.
Leebrick, J. R. U.S. Patent 3 239 411, 1966a.
Leebrick, J. R. U.S. Patent 3 247 050, 1966b.
Leebrick, J. R. U.S. Patent 3 530 158, 1970.
Leebrick, J. R. U.S. Patent 3 558 783, 1971.
Manten, A.; Klopping, H. L.; Van der Kerk, G. J. M. *Antonie van Leeuwenhoek* **1950**, *16*, 282.
Ulfvarson, V. In "Fungicides"; Jorgeson, D. C., Ed.; Academic Press: New York, 1969; Vol. II, Chapter 6.
Venugopal, B.; Luckey, T. D. "Metal Toxicity in Mammals"; Plenum Press: New York, 1978; p 205.

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