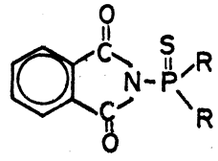
Fungicidal Phthalimidophosphonothionates

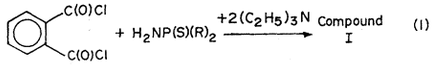
Abstract. *The fungicidal activity, mammalian toxicity, and methods of synthesis of phthalimidophosphonothionates, a novel type of organophosphoramide compound, have been investigated. The findings led to development of the compound diethyl phthalimidophosphonothionate as a new bioproduct. This diester protects a variety of crops from certain plant diseases, such as powdery mildew, apple scab, leaf spot, brown rot, and black spot.*

Results of an investigation of the polarizability of the phosphoramidothionate group led us into a study of the nucleophilicity of this group toward carbon (*1*). During this work we were able to synthesize some new types of structure, the availability of which made it possible to test the biological activity of *N*-acylated phosphoramides (*2–4*). These developments resulted in the discovery of the rather remarkable biological activity of a novel type of peracylated amide, the phthalimidophosphonothionates, of structure I (*4, 5*).



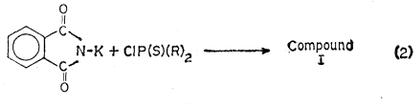
Compound I

The methods we used in the preparation of these compounds involved reactions 1, 2, and 3, the conditions of which have been described (*2, 5, 6*). The novel reaction 1 emerged from the fundamental studies mentioned above that first provided us with compounds of structure I.



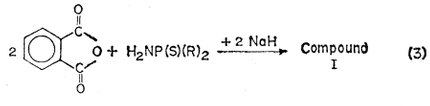
(1)

with the loss of $2(C_2H_5)_3N{\cdot}HCl$,



(2)

with the loss of KCl, and

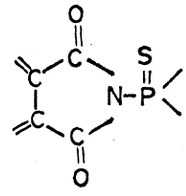


(3)

with loss of $2H_2$ and $C_6H_4(COONa)_2$.

The symmetric structure assigned to I was proved by the following facts. The main products obtained from all three reactions for a given pair of R-groups were identical with each other in regard to their physical and spectroscopic characteristics and their chemical reactivity (*6, 7*). They were also identical with the main product formed in reaction 1 if phthaloyl chloride was replaced by its unsymmetric isomer, *as*-phthalyl chloride. Infrared spectra of compounds with structure I contained two main bands that correspond to the two molecular vibrations involving symmetric and antisymmetric stretching of the carbonyl group. The same bands were also observed in the Raman spectrum where, as was to be expected, their intensities were reversed. For the compound containing ethoxyl groups in place of the R-groups, bands produced by symmetric and antisymmetric carbonyl stretching were near 1785 cm^{-1} and 1740 cm^{-1}, respectively. These data indicated the presence of a phthalimide group in compounds with structure I. Proton magnetic resonance data provided additional evidence for the symmetric structure of I.

Compounds of structure I possess a combination of useful biological properties that is unique among the variety of organophosphorus compounds synthesized previously, namely, high fungicidal activity and very low toxicity to mammals. Results obtained from an extensive study strongly indicate that this combination is tied in with the *N*-phosphorothionylated dicarboximide moiety (II), present in compounds with structure I (*5*). Substitution of oxygen for sulfur at phosphorus or insertion of a methylene group between nitrogen and phosphorus destroys fungicidal activity. Insertion of an oxygen or sulfur atom between those two atoms has the same effect and also markedly increases toxicity to mammals (*3*). Fungicidal activity is greatly reduced if the carbon atoms connecting the carbonyl groups are tetrahedral.



Compound II

According to these results, the presence of an *N*-phosphorothionylated phthalimido moiety in I was essential for obtaining the desired combination of good fungicidal activity with low toxicity to mammals. Variation of fungicidal activity, particularly the control of powdery mildew and late blight, with compounds of structure I revealed the following. An unsubstituted phthalimido group in I was more effective than a ring-substituted phthalimido group and far more effective than a nonaromatic dicarboximide group. In regard to the R-groups, a variety of aliphatic and aromatic substituents were investigated; most effective were alkoxyl and alkylthiolyl groups that consisted of unbranched carbon chains with fewer than four carbon atoms. Maximum activity was observed with C_2H_5O groups, n-C_3H_7O groups, or C_2H_5S groups

Table. 1 Activity of diethyl phthalimidophosphonothionate (IA).

Crop	Disease	Spray conc. of IA (% by wt.)	Rating of disease control
Apple	Apple scab *(Venturia inaequalis)*	0.03 to 0.06	Good
	Powdery mildew *(Podosphaera leucotricha)*	.023 to .045	Excellent
	Frogeye leaf spot *(Physalospora malorum)*	.045	Excellent
Red cherry	Leaf spot *(Coccomyces hiemalis)*	.03 to .06	Good
	Powdery mildew *(Podosphaera oxyacanthae)*	.023 to .045	Excellent
Peach	Brown rot *(Monilinia fructicola)*	.03 to .045	Good
	Rhizopus nigricans	.03 to .045	Excellent
Cucumber squash	Powdery mildew *(Erysiphe cichoracearum)*	.015 to .03	Excellent
Rose	Black spot *(Diplocarpon rosae)*	.03 to .06	Good
	Powdery mildew *(Sphaerotheca humuli)*	.015 to .03	Excellent
Turf	Powdery mildew *(Erysiphe graminis)*	.023 to .045	Good

in place of R in compound I. The *O,O*-diethyl compound was found to give complete control of powdery mildew and late blight at concentrations of about 10 and 200 parts per million (ppm), respectively; the *O,O*-di-*n*-propyl ester was somewhat more effective against these diseases. The *S,S*-diethyl compound, at a concentration of 150 ppm, almost completely controlled late blight but had no effect on powdery mildew.

Fungicidal activity was tested in the laboratory by spraying test plants to run-off with the formulated test chemicals. Plants were allowed to dry for 3 to 4 hours and were then inoculated with viable spores of the fungus either by spraying with a spore suspension or by direct contact with leaves of infected plants. Disease was allowed to develop in the proper environment for 5 to 7 days before readings on activity were taken.

The overall result of our investigation was the emergence of two potential fungicides, IA (R is OC_2H_5; m.p., 83° to 84°C) and IB (R is OC_3H_7; m.p., 53° to 54°C). Both were white crystalline substances of low toxicity to mammals. Compound IB, however, was about three times as toxic to mammals as was IA, which showed an LD_{50} (lethal dose, 50 percent effective) of 5600 mg/kg when administered orally to male rats.

Field experiments showed that the diethyl ester IA consistently controlled a variety of diseases satisfactorily (Table 1). Concentrations of the spray given in Table 1 were for protective spray schedules in which applications were made every 7 to 14 days, depending on the type of disease to be controlled. At these concentrations the diethyl ester IA is safe on both fruit and foliage.

HENRY TOLKMITH
H. O. SENKBEIL
Edgar C. Britton Research Laboratory, Dow Chemical Company, Midland, Michigan
DORSEY R. MUSSELL
Bioproducts Department, Dow Chemical Company

References and Notes

1. H. Tolkmith, *Ann. N.Y. Acad. Sci.* **79**, 187 (1959); *J. Amer. Chem. Soc.* **84**, 2097 (1962); *ibid.* **85**, 3246 (1963).
2. ———, U.S. patent application filed.
3. ———, *Ann. N.Y. Acad. Sci.* **136**, 59 (1966).
4. ———, *Nature* **211**, 522 (1966).
5. ——— and H. O. Senkbeil, Belgian patent No. 661,891 (1965).
6. D. W. Osborne, H. O. Senkbeil, J. L. Wasco, *J. Org. Chem.* **31**, 192 (1966).
7. D. W. Osborne, *ibid.*, p. 197.

22 September 1966

Diagnosis of Gaucher's Disease and Niemann-Pick Disease with Small Samples of Venous Blood

Abstract. *Enzymes which catalyze the hydrolysis of glucocerebroside and sphingomyelin have been demonstrated in preparations of washed human white blood cells. The level of activity of these respective enzymes is markedly decreased in leukocyte preparations obtained from patients with Gaucher's and Niemann-Pick diseases. Assay of these enzymes may be useful in the differential diagnosis of the sphingolipidoses.*

The metabolic defect in Gaucher's disease has been shown to be a deficiency of the enzyme which catalyzes the cleavage of glucose from glucocerebroside, the substance which accumulates in various tissues in patients with this disease (*1*). In like manner, the metabolic lesion in Niemann-Pick disease is a deficiency of the enzyme which catalyzes the hydrolysis of phosphorylcholine from sphingomyelin which accumulates in Niemann-Pick disease (*2*). These enzymes are present in a number of tissues in the body (*3*), and there appears to be a generalized attenuation of these enzymes in several organs in patients with these diseases (*2, 4*). Since human white blood cells contain a number of hydrolytic enzymes (*5*), it was considered of interest to develop methods for determining the quantity of glucocerebroside-cleaving enzyme and sphingomyelin-cleaving enzyme in human leukocytes. We now describe procedures for determining the activity of these sphingolipid hydrolases in human leukocytes. Our data show that the activity of these enzymes in leukocyte preparations from patients with Gaucher's disease and Niemann-Pick disease is very much lower than in preparations from normal human beings and patients with other diseases.

Glucocerebroside labeled with ^{14}C in the D-glucose portion of the molecule was prepared as described (*6*). Sphingomyelin was labeled with ^{14}C in the methyl carbon atoms of the choline portion of the molecule (*3*). Leukocytes were separated from erythrocytes by differential sedimentation in the following fashion. Ten milliliters of venous blood were added to a test tube containing 2 ml of a solution containing per 100 ml: 5 g of dextran, 0.7 g of sodium chloride, and 50 mg of heparin. The contents were mixed, and the red blood cells were allowed to settle (45 minutes at room temperature). The plasma containing the suspended leukocytes was removed with a capillary pipette, and the suspension was centrifuged for 10 minutes at 600*g*. The supernatant was discarded and the leukocyte pellet was suspended and washed twice with 0.85 percent sodium chloride solution. The white blood cells were suspended in a fresh portion of this isotonic sodium chloride, and the cells were counted with a Coulter counter. The suspensions were adjusted with saline so that they contained from 20,000 to 60,000 leukocytes per microliter. The incubation mixtures for determination of glucocerebroside-cleaving enzyme activity contained 50 to 100-μl portions of the leukocyte suspension, 15 μmole of potassium phosphate buffer (*p*H 6.0), 300 μg of sodium cholate, 200 μg of Cutscum (isooctylphenoxypolyoxyethanol, Fisher Chemical Co.), and 125

Table 1. Glucocerebroside-cleaving enzyme in human white blood cells. One unit of enzymatic activity is defined as the amount of enzyme required to catalyze the hydrolysis of 1 mμmole of glucocerebroside per hour under the conditions of incubation described.

Age (yr)	Sex	Units of enzymatic activity: Per mg of protein	Per 10^9 leukocytes
		Normal individuals	
40	F	3.4	480
20	F	3.7	440
32	M	2.7	530
48	M	4.1	550
39	M	3.8	480
37	M	4.2	420
31	M	3.4	530
17	M	3.4	610
21	M	6.2	640
31	F	4.6	690
48	F	3.1	590
		Familial hyperlipoproteinemia Type II	
17	M	3.8	490
32	M	2.8	450
		Tangiers disease	
12	F	3.3	490
		Refsuns syndrome	
27	F	3.0	450
28	M	3.0	480
		Niemann-Pick disease	
18	M	3.4	400
3	M	4.4	460
		Lipidosis of unknown etiology	
8	F	3.4	410
8	M	3.1	380
	Mean ± SE	3.64 ± .18	498 ± 18
		Gaucher's disease	
40	F	.47	120
48	M	.77	162
47	F	.78	119
10	M	.46	68
14	M	.57	68
	Mean ± SE	.61 ± .09	107 ± 18
	P	<.001	<.001