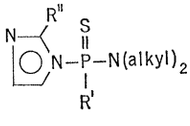
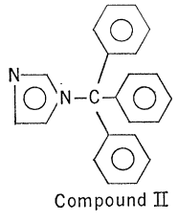
Imidazole: Fungitoxic Derivatives

Abstract. *Study of several new types of fungitoxic derivatives of imidazole reveals that imidazoles substituted on the imine nitrogen atom are likely to be active if the substituent is electron-attracting, and if the atom connecting it to the imidazolyl moiety has tetrahedral geometry. Fungitoxicity is high with phosphinamidothionate and triarylmethyl groups as substituents. The presence of an asymmetric phosphorus atom in the substituent has no effect on fungitoxicity, but affects mammalian toxicity.*

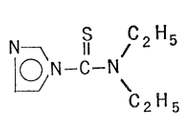
Our discovery of the rather remarkable biological properties of *O,O*-dialkyl phthalimidophosphonothionates (*1*, *2*) prompted some theoretical speculations leading to a study of various phosphorus derivatives of imidazole. We found that imidazol-l-yl-phosphinamidothionates (I) generally were quite active as foliage fungicides. The most interesting member of this novel class of biologically active compounds was *N,N*-diethyl imidazol-l-yl phenylphosphinamidothionate (Ia; R′, phenyl; R″, hydrogen; alkyl, ethyl), a white, crystalline product (melting point, 43°C) having marked resistance to nucleophilic attack and to hydrolysis in nonacidic media; it showed high fungitoxicity, low mammalian toxicity, and very little anticholinergic activity (*3*). For example, Ia controlled *Erysiphe cichoracearum* on cucumbers and *Phytophthora infestans* on potato plants at concentrations of 10 and 18 parts per million (ppm), respectively; its LD_{50} (lethal dose, 50 percent effective) was about 1000 mg/kg when administered orally to rats (*4*). Its oxygen analog was less than one-tenth as active fungicidally, somewhat more toxic to mammals (LD_{50}, about 750 mg/kg orally on rats), and mildly anticholinergic.



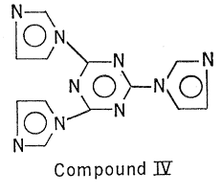
Compound I



Compound II



Compound III



Compound IV

In view of these findings it appeared doubtful that the fungitoxicity of I was dependent on the phosphorylation ability of imidazol-l-yl phosphinamidothionates; fungitoxic action seemed more likely to result from the nucleophilicity of I, that is, from the power of the azole nitrogen of the imidazolyl group to attack an electrophilic site in the fungus organism by donating electrons to this site. Such a concept of the mechanism of fungitoxicity of I would resemble the concept that the function of biologically important imidazole compounds—like *N*-acetylimidazole, dihydrogen imidazol-l-yl phosphonate, and histidine—is intimately related to the electron-donating power of their azole nitrogens. According to molecular-orbital calculations, this power is but little affected by the nature of the substituent at the other nitrogen atom of imidazole (*5*). Therefore our hypothesis on the fungitoxic action of I would imply that replacement of the entire phosphinamidothionate group in this structure by a phosphorus-free substituent, of equivalent stereoelectronic nature, should not drastically change fungitoxicity. This conclusion was examined by testing the fungitoxic behavior of II, III, and IV; the results appear in Table 1.

We found that III and IV were markedly less fungicidal than Ia, while II was nearly as active as this reference compound (*6*, *7*). In addition, *N*-trityl-imidazole (II; colorless needles; m.p., 226° to 228°C) showed moderate mammalian toxicity (LD_{50}, about 800 mg/kg orally on rats) and had a hydrolytic stability that was intermediate between the stabilities of *N*-acetylimidazole and Ia. The proton magnetic resonance spectra of II were consistent with the stucture assigned to this compound, and showed a complex splitting pattern, with a coupling constant of 1 to 2 cycles for both the phenyl protons and the three protons of the imidazole ring.

The results obtained with II prove that the presence of a phosphinamidothionate group at the imine nitrogen atom of imidazole, as in I, is not in fact critical for high fungicidal activity. Moreover, it should be noted that the exocyclic atoms connected to the imine nitrogen atoms in I and II (that is, the phosphorus atom and the central carbon atom of the trityl group, respectively) possess tetrahedral geometry. In contrast, the less-active compounds III and IV contain nearly planar, exocyclic carbon atoms at their imine nitrogens. Thus it appears that with regard to fungitoxicity the geometry of the exocyclic atom at the imine nitrogen atom of imidazole is more important than the chemical nature of the attached atom. Evidently imidazoles substituted at the imine nitrogen atom are apt to be fungicidal if the substituent is to a certain degree electron-attracting, and if the atom connecting it to the imidazolyl moiety is essentially sp^3-hybridized.

This theoretical aspect focused our attention on the possible effect that an asymmetrically substituted tetrahedral atom, at the imine nitrogen atom of imidazole, may have on biological activity; that is to say, the enantiomeric forms of the toxicant could differ in activity. A study of this question appeared to be particularly interesting with I, and a fundamentally new method of resolution made the individual optical isomers of this structure available (*8*). The enantiomers of Ib (R′, phenyl; R″, methyl; alkyl, ethyl) proved to be sufficiently stable for biological testing, and showed no difference in activity against the phytopathogens named in Table 1. Their physical characteristics and acute oral toxicity to white mice accorded with those in Table 2. Compound Ib is evidently the first thionophosphorus compound with which a difference in mammalian toxicity between its optical antipodes has been demonstrated; such difference is known with some asymmetric phosphoryl compounds (*9*).

The biological behavior of I is con-

Table 1. Minimum concentrations (ppm) of derivatives of imidazole necessary for complete control of two phytopathogens.

Compound	*Erysiphe cichoracearum*	*Phytophthora infestans*
Ia	10	18
II	>10	~37
III	150	>150
IV	>150	~75

Table 2. Optical activity and mammalian toxicity (to white mice) of *N,N*-diethyl-2-methylimidazol-1-yl phenylphosphinamidothionate (Ib).

Optical form	$[\alpha]_D^{25}$ in $CHCl_3$ (deg)	LD_{50} (mg/kg)
Racemate		147
d-Isomer	+7.9	316
l-Isomer	−9.3	147

sistent with the hypothesis that the phosphorylation ability of this type of compound is not essential regarding fungitoxicity, but is important in mammalian toxicity. The findings that certain organic phosphoramidothionates, such as I and the phosphonothionates mentioned in the opening sentence of this report, show high fungitoxicity without being effective phosphorylating agents, and that they are less active when converted to their oxygen analogs (phosphoramidates), represent fundamentally new results in the area of pesticidal organophosphorus compounds.

Addendum: Compounds of structures I and IV were prepared by reacting the corresponding intermediates, *N,N*-dialkyl phenylphosphonamidochloridothionate and cyanuric chloride, with imidazole in the presence of a tertiary base as HCl-acceptor (*6, 10*). Compounds of structures II and III were prepared similarly by reacting the respective intermediates, trityl chloride and diethylthiocarbamoyl chloride, with imidazole (*11*). The fungicidal and toxicological test methods used have been reported (*2, 12*).

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References and Notes

1. H. Tolkmith, *Ann. N.Y. Acad. Sci.* **136**, 59 (1966); *Nature* **211**, 522 (1966).
2. ———, H. O. Senkbeil, D. R. Mussell, *Science* **155**, 85 (1967).
3. H. Tolkmith, P. B. Budde, D. R. Mussell, R. A. Nyquist, *J. Med. Chem.*, in press.
4. H. Tolkmith and D. R. Mussell, *World Rev. Pest Control,* in press.
5. B. Pullman and A. Pullman, *Proc. Nat. Acad. Sci. U.S.* **44**, 1197 (1958); *Quantum Biochemistry* (Interscience, New York, 1963); A. Szent-Györgyi, *Introduction to a Submolecular Biology* (Academic Press, New York, 1960).
6. H. Tolkmith, U.S. patent 3,308,122 (1967).
7. D. R. Mussell and H. Tolkmith, U.S. patent 3,321,366 (1967).
8. J. N. Seiber and H. Tolkmith, *Tetrahedron Letters* **1967**, 3333 (1967).
9. H. S. Aaron, H. O. Michel, B. Witten, J. I. Miller, *J. Amer. Chem. Soc.* **80**, 456 (1958); G. Hilgetag and G. Lehmann, *J. Prakt. Chem.* **8**, 224 (1959); T. R. Fukuto and R. L. Metcalf, *J. Econ. Entomol.* **52**, 739 (1959); H. L. Boter, A. J. J. Ooms, G. R. Van Den Berg, C. Van Dijk, *Rec. Trav. Chim.* **85**, 147 (1966).
10. P. B. Budde and H. Tolkmith, U.S. patent 3,323,990 (1967).
11. H. Giesemann and G. Hälschke, *Chem. Ber.* **92**, 92 (1959); D. E. Rivett and J. F. K. Wilshire, *Australian J. Chem.* **19**, 165 (1966).
12. T. B. Gaines, *Toxicol. Appl. Pharmacol.* **2**, 88 (1960).
13. Presented in part before the 6th International Congress of Plant Protection, Vienna, Austria, Sept. 1967.

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Isoprenoid Acids in Recent Sediments

Abstract. *Phytanic acid, pristanic acid, and 4,8,12-trimethyltridecanoic acid have been isolated from three recent marine sediments. The ratio of palmitic to pristanic acid is similar to that encountered in typical marine lipids. This suggests a biochemical origin of these sedimentary acids; phytol is their presumed biochemical precursor. Other isoprenoid acids between C_{11} and C_{22} which are common in ancient sediments have not been found. They are probably geochemical products formed slowly and at a greater depth.*

Biochemical and geochemical conversion products of phytol are among the most ubiquitous compounds in nature. Isoprenoid hydrocarbons occur in terrestrial (*1*) and marine (*2*) plants and animals. They are incorporated into recent sediments (*3*) and persist for geological time spans in sediments (*4*) and petroleum (*5*). Isoprenoid acids are common in zooplankton (*6*), in fishes (*7*), and in marine (*8*) and terrestrial (*9*) mammals. They occur in ancient sediments (*10*) and in petroleum (*11*) but—in contrast to straight-chain acids (*12*) and those with one side chain (*13*)—they have not previously been isolated from recent sediments.

To search for isoprenoid acids in those recent sediments from which we had previously isolated pristane (*3*), we obtained samples from the Wilkinson Basin, a depression in the shelf off Cape Cod, Massachusetts, and from Volden Fjord, Norway. These samples had been selected because of their deposition from waters rich in calanid copepods. A near-shore sample from Tarpaulin Cove, Massachusetts, was included in our study.

The sediments, preserved in the frozen state, were extracted first with methanol and then with benzene-methanol azeotrope. The residue was further digested for 24 hours at 30°C with anhydrous methanol-HCl. This extract was decanted, the residue was washed with methanol, and the washings were added to the extract. The extracts were concentrated either by partitioning between aqueous sodium chloride and chloroform or by distillation after addition of alkali; the residue was acidified and extracted with chloroform. The extracts were esterified with a mixture of methanol and boron trifluoride (*14*) and chromatographed on silica gel deactivated with 5 percent water. Pentane and mixtures of pentane and benzene served as eluents; the presence of esters in the eluates was established by gas chromatography. The chromatograms indicated the presence of many straight-chain, branched, saturated, and olefinic esters. Further concentration of the isoprenoid structures was necessary. Therefore, all ester fractions were combined and hydrogenated in isooctane with platinum oxide at 60°C. The straight-chain and most of the singly branched esters were then removed by urea clathration (*15*). Gas chromatography on unpolar (Apiezon L) and polar (FFAP) substrates (*16*) of the multibranched concentrates gave peaks with values for equivalent chain length (ECL) (*17*) agreeing with those of 4,8,12-trimethyltridecanoic, 2,6,10,14-tetramethylpentadecanoic (pristanic), and 3,7,11,15-tetramethylhexadecanoic (phytanic) acid methyl esters (Table 1). Results of gas chromatography on two different substrates, when used in conjunction with silica-gel chromatography and urea clathration, provide conclusive evidence for the presence of these esters. Isomeric isoprenoid esters whose

Table 1. Gas chromatographic equivalent chain lengths of isoprenoid methyl esters. Temperature, 2°C per minute; steel columns (3.6 m, inside diameter 1.4 mm) with 0.8 percent Apiezon L on Chromosorb G, acid-washed, silicone-treated; 1.5 m of 25 percent FFAP (*16*) on Chromosorb W, acid-washed, silicone-treated. This substrate diluted 1:3 with 100–140 mesh glass microbeads, silicone-treated. Average deviation of unknowns isolated from recent sediments from standards: 0.01 ECL unit; maximum deviation −0.03 (Apiezon L) and +0.04 ECL units (FFAP).

Methyl esters	Apiezon L	FFAP
4,8,12-Trimethyl-C_{13}	14.35	14.03
Pristanate	16.41	15.76
Phytanate	17.50	16.95

Table 2. Determination of isoprenoid and palmitic acid as methyl esters. Conditions as in Table 1. Standards: methyl palmitate and methyl pristanate. Wilkinson Basin, Gosnold Cruise No. 65; 42°22′N,69°29′W; depth approximately 225 m. Volden Fjord, Norway, Chain Cruise No. 13; 63°09.5′N,5°59.8′E; depth 659 m. Tarpaulin Cove, Massachusetts; 41°28′N,70°45′W; depth approximately 18 m. Values include any olefinic acids present.

Methyl esters	Concentration (ppm)		
	Wilkinson Basin	Volden Fjord	Tarpaulin Cove
4,8,12-Trimethyl-C_{13}	0.5	0.7	4
Pristanate	1.7	0.8	7
Phytanate	0.9	1.3	1.8
Palmitate	26	25	150