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Phosphorus–Nitrogen Compounds XVIII: Hydrazides and Thiosemicarbazides

L. A. CATES[x] and T. L. LEMKE

Abstract □ Phenylphosphono(thio)dihydrazides and phosphorylated thiosemicarbazides were synthesized for anticancer testing, and some of these agents displayed chelation properties. Reactions involving methylhydrazine resulted in substitution of N^1-protons. A study of diphenylphosphoro- and phosphoramidomethylhydrazides also indicated that the N^1-phosphorylated isomers are predominately formed.

Keyphrases □ Phosphorus–nitrogen compounds—synthesis of hydrazides and thiosemicarbazides as potential anticancer agents □ Nitrogen–phosphorus compounds—synthesis of hydrazides and thiosemicarbazides as potential anticancer agents □ Thiosemicarbazides, phosphorylated—synthesis as potential anticancer agents □ Hydrazides—synthesis of phenylphosphono(thio)dihydrazides as potential anticancer agents □ Anticancer agents, potential—synthesis of phenylphosphono(thio)dihydrazides and phosphorylated thiosemicarbazides

The hydrazino moiety frequently appears in agents possessing antitumor activity. The presence of this grouping in some of these compounds, such as procarbazine, indole-3-propionic acid hydrazide, α-hydrazino-ω-cyclohexylbutyric acid, 5-hydrazide 1-glutamic acid, and 2-(4-nitroso-7-oxo-1,3,5-cycloheptatrien-1-yl)isonicotinic acid hydrazide (1), is obvious, while in other agents its occurrence is less conspicuous. Various thiadiazole, pyrazole, triazeno, and azapurine oncolytics also contain the N—N bond. In a few cases, such as procarbazine and heterocyclic 2-carboxaldehyde thiosemicarbazones, the hydrazino grouping is believed to be involved in the cytotoxic process *via* redox reactivity (2) or chelation (3), respectively. Even hydrazine sulfate inhibits Walker carcinosarcoma by 28–94%, possibly through gluconeogenesis interference (4).

The confirmed activity of 4,4′,4″-phosphinylidyne trisemicarbazide[1] (5) in Walker carcinosarcoma encouraged the preparation of additional hydrazine compounds (I–XII) as potential antitumor agents. Six of the products can be classified as thiosemicarbazides, including a derivative (XII) of the anticancer agent 2-amino-1,3,4-thiadiazole, which contains a comparable structure in cyclic form. The remaining compounds are hydrazides (I–V and XI), including one containing a urethan portion (V) and a water-soluble dihydrazide (XI). This latter product may find interest in Walker carcinosarcoma in view of the probable greater sensitivity of this tumor toward hydrophilic drugs (6), while the methylhydrazides, I and II, were considered of interest in view of the established activity of procarbazine.

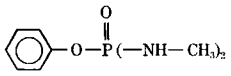
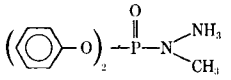
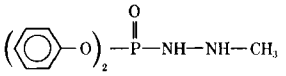
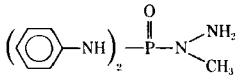
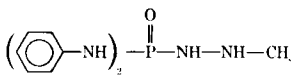
DISCUSSION

During this investigation, particular attention was directed toward the reaction involving the monophosphorylation of methylhydrazine. This reactant possesses two nitrogens with replaceable hydrogens, and both N^1- or N^2-substitution products are possible (Scheme I). Theoretically, the more nucleophilic N^1 should preferentially undergo substitution. Debo (7), however, reported that N^2-phosphorylation occurred when phosphorochloridates were reacted with methylhydrazine in sodium carbonate solution. Support for this assignment is found in work with the reactions of methylhydrazine with esters and anhydrides of carboxylic acids (8). This study showed that, while anhydrides form mainly 1-acyl-1-methylhydrazides, esters yield chiefly 1-acyl-2-methylhydrazides, with the percentage of these latter isomers increasing with the size of the acyl group of the esters.

Debo's synthesis using diphenylphosphorochloridate was repeated during this study and the reaction products were examined by NMR spectroscopy. Of the three methylhydrazides reported by

[1] This agent has subsequently been found to possess a low therapeutic index.

Table I—NMR Spectra of Isomeric Hydrazides and Phenyl *N,N'*-Dimethylphosphorodiamidate[a]

Compound	Structure	δ, ppm	Assignment	Signal[b]	Protons	*J*, Hz
XIII		2.50 7.22	N-CH_3 Ar-H	d s	6 5	12 —
XIV		2.97 7.35	N-CH_3 Ar-H	d s	3 10	8 —
XV		2.53 7.35	N-CH_3 Ar-H	s s	0.3 10	— —
XVI		2.87 6.28 7.04	N-CH_3 Ar-N-H Ar-H	d d s	3 2 10	12 14 —
XVII		2.34 6.28 7.04	N-CH_3 Ar-N-H Ar-H	s d s	0.3 2 10	— 14 —

[a] Taken on Varian T-60 or EM 360 spectrometer using deuterated chloroform as the solvent and tetramethylsilane as the internal reference. The N-H signals, other than anilino-H, are generally broad and poorly defined. [b] Singlet and doublet are abbreviated s and d, respectively.

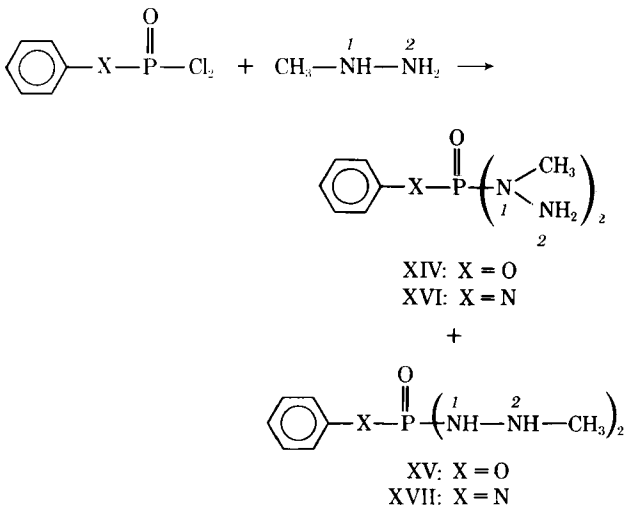
Debo, the bulkier diphenyl ester derivative was chosen as more likely to result in the N^2-substituted product. Interpretation of the NMR findings is based on the investigation of Nielsen and Sisler (9), who reported the spectra of phosphorus hydrazides but confined their study to unsymmetrical dimethyl-, trimethyl-, and dimethylethylhydrazines, each of which permits only one product.

Hydrazides resulting from monophosphorylation of dimethylhydrazine possess the N^1-H system which gives doublets (δ3.7, 3.8, 4.0, and 4.5). The N^2-methyl protons, however, were not split by phosphorus and showed singlets at δ2.4, 2.5, 2.5, 2.6, and 3.2. However, when a methyl or methylene is attached to N^1, as in the case of one 1,1,2-trimethyl- and two 1,1-dimethyl-2-ethylhydrazides, these protons gave a doublet (δ2.5) and quadruplets (δ3.3 and 3.3), respectively. Phenyl *N,N'*-dimethylphosphorodiamidate (XIII) was prepared for NMR study and showed similar splitting (Table I). Therefore, N^1-H, N^1-CH_2-, and N^1-CH_3 protons are split by phosphorus while N^2-methyl protons are not.

When the unpurified, water-insoluble oil product from the Debo synthesis was examined, it showed a doublet, assigned to XIV, and a nonreplaceable singlet (Table I and Fig. 1). This latter signal was assigned to the methyl protons of the N^2-substituted product, XV, with the amount of this material estimated by integration of the signals to be 10%. The same results were obtained from the crude products synthesized by the reaction of diphenylphosphonate–carbon tetrachloride–methylhydrazine and of diphenylphosphorodiamidic chloride–methylhydrazine (XVI and XVII, Table I). TLC of the mixture of XIV and XV gave two spots attributable to each isomer. Column chromatographic separation was only partially successful and provided for the isolation of only XIV. In addition, spectra of the pure products isolated from the reaction of phenylphosphonic dichloride (I), phenylphosphonothioic dichloride (II), and methylhydrazine showed only doublets for the methyl protons. Unsymmetrical hydrazide XI provides a compound containing both P—N—CH_3 and P—N—N—CH_3 systems, and its NMR spectrum shows a doublet (two methyls) and a singlet (four methyls) (Table II).

These data demonstrate that the percent composition of a mixture of isomers can readily be estimated by NMR spectroscopy. With regard to selective phosphorylation of a monosubstituted hydrazine, a similar situation was encountered when *N,N*-dimethylphosphoramidic dichloride and phenylphosphorodichloridate were reacted with 2-hydroxyethylhydrazine. The 3-*N*-amino-1,3,2-oxazaphospholidines were shown, *via* IR and NMR spectroscopy and derivatization, to result from substitution on the secondary nitrogen (10).

The new hydrazides (I–V and XI) and thiosemicarbazides (VI–X and XII) were synthesized *via* conventional substitution and condensation reactions, respectively. The dihydrazide V was the product from an attempt at the synthesis of the monohydrazide, a



XIV: X = O
XVI: X = N

+

XV: X = O
XVII: X = N

Scheme I

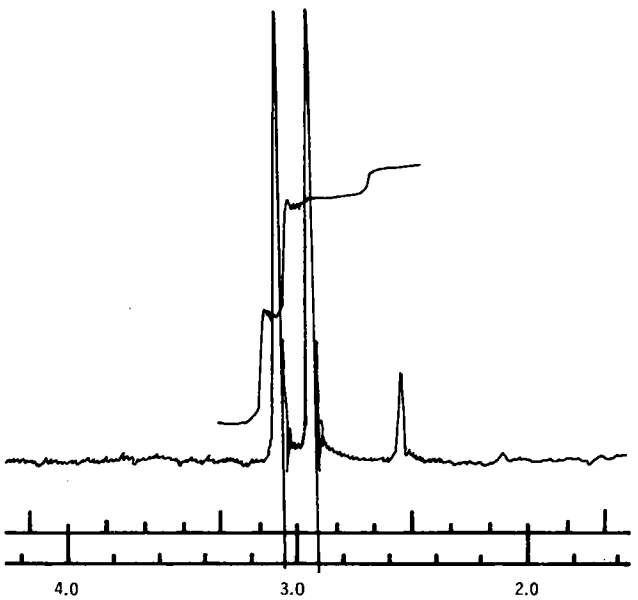
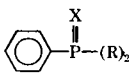
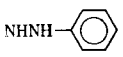
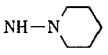
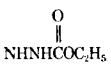
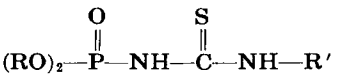
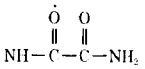
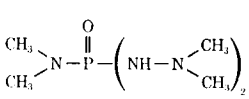
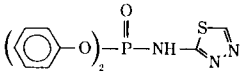


Figure 1—*Portion of an NMR spectrum produced by a mixture of isomers XIV and XV. The solvent was $CDCl_3$ and D_2O.*

Table II—Hydrazides and Thiosemicarbazides

Compound	X	R	Melting Point[a]	Formula	Analysis, %[b]		NMR Spectra[c]				
					Calc.	Found	δ, ppm	Assignment	Signal	Protons	J, Hz
											
I	O		139–143° dec.	$C_8H_{15}N_4OP$	C 44.86 H 7.06 N 26.16	45.10 7.07 26.06	2.91 7.50 to 8.14	$N\text{-}CH_3$ Ar-H	d m	6 5	9 —
II	S		96–99°	$C_8H_{15}N_4PS$	C 41.73 H 6.57 N 24.33	41.92 6.50 24.37	2.82 7.31 to 8.32	$N\text{-}CH_3$ Ar-H	d m	6 5	12 —
III	O		188–190° dec.	$C_{18}H_{19}N_4OP$	C 63.90 H 5.66 N 16.56	63.60 5.66 16.50	6.94 to 7.72	Ar-H	m	—	—
IV	O		179–180°	$C_{16}H_{27}N_4OP$	C 59.61 H 8.44 N 17.38	59.78 8.55 17.22	1.59 2.62 7.17 to 7.62 7.75 to 8.26	C-H C-H Ar-H Ar-H	m m m m	12 8 3 2	— — — —
V	O		197–198° dec.	$C_{12}H_{19}N_4O_5P$	C 43.64 H 5.80 N 16.96	43.70 5.88 16.91	1.22 4.07 7.26 to 8.13	C-H C-H Ar-H	t q m	6 4 5	7 6 —
											
	R	R′									
VI	CH_3CH_2		153°	$C_9H_{20}N_3C_4PS$	C 36.36 H 6.78 N 14.13	36.47 6.81 14.05	1.32 2.88 3.74 3.89 to 4.99	C-H C-H[d] C-H[d] C-H	t m m m	6 4 4 4	7 — — —
VII			167–168° dec.	$C_{17}H_{20}N_3O_4PS$	N 11.13	10.71	2.60 3.71 7.30	C-H[d] C-H[d] Ar-H	m m s	4 4 10	— — —
VIII	CH_3CH_2		129°	$C_{10}H_{22}N_3O_3PS$	C 40.67 H 7.51 N 14.23	40.79 7.55 14.16	0.99 to 1.58 1.71 2.86 3.93 to 4.51	C-H C-H[e] C-H[e] C-H	m m m m	6 6 4 4	— — — —
IX			161–162° dec.	$C_{18}H_{22}N_3O_3PS$	C 55.23 H 5.67 N 10.73	55.34 5.72 10.65	1.55 2.72 7.31	C-H C-H Ar-H	m m s	6 4 10	— — —
X			153–154° dec.	$C_{15}H_{15}N_4O_5PS$	C 45.69 H 3.83 N 14.21	45.47 3.98 14.04	6.63 to 7.82	Ar-H	m	—	—
				Miscellaneous							
XI			96–98°	$C_6H_{20}N_3OP$	C 34.44 H 9.63 N 33.47	34.32 9.72 33.59	2.58 2.75	$N\text{-}CH_3$ $N\text{-}CH_3$	s d	12 6	— 9
XII			158° dec.	$C_{14}H_{12}N_3O_3PS$	C 50.45 H 3.63 N 12.61	50.80 3.68 12.62	7.37 to 7.73 8.23	Ar-H C-H	m d	10 1	— 2

[a] Performed on a Fisher-Johns apparatus and are uncorrected. [b] Performed by Atlantic Microlab, Inc., Atlanta, Ga. [c] Taken on Varian T-60 or EM 360 spectrometer using deuterated chloroform (II, V, VIII, X, and XII) or deuterated dimethyl sulfoxide (I, II, IV, VI, VII, IX, and XI) as the solvent and tetramethylsilane as the internal reference. Generally, N-H signals were indistinct and are not reported. Singlet, doublet, triplet, quartet, and multiplet splittings are abbreviated s, d, t, q, and m, respectively. [d] Morpholino-H. [e] Piperidino-H.

proposed candidate for intramolecular dehydrohalogenation to the imide derivatives.

In view of the antitumor activity associated with chelation properties (3), the ability of six derivatives to complex Co^{+2}, Cu^{+2}, Fe^{+3}, and Ni^{+2} ions was demonstrated. Those agents displaying ligand properties were IV–VI (Co, Cu, Ni), VII and IX (Co, Ni), and VIII (Co, Fe, Ni). The thioic analog of III (X = S) was reported (11) to have some activity against E0771 tumor in mice.

Samples of Compounds I–XII have been submitted to the National Cancer Institute for antitumor testing.

EXPERIMENTAL

Phenylphosphono(thio)dihydrazides (I–V)—Solutions of 1 mole of phenylphosphonic dichloride or phenylphosphonothioic dichloride in ether were added to ethereal solutions of 4 moles of methylhydrazine (I and II), a mixture of 2 moles of phenylhydrazine (III) or *N*-aminopiperidine (IV) and 2 moles of triethylamine, or 2 moles of ethyl carbazate (V) placed in a three-necked, round-bottom flask equipped with dropping funnel and condenser fitted with a drying tube. The dichlorides were added at a rate sufficient to maintain reflux conditions. The reaction mixtures were filtered, the precipitates were washed with water, and any insoluble material was added to the filtrates which were then spin evaporated. The residues were crystallized from petroleum ether (bp 30–60°) for I, benzene for II, or ethanol for III–V.

Phosphorylated Thiosemicarbazides (VI–X)—Equal molar quantities of either diethyl- or diphenylphosphoroisothiocyanatidate were added to solutions of *N*-aminomorpholine (VI and VII), *N*-aminopiperidine (ether) (VIII and IX), or oxamic dihydrazide (acetonitrile) (X). After remaining at ambient temperature for 24 hr, the precipitates that formed were collected on a filter and crystallized from ethanol.

***N,N*-Dimethyl-N^2,N^2-dimethylphosphoramidodihydrazide (XI)**—A mixture of 1 mole of *N,N*-dimethylphosphoramidic dichloride (12) and 4 moles of 1,1-dimethylhydrazine in ether was refluxed for 4 hr and then filtered. The filtrate was spin evaporated to yield an oil, which precipitated as crystals from its ethereal solution. An IR spectrum[2] of XI showed absorptions consistent with this structure.

Diphenyl *N*-2-(1,3,4-Thiadiazolyl)phosphoramidate (XII)—One mole of diphenylphosphorochloridate was added to a dioxane solution of 1 mole each of 2-amino-1,3,4-thiadiazole and triethylamine. The filtrate from the reaction mixture was treated with water to yield a precipitate, which was collected on a filter and crystallized from ethanol.

Isomeric Diphenylphosphoro- and Phosphoramidomethylhydrazides (XIV–XVII)—The reaction between diphenylphosphorochloridate and methylhydrazine in 20% sodium carbonate solution was conducted according to the procedure of Debo (7). The resultant semisolid was dissolved in chloroform, and the solution was dried over calcium sulfate and filtered. The filtrate was spin evaporated; samples of the residue were then used for NMR spectroscopic examination, TLC, column chromatography, and purification. An NMR spectrum of the crude product was obtained, and the pertinent absorptions are shown in Fig. 1 and Table I.

Spectra of both starting materials, chloroform solvent, deuterated chloroform, and deuterium oxide were run separately and showed no peaks in this region. TLC [methanol–chloroform (1:10)] of the crude product and reactants revealed two spots not attributable to the latter. A sample placed on a silica gel column was eluted with chloroform to yield XIV and then with methanol. The latter eluate gave a material whose NMR spectrum failed to show aromatic proton absorption. Crystallization of the crude product from ether gave XIV, mp 53–54°. The unpurified product obtained from the reaction between diphenylphosphonate, carbon tetrachloride, and dimethylamine (13) gave results identical to those reported here.

Methylhydrazine (2 moles) in acetonitrile was added dropwise with stirring and cooling at 0–5° to an acetonitrile solution of *N,N*-diphenylphosphorochloridate (14). After remaining at ambient temperature for 48 hr, the reaction mixture was filtered. Spin evaporation of the filtrate produced a brown semisolid. NMR spectroscopic examination (Table I) and TLC [methanol–chloroform (1:10)] of this material indicated that it consisted of XVI and XVII. Crystallization of the crude product from ethanol–water yielded white crystals of XVI.

Phenyl *N,N*-Dimethylphosphorodiamidate (XIII)—This compound was synthesized according to the procedure of Audrieth and Toy (15).

Chelation Studies—To 2 ml of 1% ethanol solutions of V–IX were added 0.2 ml of 1% ethanol solutions of cobaltous, cupric, ferric, or nickelous chloride followed by 0.05 ml of 10% alcoholic potassium hydroxide. Solutions of ethyl carbazate, *N*-aminopiperidine, and *N*-aminomorpholine were treated in the same manner. The formation of colors in the former tests, prior to and after the addition of base, as compared to those involving starting materials was interpreted as the ability to chelate the metal ions.

[2] Beckman IR-8 spectrometer.

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