respectively; 4-, 10-, and 13-CH_3 signals of the isomer mixture), 6.44 (1 H, d, J = 2 Hz, 12-H), 6.31, 6.03 (each 1 H, d, J = 9 Hz, $-OCH_2-$), 5.90 (1 H, br s, 1-H), 5.31 (1 H, br s, 7-H), 4.16 (1 H, d, J = 11 Hz, 15-H), 2.50 (3 H, m, A_2B part of an A_2BX_2 system), 1.91 (2 H, dd, J = 6, 1 Hz, X_2 part of an A_2BX_2 system); mass spectrum m/e 500 (M^+), 482, 379, 249, 105. Anal. ($C_{27}H_{32}O_9$) C, H.

On ChromAR **15** was clearly resolved into two components using ethyl acetate–cyclohexane (1:1) whereas on SilicAR best resolution was observed in 10% acetone–ether. Column chromatography (138 g, SilicAR CC-7, Mallinckrodt; 2.5 × 60 cm column) of the reduction product from 498 mg of **11** using anhydrous ether (Baker AR) as eluent gave an early fraction (62.3 mg) of the high R_f component (**15a**), and crystallization from acetone gave an analytical sample. Chromatography on ChromAR (17 plates; double development with 1:1 ethyl acetate–cyclohexane) of 21.4 mg of a column fraction containing approximately equal portions of **15a** and **15b**, followed by rechromatography of each of the components under identical conditions, gave 9.1 mg of **15a** and 8.7 mg of **15b**. The two components, which showed identical ir (KBr) and mass spectra, were easily differentiated by NMR: **15a** ($CDCl_3$) τ 8.99 (3 H, d, J = 7 Hz, 4-CH_3), 8.88 (3 H, d, J = 7 Hz, 13-CH_3), 8.76 (3 H, s, 10-CH_3); **15b** ($CDCl_3$) τ 8.91, 8.85, 8.75 (br, CH_3) and ($CDCl_3$ + C_6D_6) τ 9.21 (3 H, d, J = 5 Hz), 9.08 (3 H, s, 10-CH_3), 8.95 (3 H, d, J = 6.4 Hz).

Dihydroglaucarubinone (16). A solution of **6** (50 mg, 0.101 mmol) in absolute ethanol (50 ml) was subjected to atmospheric hydrogenation at room temperature for 16 h using 5% palladium on charcoal (50 mg) as catalyst. Filtration, concentration, and crystallization from acetone–hexane afforded **16** (34 mg, 67%) as small needles: mp 220–223°; uv (EtOH) λ 239 nm (ϵ 69); ir (KBr) 3.03, 5.75, 8.78, 9.54 μ; NMR ($CDCl_3$) τ 9.03 (3 H, t, J = 7 Hz, $-CH_2CH_3$), 8.91 (6 H, m, 4-CH_3, 13-CH_3), 8.77 (3 H, s, 10-CH_3), 8.55 (3 H, s, 2′-CH_3), 6.46 (1 H, br s, 12-H), 6.33, 6.08 (each 1 H, d, J = 9 Hz, $-OCH_2-$), 5.93 (1 H, br s, 1-H), 5.39 (1 H, br s, 7-H), 4.33 (1 H, d, J = 12 Hz, 15-H); mass spectrum m/e 496 (M^+), 478, 379, 250, 231, 73. Anal. ($C_{25}H_{36}O_{10}$) C, H.

Chromatography of a sample of **16** (68.5 mg) on ChromAR (21 plates developed three times with 3:2 ethyl acetate–cyclohexane) resolved the product into two bands which were each rechromatographed on ChromAR (20 plates developed three times with 3:1 ethyl acetate–cyclohexane) to give a higher R_f component (**16a**, 28.4 mg) and a lower R_f component (**16b**, 17.0 mg). Although the two products were indistinguishable by ir (KBr) and mass spectra, the NMR spectra (acetone-d_6) showed 10-CH_3 signals at τ 8.74 for the higher R_f component and at 8.85 for the lower R_f component.

References and Notes

(1) (a) Tumor Inhibitors. 115. For part 114, see S. M. Kupchan and A. Karim, *Lloydia*, in press. (b) The work was supported by a contract with the Division of Cancer Treatment, National Cancer Institute (N.C.I.), National Institutes of Health, Department of Health, Education and Welfare (NO1-CM-12099), research grants from the N.C.I. (CA-11718) and the American Cancer Society (CI-102J), and a Postdoctoral Fellowship (G.A.H., CA-02060-01) from the N.C.I.

(2) S. M. Kupchan, R. W. Britton, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.*, **38**, 178 (1973).

(3) S. M. Kupchan, R. W. Britton, J. A. Lacadie, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.*, **40**, 648 (1975).

(4) A. Gaudemer and J. Polonsky, *Phytochemistry*, **4**, 149 (1965).

(5) J. Polonsky, *Fortschr. Chem. Org. Naturst.*, **30**, 101 (1973).

(6) S. M. Kupchan, M. Maruyama, R. J. Hemingway, J. C. Hemingway, S. Shibuya, and T. Fujita, *J. Org. Chem.*, **38**, 2189 (1973).

(7) S. M. Kupchan, S. P. Erikson, and M. Friedman, *J. Am. Chem. Soc.*, **88**, 343 (1966).

(8) S. M. Kupchan and G. Tsou, *J. Org. Chem.*, **38**, 1055 (1973).

(9) Antileukemic activity and cytotoxicity were assayed under the auspices of the National Cancer Institute, by the procedures described by R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott [*Cancer Chemother. Rep., Part 3*, **3** (2), 1 (1972)].

(10) S. M. Kupchan, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **33**, 2288 (1974).

(11) P. Chamberlain and G. H. Whitham, *J. Chem. Soc., Perkin Trans. 2*, **72**, 130 (1972).

(12) S. M. Kupchan and J. A. Lacadie, *J. Org. Chem.*, **40**, 654 (1975).

Phosphorus–Nitrogen Compounds. 20. Thiophosphorus Hydrazones

L. A. Cates,* Y. M. Cho, L. K. Smith, L. Williams, and T. L. Lemke

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Houston, Houston, Texas 77004.

Received October 31, 1975

Six pyridine-2-carboxaldehyde, one pyridine *N*-oxide 2-carboxaldehyde, and five diketone thiophosphoric hydrazones, three thiophosphoric hydrazides, and two cupric chelates were synthesized. The chelates and nine of the hydrazones were tested against Ehrlich ascites carcinoma. Seven of these latter agents were administered concurrently with either cupric and/or ferrous salts to mice bearing this tumor. The greatest activity was found with the chelate, dimethyl pyridine-2-carboxaldehyde phosphorothioic hydrazone–copper (1:1). The hydrazone portion of this chelate also formed a ligand–copper (2:1) complex. Although all of the hydrazones but one were inactive when evaluated alone, the concurrent injection of cupric ion increased survival times by an average of 145% T/C in four compounds so tested. *Escherichia coli* alkaline phosphatase was found to be inhibited by two thiosemicarbazones in a manner similar to that previously reported by these agents against alkaline phosphatase derived from Sarcoma 180 6-thiopurine resistant ascites cells. None of the 14 hydrazides or hydrazones tested against *E. coli* enzyme displayed significant inhibition.

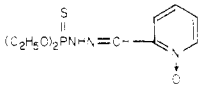
Thiosemicarbazones and their metal chelates have been investigated as medicinal agents since Domagk[1] discovered antitubercular activity in this class of chemicals and, subsequently, they have been studied for antifungal, antiviral, and oncolytic properties.[2]

It is generally considered that these compounds function as ligands in chelation and their role in cancer chemotherapy has been recently reviewed.[3] In the past few years, heterocyclic carboxaldehyde and aliphatic diketone thiosemicarbazones and their metal chelates have been tested in animal model tumor systems, as well as ribonucleoside diphosphate reductase (RDR) and alkaline phosphatase (AP) inhibition studies.[2,4,5]

This paper reports the synthesis of phosphorus analogues of pyridine-2-carboxaldehyde, one *N*-oxide analogue, and aliphatic diketone thiosemicarbazones and their effects as inhibitors of the action of *Escherichia coli* AP and the growth of Ehrlich ascites carcinoma cells in mice. Selected agents were tested by this latter method as cupric complexes and via concurrent administration of cupric and ferrous ions.

Chemistry. Three previously unreported thio-

Table I. Thiophosphorus Hydrazides, Hydrazones, and Chelates

Compd	R	R′	Mp, °C	Formula	Analyses
		$RP(S)(NHNH_2)_2$			
1	C_2H_5O		75-76	$C_2H_{11}N_4OPS$	C, H, N
2	C_6H_5		110-112	$C_6H_{11}N_4PS$	C, H, N
		$(R)_2P(S)NHN{=}CH$-2-Py			
3	CH_3O		106-108	$C_8H_{12}N_3O_2PS$	C, H, N
4	C_2H_5O		56-57	$C_{10}H_{16}N_3O_2PS$	C, H, N
5	C_3H_7O		39-42	$C_{12}H_{20}N_3O_2PS$	C, H, N
6	c-$O(CH_2CH_2)_2N$		182-183	$C_{14}H_{22}N_4O_2PS$	C, H, N
		$CH_3C(R){=}NNHP(S)(OR')_2$			
7	H	C_2H_5	49-53	$C_{11}H_{26}N_4O_4P_2S_2$	C, H, N
8	CH_3	C_2H_5	99-101	$C_{12}H_{28}N_4O_4P_2S_2$	C, H, N
9	CH_3	C_3H_7	124-125	$C_{16}H_{36}N_4O_4P_2S_2$	C, H, N
		Miscellaneous			
10	$CH_3C(NOH){=}NNH$-$P(S)(OC_3H_7)_2$		89-90	$C_{10}H_{22}N_3O_3PS$	C, H, N
11	$C_2H_5OP(S)(NHN{=}CH$-2-Py$)_2$		140-142	$C_{14}H_{17}N_6OPS$	C, H, N
12	$C_6H_5P(S)(NHN{=}CH$-2-Py$)_2$		178-180	$C_{18}H_{17}N_6PS$	C, H, N
13	p-Br-C_6H_4-$C(O){-}CH{=}NNHP(S)$(c-$N(CH_2CH_2)_2O)_2$		180	$C_{16}H_{22}BrN_4O_3PS$	C, H, N
14	(c-$N(CH_2CH_2)_2O)_2P(S)NHNH_2$		81-84	$C_8H_{19}N_4O_2PS$	C, H, N
15			138-140	$C_{10}H_{16}N_3O_3PS$	C, H, N
3-Cu(II)			>300	$C_8H_{12}N_3O_2PS{\cdot}CuCl_2$	C, H, N; Cl,[a] Cu[b]
4-Cu(II)			>300	$C_{10}H_{16}N_3O_2PS{\cdot}CuCl_2$	C, H, N

[a] Cl: calcd, 18.67; found, 17.28. [b] Cu: calcd, 16.73; found, 17.36.

phosphoric hydrazides, **1**, **2**, and **14**, were synthesized and used to prepare dihydrazones **11** and **12** and monohydrazones **6** and **13** (Table I). Known hydrazides were similarly condensed with pyridine-2-carboxaldehyde, the *N*-oxide of this compound, pyruvaldehyde, 2,3-butanedione, or 2,3-butanedione oxime to yield mono- and dihydrazones **3**–**10** and **15** (Table I). The lower melting pyridine monohydrazones **3**–**5** and pyruvaldehyde dihydrazone **7** proved difficult to obtain in crystalline form. Pure crystals of the former were, therefore, obtained by means of high-pressure liquid chromatography and used to seed oils from the reaction mixtures, while **7** was purified using column chromatography.

The addition of an alcoholic solution of cupric chloride to a solution of **3** in ethanol gave a precipitate of a water-soluble complex **3**–Cu(II) consisting of a 1:1 ratio of reactants (Table I). The filtrate from this reaction mixture contained an alcohol-soluble complex of **3** and Cu(II). Spectral evidence that **3** can form a coordination complex with copper is shown in Figure 1. Addition of copper in increasing increments caused a hypsochromic shift until the ratio of **3** to copper was 2:1. Further addition of copper did not cause any change in spectrum from that produced by the mixture containing 9.45×10^{-5} M **3** and 4.73×10^{-5} M copper. This suggests that optimum interaction of ligand and metal occurred at a ratio of 2:1. Attempts at isolating this complex were unsuccessful; chemical lability in related chelates has also been reported.[6] Gingras et al.[7] found that elemental analyses of copper chelates with low solubility were unsatisfactory and Van Giessen and Petering[8] expressed similar difficulties which they attributed to the presence of liganding solvent molecules. Even with **3**–Cu(II) the Cl and Cu analyses were not in complete agreement with the theoretical (±0.4%) but results for the other elements (C, H, and N) examined precluded another structural assignment. Similarly prepared **4**–Cu(II) gave correct analysis for these latter elements and may be of the same nature; **11**–Cu(II) was not analyzed but tested per se.

Aliphatic diketone thiosemicarbazones[9] chelate cupric ion in a 2:1 ligand–metal ratio with regard to the number of thiosemicarbazide residues present in the molecule. Heterocyclic 2-carboxaldehyde thiosemicarbazone–copper chelates, however, have been reported to form in a 1:1 ligand–metal ratio.[10] The reaction of benzaldehyde 4′,4′-dimethylthiosemicarbazone with cuprous chloride in ammonia yields an acetone-soluble chelate and a complex insoluble in acid, base, acetone, ether, and alcohol, which were assigned ligand–metal ratios of 2:1 and 1:1, respectively.[11] The 1:1 complex is considered to be a polymeric

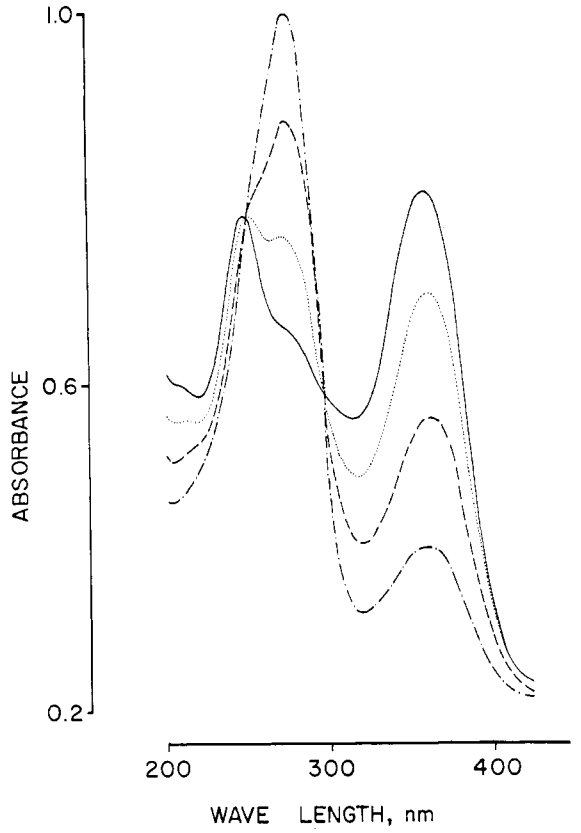


Figure 1. Spectrum of III and its cupric complexes. The concentration of **3** was 9.46×10^{-5} M in ethanol. Cupric chloride dihydrate was added in increments and the spectra of the formed complexes were measured: (———) **3** + 4.73×10^{-5} M Cu; (· · · · · ·) **3** + 3.53×10^{-5} M Cu; (- - - - - -) **3** + 2.36×10^{-5} M Cu; (- · - · - · -) **3** + 1.18×10^{-5} M Cu.

substance. Although none of this related work explains the nature of **3**–Cu(II) (1:1 complex) and the 2:1 complex, it is possible that in the former the ligand exists in the *Z* conformation with chelation involving only the N^2 and S atoms, while the ligand in the latter possesses the *E* form and gives complexes utilizing these atoms and/or the hetero nitrogen. Compounds **4** and **11** also gave similar precipitates when treated with cupric chloride in ethanol and these materials, **4**–Cu(II) and **11**–Cu(II), were tested in the murine ascites system. It was also noted that **3**, **4**, and **11** did not yield alcohol-insoluble precipitates with cupric sulfate, acetate, or nitrate.

Biological Results. Ligands **3**–**5**, **7**, **9**–**12**, and **15** were tested against Ehrlich ascites carcinoma with only the pyruvaldehyde derivative **7** showing interesting activity at the maximum dose used in the study (Table II). These agents are essentially inactive against this system with no 50-day survivors being observed.

Since the principal objective of this investigation was to ascertain the potential oncolytic effect of a new type of ligand, it was decided to test certain of these compounds by concurrent ip administration of cupric and ferrous salts (Table II). This method was suggested by the work of Cappuccino et al.[12] who tested pyruvaldehyde bis(thiosemicarbazone) alone and in combination with dietary cupric sulfate on ten tumor systems. These workers found that Cu(II), as well as Ca(II) and Zn(II), enhanced the antitumor activity of subinhibitory doses of this compound in the case of two solid tumors. This present study yields similar results with regard to Cu(II), but not Fe(II), ion. The percent T/C values of **5**, **7**, **9**, and **12** in combination with cupric chloride, compared to those for the ligands alone, increased 167, 152, 185, and 75%, respectively. Thus, the pyridine carboxaldehyde monohydrazone and glyoxal hydrazones, administered concurrently with cupric ion, have comparable effects, whereas the effect of cupric ion on the latter is less.

Table II. Antitumor Effects of Ligands, Concurrently Administered Ligands–Metal Salts, and Cupric Complexes

Compd	Dose, mg/kg[a]	50-Day survivors	T/C, %[b]
3	100	0/6	93
4	100	0/6	80
4	50 + Fe(II)[c]	0/6	108
5	100	0/6	94
5	50 + Fe(II)[d]	0/6	104
5	50 + Cu(II)[e]	4/6	261
7	100	0/6	146
7	50 + Cu(II)[e]	0/6	298
8	50 + Fe(II)[f]	0/6	91
9	50	0/6	109
9	50 + Fe(II)[g]	0/6	93
9	50 + Cu(II)[e]	2/6	294
10	50	0/6	115
10	50 + Fe(II)[d]	0/6	92
11	100	0/6	101
12	100	0/6	103
12	50 + Cu(II)[e]	0/6	178
15	100	0/6	119
15	50 + Cu(II)[e]	0/6	111
3-Cu(II)	5.0	3/6	198
	7.5	1/6	119
4-Cu(II)	5.0	1/6	130
	7.5	1/6	154
11-Cu(II)	5.0	0/6	137
	7.5	0/6	135

[a] Injections of each indicated dose were administered ip to six animals on days 1, 3, and 5 with day 1 being 24 h after implantation of 4×10^6 Ehrlich carcinoma cells. [b] Mean survival time of test animals (T)/mean survival time of control animals (C) × 100. [c] 25.5 mg/kg of $FeSO_4 \cdot 7H_2O$. [d] 23.1 mg/kg of $FeSO_4 \cdot 7H_2O$. [e] 8.5 mg/kg of $CuCl_2 \cdot 2H_2O$. [f] 16.6 mg/kg of $FeSO_4 \cdot 7H_2O$. [g] 14.6 mg/kg of $FeSO_4 \cdot 7H_2O$.

In view of the antitumor activity shown by cupric chelates of 1-formylisoquinoline thiosemicarbazone,[13] 3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazones),[8] and pyruvaldehyde bis(thiosemicarbazones),[12] **3**–Cu(II), **4**–Cu(II), and **11**–Cu(II) were included in the Ehrlich ascites study. In previous studies where comparisons were made,[8-12] the copper chelates were significantly more active than the corresponding ligands or even the concurrently administered ligands and ion.[12] This situation prevails for the phosphorus compounds with **3**–Cu(II) giving a percent T/C of 198 with three out of six 50-day survivors at 5 mg/kg, the greatest activity found in this study (Table II). This agent was the only one subsequently tested against murine lymphoid leukemia and was found ineffective under the experimental conditions. **3**–Cu(II) injected on days 1 and 5 at a dose of 12.5 mg/kg in BDF_1 mice bearing L1210 ascites cells gave a percent T/C of 86.

The inclusion of ferrous ion in this study is based on the inhibition of RDR, and thus DNA biosynthesis, through the complexing of Fe(II) by pyridine and isoquinolinecarboxaldehyde thiosemicarbazone ligands or by the preformed iron chelates binding to the enzyme.[14] Although **4**, **5**, **9**, and **10** complexed with ferrous ion, as evidenced by a color change when a dilute aqueous ferrous sulfate solution was added to dilute ethanol solutions of the ligand, concurrent administration failed to give survival time increases (Table II). This lack of effect may be attributed to tumor systems responding differently to various metals as experienced by Cappuccino and co-workers.[12]

2-Formylpyridine *N*-oxide arylsulfonylhydrazones have been reported to possess potent anticancer properties, including the ability to inhibit a subline of Sarcoma 180 resistant to α-N-heterocyclic carboxaldehyde thiosemicarbazones, and preliminary structure–activity relationships were indicated.[15] This study prompted the synthesis of **15**, a phosphorus analogue of the sulfonyl hydrazones, and the testing of this ligand alone and in combination with cupric ion (Table II). Neither **15** nor **15**–Cu mixture extended survival times of tumor-inoculated mice, similar results being found for **4** which lacks the *N*-oxide. Additional compounds need to be prepared, including those with bulky P substituents, in order to ascertain the potential of these agents.

The investigation of potential inhibitors of AP has recently been a subject of interest in cancer chemotherapy, since it has been noted that there is an increase in concentration of this enzyme in tumors resistant to 6-thiopurines with subsequent reduction in amount of these agents in their active nucleotide form.[16] There have been few potent inhibitors of AP reported, but certain members of a series of 4′-substituted pyridine-2-carboxaldehyde thiosemicarbazones, which are considered to act via chelation of zinc ion, have shown good activity against AP obtained from Sarcoma 180 6-thiopurine (Sarcoma 180/TG) resistant ascites cells. The nature of the 4′ substituent is important in providing potent compounds. As opposed to active oncolytic agents, which require small substituents in this position,[14] the greatest AP inhibition occurs when the 4′ N is replaced with a pyrrolidinyl[4] or morpholino[17] grouping. Following the synthesis and testing of **3–5**, **7**, and **9–12** against Ehrlich ascites carcinoma, these agents, since they possess the requisite bulk in the terminal portion of the side chain, were tested against AP obtained from *E. coli*. Their lack of inhibitory effect then prompted the preparation and testing of **6** and **13**, which contain the morpholino moiety. Since *E. coli* AP had not previously been used in this type of testing, its sensitivity to chelating inhibitors was first investigated with two compounds that have been shown to inhibit AP from Sarcoma 180/TG cells.[17] The concentrations of 1-formylisoquinoline 4′-diethyleneoxythiosemicarbazone **16** and its 5-hydroxy derivative **17** required to inhibit the Sarcoma AP by 50% were 9×10^{-6} and 8×10^{-6} M, respectively. Against *E. coli* AP at pH 8.0 and 25° the concentrations of **18** and **19** required to inhibit enzyme activity by 50% were 4.7×10^{-5} and 2.3×10^{-5} M, respectively. However, when the pH was increased to 9.0 with incubation at 37°, the concentrations required to inhibit enzyme activity by 50% decreased to 7.3×10^{-6} and 3×10^{-6} M, respectively. It appears, therefore, that the sensitivity of *E. coli* AP is similar to that observed with the Sarcoma enzyme. The pattern of replacing both terminal protons in thiosemicarbazones to enhance their inhibitory effect was also observed when *E. coli* AP was used. The inhibitory activity of pyridine-2-carboxaldehyde thiosemicarbazone[18] was so low that the concentration required to inhibit enzyme activity by 50% could not be determined. However, the diethyleneoxy derivative **18** inhibited enzyme activity by 50% at a concentration of 1.4×10^{-5} M. This latter agent, which is a pyridine derivative and, therefore, more closely related to several of the test compounds than **16** and **17**, was used as a standard reference. The results of this study indicate a low order of inhibitory effect when compared to **18**. In some cases, insufficient solubility precluded a complete evaluation of test compounds.

Experimental Section

Spectra of new compounds (NMR), 3–Cu ratio (uv), and enzyme preparations (visible) were taken on Varian EM 360, Beckman recording DB-GT, and Beckman DU-2 spectrophotometers, respectively. NMR spectra were run on all compounds and are in agreement with assigned structures. Deuterated chloroform (**3–11**, **13–15**), deuterated dimethyl sulfoxide (**2** and **12**), and deuterium oxide (**1**) were the solvents with tetramethylsilane as the internal or external reference. Chromatographic separations were achieved by means of a Waters Associates Model ALC-201 with 6000 solvent delivery system. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga., and are within ±0.4% of theoretical values unless otherwise indicated. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected.

Syntheses. The hydrazides, hydrazones, and chelates were prepared by modifications of procedures described by Tolkmith,[19] Agrawal,[18] and Gingras,[11] respectively.

Thiophosphoric Dihydrazides (1 and 2). Ethyl phosphorodichloridothionate or phenyl phosphonothioic dichloride (1 mol) in ether was added dropwise to hydrazine (4.5 mol) in ether; the mixture was refluxed 2 h and allowed to remain ca. 12 h at 25°. The white residue containing **1** was dissolved in warm EtOH and the solution cooled to precipitate hydrazine hydrochloride, which was removed by filtraton. Upon concentration, the filtrate yielded **1**. The white solid formed in the reaction mixture containing **2** was collected, washed successively with $CHCl_3$, warm H_2O, and absolute EtOH, and dried to give **2**.

***N,N′*-Diethyleneoxyphosphorodiamidothioic Hydrazide (14).** A CH_2Cl_2 solution of morpholine (2.2 mol) was added dropwise with stirring to freshly distilled thiophosphoryl chloride (0.5 mol) in CH_2Cl_2 with temperature maintained at 5–10°. After remaining ca. 12 h at 25° the mixture was filtered and the filtrate concentrated in vacuo to yield white crystals. An ether suspension of the crystals (34 mmol based on the monochloride) was added to an ether solution of hydrazine (125 mmol) and the mixture refluxed for ca. 12 h. The ether layer was collected and the solvent removed in vacuo. Upon H_2O treatment the residue gave a white precipitate which was collected and recrystallized from $CHCl_3$. Additional product was obtained from the nonethereal reaction mixture layer by H_2O treatment to give a white precipitate which was recrystallized from $CHCl_3$.

Pyridine-2-carboxaldehyde Monohydrazones (3–5). Dimethyl-[19] ($CHCl_3$), diethyl-[19] ($CHCl_3$), or di-*n*-propyl-[20] (no solvent) phosphoramidothioic hydrazide (56 mmol) was added to pyridine-2-carboxaldehyde (60 mmol) and the mixture heated for 0.5 h at 50–60°. Samples of the reaction mixtures were chromatographed on a high-pressure liquid chromatograph using $CHCl_3$ eluent and Corasil column to yield solids which were used to seed the original reaction mixtures. The resulting solid was recrystallized from EtOH–H_2O to give pure products.

Pyridine-2-carboxaldehyde-*N,N′*-diethyleneoxyphosphorodiamidothioic Hydrazone (6). Equimolar EtOH solution of pyridine-2-carboxaldehyde and **14** to which was added HOAc (3 ml) were allowed to remain ca. 12 h at 25°. Addition of H_2O and concentration of solvents gave the white solid product which was washed with water and dried.

1,2-Diketone Dialkylphosphorothioic Hydrazones (7–10). EtOH solutions of diethyl-[19] or di-*n*-propyl-[20] phosphoramidothioic hydrazide (13 mmol) were added to 40% aqueous pyruvaldehyde (7.3 mmol), 2,3-butanedione (7.5 mmol in EtOH), or 2,3-butanedione oxime (13 mmol in EtOH). HOAc (0.1 ml) was added; the mixture was boiled for 10 min and then cooled to 25°. **7** was obtained by concentrating the reaction mixture and chromatographing on silica gel with a $CHCl_3$–MeOH (95:5) eluent. The colorless oil from the reaction mixture containing **8** was washed with hot H_2O and dissolved in EtOH. H_2O addition gave an oil which solidified to a waxy solid upon cooling. Recrystallization from ether–petroleum ether (bp 38–49°) afforded **8**. **9** and **10** appeared as crystals in their reaction mixtures upon cooling and were recrystallized from EtOH.

Pyridine-2-carboxaldehyde Dihydrazones (11 and 12). EtOH solutions of **1** or **2** (12 mmol) and pyridine-2-carboxaldehyde (33 mmol) were mixed and HOAc (0.15 ml) was added. After remaining at ca. 12 h at 25°, the white precipitates were collected, washed with water and EtOH, and dried.

2-(4-Bromophenyl)-1-(*N,N′*-diethyleneoxyphosphorodiamidothioic Hydrazone) Glyoxal (13). Equimolar EtOH

solutions of 4-bromophenylglyoxal[21] and **14** were mixed and HOAc (1 ml) was added. During 0.5 h the solution turned yellow, water (10 ml) was added, and the mixture allowed to remain ca. 12 h at 25°. The resultant yellow precipitate was collected and recrystallized from EtOH–water.

Pyridine *N*-Oxide 2-Carboxaldehyde-*N*,*N*'-diethoxyphosphorodiamidothioic Hydrazone (15). Equimolar EtOH solutions of pyridine *N*-oxide 2-carboxaldehyde[22] and diethyl phosphoramidothioic hydrazide[19] were mixed and HOAc (1 ml) was added. The mixture was heated (50–60°) for 0.5 h. The resultant oil was stirred with H_2O to yield a solid. The pale yellow crystalline product was obtained by recrystallization (EtOH–H_2O).

Cupric Chelates [3-Cu(II), 4-Cu(II), and 11-Cu(II)]. A saturated solution of cupric chloride dihydrate in absolute EtOH was added dropwise to an EtOH solution (500 mg in 10 ml) of **3**, **4**, or **11** until no additional precipitate formed. The reaction mixture was then stirred 15 min. The precipitates were collected, washed with absolute EtOH, and dried in vacuo.

Alkaline Phosphatase Assay. *E. coli* AP was assayed in triplicate by the method of Garen and Levinthal[23] with several modifications. The reaction mixture contained 1 mmol of *p*-nitrophenyl phosphate (PNPP) in 3.0 ml of 1 M Tris buffer (pH 9.0), test compound in 50% dimethyl sulfoxide (0.1 ml), and 0.1 ml of enzyme (0.03 unit) dissolved in 1 M Tris buffer (pH 9.0). The AP (Sigma Chemical Co.) consists of 12.0 mg of protein/ml and 12 units/mg of protein suspended in 2.5 M $(NH_4)_2SO_4$; 1 unit will hydrolyze 1 μmol of *p*-nitrophenyl phosphate per minute at pH 10.4 (37°). The reaction was initiated by adding substrate to a mixture of enzyme and test compound which had been allowed to incubate for 15 min at 37°. The solubility limit of **5** under the assay conditions was 0.1 mmol. **9** had to be dissolved in 60% Me_2SO solution due to its lack of solubility and could not be assayed at concentrations above 0.05 mmol. The reaction rate was determined spectrophotometrically by measuring the initial increase in absorption at 410 nm, resulting from the hydrolysis of PNPP to *p*-nitrophenol, at room temperature in a cell with a 1-cm path length. For controls, enzyme was incubated for 15 min in the presence of 50% dimethyl sulfoxide solution before adding substrate.

Antitumor Evaluation. Tumor transplantation and measurement of inhibition were performed according to previously described procedures.[24] In the survival studies the animals were distributed into groups of six mice of comparable weight (±1 g) and maintained throughout on Purine Laboratory Chow pellets and water ad libitum. Compounds were administered ip beginning 24 h after tumor implantation (day 1), one injection being given on day 1, 3, and 5. All test compounds were finely ground and suspended in sterile normal saline containing 0.1% Tween 80 unless they were soluble in normal saline. All compounds were administered in volumes of 0.2–0.4 ml with controls given injections of comparable volume of vehicle. In another control experiment, treatment of mice bearing Ehrlich ascites carcinoma with the highest doses of ferrous sulfate (25.5 mg/kg) or cupric chloride (8.5 mg/kg) did not extend survival time or produce host toxicity. When compound and metal ion were administered concurrently solutions of the two were mixed in the syringe prior to injection. Mice were observed each day until death or sacrifice and the average life span was calculated.

Acknowledgment. The authors wish to thank Dr. A. C. Sartorelli, Department of Pharmacology, Yale University, New Haven, Conn., for the generous supply of compounds **16** and **17**. This investigation was supported by U.S. Public Health Service Grant CA-13123 from the National Cancer Institute to L.A.C.

References and Notes

(1) G. Domagk, R. Behnisch, F. Mietzsch, and H. Schmidt, *Naturwissenshaften*, **33**, 315 (1946).
(2) B. Prescott, *J. Med. Chem.*, **10**, 484 (1967), and papers cited therein.
(3) D. R. Williams, *Chem. Rev.*, **2**, 203 (1972).
(4) K. C. Agrawal, B. A. Booth, S. M. DeNuzzo, and A. C. Sartorelli, *J. Med. Chem.*, **18**, 368 (1975), and papers cited therein.
(5) H. G. Petering, H. H. Buskirk, and G. E. Underwood, *Cancer Res.*, **24**, 367 (1964), and papers cited therein.
(6) J. G. Cappuccino, M. Arakawa, and M. E. Balis, *J. Med. Chem.*, **11**, 399 (1968).
(7) B. A. Gingras, R. W. Hornal, and C. H. Bayley, *Can. J. Chem.*, **38**, 712 (1960).
(8) G. J. Van Giessen and H. G. Petering, *J. Med. Chem.*, **11**, 695 (1968).
(9) B. A. Gingras, T. Suprunchuk, and C. H. Bayley, *Can. J. Chem.*, **40**, 1053 (1962).
(10) K. C. Agrawal, B. A. Booth, R. L. Michand, E. C. Moore, and A. C. Sartorelli, *Biochem. Pharmacol.*, **23**, 2421 (1974).
(11) B. A. Gingras, R. L. Somorjai, and C. H. Bayley, *Can. J. Chem.*, **39**, 973 (1961).
(12) J. G. Cappuccino, S. Banks, G. Brown, M. George, and G. S. Tarnowski, *Cancer Res.*, **27**, 968 (1967).
(13) K. D. Agrawal, B. A. Booth, E. C. Moore, and A. C. Sartorelli, 10th Annual Meeting of the American Society of Clinical Oncology and 65th Annual Meeting of the American Association for Cancer Research, March 27–30, 1974, Abstracts, p 14.
(14) P. D. Mooney, B. A. Booth, E. C. Moore, K. C. Agrawal, and A. C. Sartorelli, *J. Med. Chem.*, **17**, 1145 (1974), and references cited therein.
(15) K. C. Agrawal, B. N. Booth, and A. C. Sartorelli, 170th National Meeting of the American Chemical Society, Chicago, Ill., August 1975, Abstracts, MEDI 47.
(16) K. C. Agrawal, M. H. Lee, B. A. Booth, E. C. Moore, and A. C. Sartorelli, *J. Med. Chem.*, **17**, 934 (1974), and references cited therein.
(17) M. H. Lee, E. Sznycer-Bochner, K. C. Agrawal, M. K. Volpert, and A. C. Sartorelli, *Biochem. Pharmacol.*, **22**, 1477 (1973).
(18) K. C. Agrawal, B. A. Booth, and A. C. Sartorelli, *J. Med. Chem.*, **16**, 715 (1973).
(19) H. Tolksmith, *J. Am. Chem. Soc.*, **84**, 2097 (1962).
(20) N. N. Mel'nikov and A. G. Zenkevich, *Zh. Obshch. Khim.*, **25**, 828 (1955); *Chem. Abstr.*, **50**, 2415*d* (1956).
(21) N. Kornblum and H. W. Frazier, *J. Am. Chem. Soc.*, **88**, 865 (1966).
(22) D. Jerchel, J. Heider, and H. Wagner, *Justus Liebigs Ann. Chem.* **613**, 153 (1958).
(23) A. Garen and C. Levinthal, *Biochim. Biophys. Acta*, **38**, 470 (1960).
(24) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacker, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3 (1972).