Phosphorus–Nitrogen Compounds XXIII: Oncolytic Phosphorylated Imines

LINDLEY A. CATES ˣ and VEN-SHUN LI

Received March 18, 1981, from the *Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Houston, Houston, TX 77004.* Accepted for publication July 22, 1981.

Abstract □ A series of 15 amidine, iminopiperidine, iso(thio)urea, and guanidine derivatives and six 2,5-dihydro-1,3,5,2-triazaphosphorines were synthesized. Most of the compounds were tested for ability to react with L-cysteine and for antitumor activity against sarcoma 180 and P-388 murine tumor systems. Three acyclic phosphorylated imines and one triazaphosphorine showed activity in the former model to indicate that the P(O)N=C grouping serves as an oncolytic moiety. All agents condensed with L-cysteine with the active antitumor compounds displaying a tendency for relatively higher reactivity with this amino acid.

Keyphrases □ Phosphorus–nitrogen compounds—oncolytic phosphorylated imines □ Antitumor agents, potential—phosphorylated imines □ Phosphorylated imines—amidine, iminopiperidine, iso(thio)-urea, and guanidine derivatives, synthesis and screening for anticancer activity

A number of synthetic and naturally occurring products possessing α,β-unsaturated carbonyl moieties or their congeners are reported to produce an oncolytic effect *via* a type of Michael-condensation with biologically important nucleophiles. Since compounds that contain a P(O)-N=C grouping would be expected to undergo a similar reaction, a series of cyclic and acyclic phosphorylated imines were synthesized and tested for their ability to condense with L-cysteine and for anticancer activity.

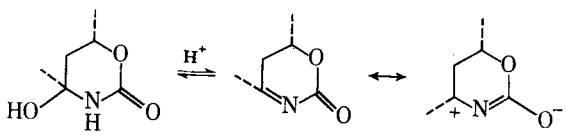
BACKGROUND

Recently, there have been several investigations of conjugated systems that possess anticancer properties. Among those reported are phenyl nonenones (1, 2), styryl ketones (3–6), α-methylene lactams and lactones (7–9), and cyclopentenones (10, 11). Each compound contains a carbonyl function with a lateral overlap between two π-bond systems to produce a molecular orbital encompassing all four atoms. A valence bond representation of the system is $^{+}C—C=C—O^{-}$ where the β-carbon bond carries a positive charge and is subject to nucleophilic attack.

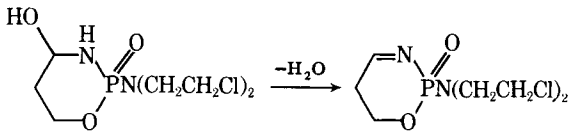
A related system contains an azomethine lactone grouping (IV) arising from the dehydration of carbinolamide derivatives (III) which were synthesized during an investigation of the "eastern zone" of maytansine (12). The dehydration rate of these tertiary alcohols to the conjugated system correlated with their ability to react with DNA bases and to show antineoplastic activity. The carbinolamides also undergo 1,4-addition with alcohols and ethanethiol in the presence of trace acid (13).

The only reported α,β-unsaturated system containing a phosphorus atom and concerned with anticancer activity is a proposed metabolite of cyclophosphamide (14). Iminophosphamide(VI) is thought to arise from the dehydration of 4-hydroxycyclophosphamide(V), providing an alternative explanation for the presence of 4-ethoxycyclophosphamide in microsomal incubation mixtures of V treated with ethanol.

Cyclophosphamide remains the most used and investigated chemotherapeutic of this chemical type. Studies, including those involving metabolism, have not completely accounted for this superior effectiveness. It can be speculated that the P(O)N=C moiety of VI may provide alkylating properties in addition to those of the nitrogen mustard group.



III IV



V VI

This functionality may permit the *in vivo* formation of substituted derivatives as stable, and less toxic transport forms. Support for this latter hypothesis is given by studies with 4(*S,R*) sulfido cyclophosphamide derivatives as stabilized forms of activated V (15).

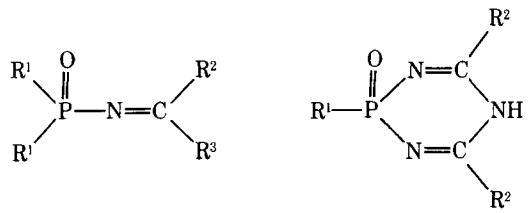
The biological nucleophile most frequently associated with 1,4-addition to α,β-unsaturated system is L-cysteine, alone or as part of a protein molecule (16). However, correlation between L-cysteine and cytotoxicity was not observed in certain sesquiterpene lactones (17) and α-methylene lactones (9).

With this in mind, a series of acyclic(I) and cyclic(II) phosphorylated imines were synthesized for an investigation of their antitumor and L-cysteine addition properties (Table I).

EXPERIMENTAL

All solvents used were spectranalyzed or reagent grade. Melting points were determined by the capillary method (oil bath) and are corrected to reference standards. IR[1] (potassium bromide or neat for Ic), UV[2], and mass[3] spectra were recorded. ^{1}H-NMR[4] (in deuterochloroform) and ^{13}C-NMR[5] (in dimethylsulfoxide-d_6) spectra were measured in parts per million with tetramethylsilane as the internal standard. Elemental microanalyses were performed[6]. Silica gel 60 (70–230 mesh) was used for column chromatography and silica gel GHLF[7] was used for TLC.

Seven compounds [Ie, If, Ij, Ik (18), Ig, Ih (19), and In (20)] were prepared using literature methods employing the usual reaction of phosphoro(di)chloridates with compounds possessing imino groups or their salts. The catalytic hydrogenolysis of Ij gave Ia (18). Compounds Ib, Ii, Il, and Im were synthesized from *N*-methyl-1-chloro-*N'*-(dichlorophosphinyl)formamidine according to the procedure of Derkach and Narbut (21), while the triazaphosphorines IIa–IId were prepared by a novel reaction involving an intramolecular cyclization of bis imides (20). Compound Ic was prepared previously (22) from triethylphosphite and ethyl *N*-chloroacetamidine and isolated by vacuum distillation (bp 49–50°, 0.08 mm Hg). Compound Ic was synthesized using 4.7 g (0.05 mole) of acetamidine hydrochloride and 8.6 g (0.05 mole) of diethyl phosphorochloridate in the presence of 11.1 g (0.11 mole) of triethylamine and the product purified by chromatography. A solution of the chloridate in 50 ml of methylene chloride was added dropwise with stirring under nitrogen to the amidine salt and triethylamine in methylene chloride (100

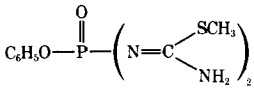
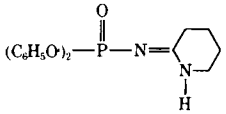


I II

[1] Perkin-Elmer 282 spectrophotometer.
[2] Perkin-Elmer 200 spectrophotometer.
[3] Hewlett-Packard 58930 GC/MS with 5933A data system.
[4] Varian Associates T-60 spectrometer.
[5] By Dr. G. E. Martin, employing a Varian Associates XL-100 spectrometer.
[6] Atlantic Microlab, Inc., Atlanta, Ga.
[7] Analtech.

0022-3549/82/0300-0308$01.00/0

Table I—Acyclic (I) and Cyclic (II) Phosphorylated Imines

Compound	R^1	R^2	R^3	Melting Point or Boiling Point/mm	Formula
I*a*	HO	CH_3	NH_2	166–167°[a]	$C_2H_7N_2O_3P$
I*b*	CH_3O	CH_3O	CH_3NH	74–75°/0.15[b]	$C_5H_{13}N_2O_4P$
I*c*	C_2H_5O	CH_3	NH_2	54–56°[c]	$C_6H_{15}N_2O_3P$
I*d*	C_2H_5O	C_6H_5	C_6H_5NH	122–123°	$C_{16}H_{21}N_2O_3P$
I*e*	C_6H_5O	NH_2	NH_2	117–118°[d]	$C_{13}H_{14}N_3O_3P$
I*f*	C_6H_5O	CH_3	NH_2	86–87°[e]	$C_{14}H_{15}N_2O_3P$
I*g*	C_6H_5O	CH_3S	NH_2	76–77°[f]	$C_{14}H_{15}N_2O_3PS$
I*h*	C_6H_5O	CH_3O	NH_2	78–79°[g]	$C_{14}H_{15}N_2O_4P$
I*i*	C_6H_5O	C_6H_5O	CH_3NH	84–85°[h]	$C_{20}H_{19}N_2O_4P$
I*j*	$C_6H_5CH_2O$	CH_3	NH_2	90.5–91.5°[i]	$C_{16}H_{19}N_2O_3P$
I*k*	$C_6H_5CH_2O$	H	NH_2	110–112°[j]	$C_{15}H_{17}N_2O_3P$
I*l*	n-C_4H_9NH	n-C_4H_9NH	CH_3NH	95–97°	$C_{14}H_{34}N_5OP$
I*m*	C_6H_5NH	C_6H_5NH	CH_3NH	163–166°[k]	$C_{20}H_{22}N_5OP$
I*n*				114–114.5°[l]	$C_{10}H_{15}N_4O_2PS_2$
I*o*				131.5–132°	$C_{17}H_{19}N_2O_3P$
II*a*	C_2H_5O	CH_3		231–232[l]	$C_6H_{12}N_3O_2P$
II*b*	C_2H_5O	C_6H_5		198–200°[l]	$C_6H_{12}N_3O_2P$
II*c*	C_6H_5O	CH_3		303–305°[l]	$C_{10}H_{12}N_3O_2P$
II *d*	C_6H_5	CH_3		270–272°[l]	$C_{10}H_{12}N_3OP$
II *e*	$(ClCH_2CH_2)_2N$	CH_3		191–192°	$C_8H_{15}Cl_2N_4OP$
II *f*	$(ClCH_2CH_2)_2N$	C_6H_5		171–173°	$C_{18}H_{19}Cl_2N_4OP$

[a] Lit. mp 161°; ref. 18. [b] Lit. bp 100–103°/0.6 mm; ref. 21. [c] See *Experimental*. [d] Lit. mp 118°; ref. 18. [e] Lit. mp 86–87°; ref. 18. [f] Lit. mp 78°; ref. 19. [g] Lit. mp 80°; ref. 18. [h] Lit. mp 86–87°; ref. 21. [i] Lit. mp 92.5°; ref. 18. [j] Lit. mp 116; ref. 18. [k] Lit. mp 149–150°; ref. 21. [l] Reference 20.

ml) at 0°. The reaction mixture was stirred to room temperature over a 2-hr period and refluxed for 12 hr. The mixture was filtered, spin evaporated, and the residue dried under vacuum. This material was extracted with benzene, filtered, and the residue dried under vacuum. The crude product was chromatographed with 10% methanol in chloroform to yield the pure material; IR: 3380, 3180 (NH_2), 1658 (N=C), and 1200 (P=O) cm^{-1}; ^{1}H-NMR: 1.32 (t, 6H, $2CH_3$), 2.15 (s, 3H, CH_3), 4.05 (m, 4H, $2CH_2$), and 7.12, 7.40 (2bs, 2H, NH_2) ppm; mass spectrum: *m/z*, 194 (M^+) (36.8%) and 195 (M^{+1})(6.0%).

***N*-(Diethoxyphosphinyl)phenylbenzamidine (I*d*)**—To a solution of 4.9 g (0.1 mole) of *N*-phenylbenzamidine and 10.1 g (0.1 mole) of triethylamine in 250 ml of ether was added a solution of 4.3 g (0.025 mole) of diethyl phosphorochloridate in 50 ml of ether, dropwise with stirring. The mixture was refluxed for 4 hr and filtered. The filtrate was concentrated by spin evaporation to yield, after drying in a vacuum desiccator, an analytically pure product; ^{1}H-NMR: 6.69–7.69 (m, 10H, aromatic), 3.59–4.28 (p, J = 7 Hz, 4H, $2CH_2$), and 1.20 (t, J = 7 Hz, 6H, $2CH_3$).

Anal.—Calc. for $C_{13}H_{14}N_3O_3P$: C, 61.34; H, 6.37; N, 8.43. Found: C, 61.43; H, 6.39; N, 8.42.

***N*-(Diphenoxyphosphinyl)-3-iminopiperidine (I*o*)**—A solution of 26.9 g (0.1 mole) of diphenyl phosphorochloridate in 100 ml of benzene was added dropwise with stirring at 5–10° to a solution of 13.4 g (0.01 mole) of 2-iminopiperidine hydrochloride and 30.3 g (0.3 mole) of triethylamine in 150 ml of benzene. The mixture was refluxed for 3 hr, at which time only two spots developed on a TLC plate (50% benzene in chloroform) at the origin (I_2 vapor) and at R_f 0.41 (UV and I_2 vapor). The mixture was cooled and filtered. Recrystallization of the residue from benzene produced a pure product. ^{13}C-NMR: pyridine carbons, 17.8 and 19.8 (C-4 and C-5), 28.1 (J = 7.1 Hz, C-3), 42.3 (C-6), and 169.3 (J = 4.6 Hz, C-1), 125.4 (C-4), 129.9 (C-3), and 150.0 (J = 7.3 Hz, C-1).

Anal.—Calc. for $C_{17}H_{19}N_2O_3P$: C, 61.81; H, 5.80; N, 8.48. Found: C, 61.89; H, 5.81; N, 8.47.

2-(Bis-*n*-butylaminophosphinyl)-1-*n*-butyl-3-methylguanidine (I*l*)—To a solution of 6.67 g (0.066 mole) of triethylamine and 4.82 g (0.066 mole) of *n*-butylamine in 20 ml of benzene, precooled to −10° and under a nitrogen atmosphere, was slowly added a solution of 4.10 g (0.02 mole) of *N*-methyl-1-chloro-*N'*-(dichlorophosphinyl)formamidine (21) in 60 ml of benzene. The reaction mixture was stirred at room temperature for 12 hr, filtered, the filtrate washed three times with water, and the benzene layer dried over anhydrous potassium carbonate. The benzene was removed under reduced pressure to yield a glassy solid. The crude product was purified by column chromatography using chloroform–ether (9:1) as the eluent. Recrystallization from methylene chloride–ether gave a white solid; IR: 3280 (NH), 1630 (C=N), and 1120, 1090 (P=O) cm^{-1}; ^{1}H-NMR: 0.65–1.60 (m, 21H, 3 $CH_3CH_2CH_2$), 1.80–2.40 (m, 2H, 2 NHP=O), 2.40–3.30 (m, 9H, CH_3N and 3 CH_2N), and 5.30–6.25 (bd, 2H, 2 NHC=N) ppm; mass spectrum: *m/z*, 247 (100), 191 (48), 149 (44), and 319 (M^+, 41).

Anal.—Calc. for $C_{14}H_{34}N_5OP$: C, 52.62; H, 10.73; N, 21.92. Found: C, 52.56; H, 10.72; N, 21.88.

2-Bis(2-chloroethyl)amino-4,6-dimethyl-2,5-dihydro-1,3,5,2-triazaphosphorine-2-oxide (II*e*)—To a mixture of 4.7 g (0.05 mole) of acetamidine hydrochloride and 1.1 g (0.11 mole) of triethylamine in 250 ml of methylene chloride was added, with stirring, cooling at 5–10°, and under a nitrogen atmosphere, 6.5 g (0.025 mole) of *N*-bis(2-chloroethyl)phosphoramidic dichloride. The reaction mixture was refluxed for 18 hr and filtered. The filtrate was spin evaporated and the residue dried under vacuum. The remaining material was extracted with acetone, the solvent removed by spin evaporation, and the crude product chromatographed with 20% methanol in chloroform to give a crystalline solid. This material was recrystallized from chloroform to yield the pure product; IR: 3260 (NH), 1630, 1660 (C=N), and 1210 (P=O) cm^{-1}; ^{1}H-NMR: 2.16 (s, 6H, $2CH_3$) and 2.95–3.75 (m, 8H, $4CH_2$) ppm; mass spectrum: *m/z*, 284 (M^+, 1.9) and 286 (M^{+2}, 1.0).

Anal.—Calc. for $C_8H_{15}Cl_2N_4OP$: C, 33.79; H, 5.32; N, 19.71. Found: C, 33.63; H, 5.39; N, 19.64.

2-Bis(2-chloroethyl)amino-4,6,diphenyl-2,5-dihydro-1,3,5,2-triazaphosphorine-2-oxide (II*f*)—The method employed was the same as for II*e* with the use of 7.8 g (0.05 mole) of benzamidine hydrochloride instead of acetamidine hydrochloride and except for the chromatographic and recrystallization procedures. The first elution was stepwise with 100 ml of chloroform followed by 2, 5, and 10% of methanol in chloroform. Recrystallization from methylene chloride gave the pure product; IR: 1610, 1630 (C=N), 1580 (C=C), and 1200 (P=O) cm^{-1}; ^{1}H-NMR: 3.0–3.7 (m, 8H, $4CH_2$), 7.2–8.9 (m, 10H, aromatic), and 11.5 (bs, 1H, NH) ppm; mass spectrum: *m/z*, 408 (M^+, 1.8) and 410 (M^{+2}, 0.8).

Anal.—Calc. for $C_{18}H_{19}Cl_2N_4OP$: C, 52.80; H, 4.68; N, 13.69. Found: C, 52.76; H, 4.73; N, 13.66.

An improved method for the preparation of *N*-bis(2-chloroethyl)-phosphoramidic dichloride for use in the synthesis of II*e* and II*f* was devised. A previous procedure (23) employed vacuum distillation to get this product, whereas a slightly higher yield was obtained using recrystallization for the purification step. A suspension of 50.0 g (0.28 mole) of bis(2-chloroethyl)amine hydrochloride, purified by washing with acetone, in 213.8 g (1.37 moles) of freshly distilled phosphorus oxychloride was refluxed at 135–140° in an oil bath for 15 hr or until a clear mixture was obtained. Excess phosphorus oxychloride was removed under vacuum. The residue was dissolved in warm acetone, filtered, the filtrate spin

Table II—Antitumor Testing

	Sarcoma 180		P-388[a]	
	mg/kg	T/C%[b]	mg/kg	T/C%
I *a*	200	144[c]	200[d]	100
I *b*	400	93	100[e]	110
I *c*	100	132	—	—
I *d*	400	89	100[e]	99
I *f*	100	97	50[f]	104
I *g*	400	112	—	—
I *h*	400	128	200[e]	100
I *i*	400	95	100[e]	93
I *j*	100	110	200[d]	97
I *k*	50	100	100[d]	101
I *l*	50	89	25[g]	108
I *m*	400	89	200[e]	86
I *n*	400	93	—	—
I *o*	25	122	25[e]	99
II *a*	200	109	200[d]	95
II *c*	—	—	200[d]	106
II *d*	200	109	200[d]	92
II *e*	100	144[c]	500[h]	104

[a] Performed by contractors of the National Cancer Institute, National Institutes of Health, according to general screening procedures; Ref. 24. In each case the results from the highest, nontoxic dose are shown. [b] Average survival time (treated/control) × 100. [c] 1 cure/6 mice. [d] Administered five times, every other day, beginning with Day 1. [e] Administered nine times, each day, beginning with Day 1. [f] Results of the repeat study. [g] Administered three times, four days apart, beginning with Day 1. [h] Administered once on Day 1.

evaporated, and the residue dried under vacuum. This material (71.0 g) was refluxed for 0.5 hr with 400 ml of ether, the suspension filtered to remove ether insoluble substances, and the filtrate concentrated to give two crops of pure product totaling 60.8 g (83.8%) melting at 53–55° [lit. (23) mp 54–56°].

A modified procedure of Cassady *et al.* (9) was employed for the measurement of L-cysteine addition. To 2.0 ml of 1.25×10^{-4} *M* L-cysteine in 0.05 *M* phosphate buffer (pH 7.4) under nitrogen atmosphere in a 1-cm cell was added 50 μl of a 10^{-2} *M* solution of the test compound or cyclopentenone in tetrahydrofuran. The reference cell contained 2.0 ml of the L-cysteine solution and 50 μl of tetrahydrofuran. After 4 min, 0.1 ml of 10^{-2} *M* 2,2′-dithiopyridine in tetrahydrofuran was added to each cell, the cells inverted to affect mixing, and the absorbance read at 343 nm. The method was done in triplicate, the values averaged, and the deviations between the three values were calculated. The absorbances of the test compounds were converted to values relative to cyclopentenone, arbitrarily set at 100.

Pharmacological Testing—Groups of six female Swiss albino mice weighing 20 ± 2 g were injected intraperitoneally with 0.1 ml of ascites fluid from donor mice bearing 7-day tumor growths and containing ~7.5×10^6 Sarcoma 180 cells. After 24 hr (Day 1) the control group was intraperitoneally administered 0.2 ml of vehicle consisting of 2% polyoxyethylene (20) sorbitan monooleate in normal saline, and test groups were similarly dosed with 0.2-ml solutions or suspensions of the compounds in vehicle. The injections were repeated every other day for a total of five doses. Animals surviving 30 days from Day 1 were considered to be cured and were calculated as 30-day survivors in the calculation of average survival times (treated/controls × 100). Control groups survived an average of 15.4 ± 2.7 (first study) and 16.5 ± 3.2 (second study) days. Prior to tumor testing the maximum nontoxic dose of each compound, up to 400 mg/kg, was estimated by similar injections of female albino mice with the above preparations.

RESULTS AND DISCUSSION

The only chemistry deemed appropriate for discussion concerns compound Io. Although it was assumed that phosphorylation occurs at the more basic imino nitrogen of 2-iminopiperidine, this starting material also possesses a secondary amine nitrogen capable of substitution. The only product isolated was converted to the hydrochloride salt and both it and 2-iminopiperidine hydrochloride were subjected to ^{13}C-NMR spectral analysis. The pertinent chemical shifts and couplings occurred as singlets at 25.3 and 116.5 ppm, which were assigned to C-2 and C-3 of the piperidine ring, respectively, for the starting material and as doublets at 28.1 and 169.3 ppm for the same carbon atoms in the product. If the piperidino nitrogen were phosphorylated, additional splitting of the ring carbon atoms at the 5- and 6-positions would be expected.

The effect of the compounds on the survival times of mice bearing Sarcoma 180 and P-388 ascites cells is shown in Table II. The Sarcoma 180 testing was done in two sets of experiments, while the P-388 screening was done periodically over a 1-year period. The initial Sarcoma 180 study involved Id, Ig–Ii, and Ik–Im. Four compounds, Id, Ii, Il, and Im possessed bulky substituents on the imino carbon atom. The inactivity of these agents led to speculation that adverse steric or solubility factors were involved. However, Ig and Il, whose toxicity required reduction to a lower dose, also gave negative results. The positive effect of Ih and the initial presumptive activity of If against P-388 (which was not confirmed in later testing) prompted the synthesis of compounds possessing methyl or methoxy and amino or methylamino groups as the two imino carbon substituents. Subsequently, the remaining derivatives, principally those with the aforementioned substituents and several with increased hydrophilicity, were synthesized and tested. Activity was found with IIe > Ia > Ic > Ih and borderline effects with Io and IIa, based on T/C % values. Each of these four active compounds possess methyl, methoxy and amino, or NH grouping on the imino, or comparable, carbon atom. Of the three active imido derivatives, the highest activity was found in those with the greatest hydrophilicity, Ia and Ic. Compound IIe, bearing a nitrogen mustard moiety had the greatest activity, but the effects produced by Ia, Ic, and Ih establish the P(O)N=C system as a new oncolytic grouping, probably acting through an alkylating mechanism. Although seven and ten different atoms or groups were used for R1 and attached to the imino carbon atom, respectively, it is believed that substituents providing optimum electronic, steric, and solubility characteristics leading to the most active derivatives of this nature have yet to be found.

Table III—L-Cysteine Addition

Compound	Relative Reactivity
Cyclopentenone	100.0 ± 8.1
I *g*	79.4 ± 3.6
I *a*	77.3 ± 1.1
II *a*	77.1 ± 5.1
I *k*	74.0 ± 2.2
I *n*	74.0 ± 2.2
I *f*	65.0 ± 6.7
I *c*	64.5 ± 3.3
I *o*	61.7 ± 1.1
I *l*	59.2 ± 3.9
II *e*	54.9 ± 1.1
I *j*	50.4 ± 1.1
II *d*	50.4 ± 1.1
II *b*	49.3 ± 4.5
I *i*	47.1 ± 6.7
I *h*	47.1 ± 2.3
I *e*	40.4 ± 4.5
I *d*	33.6 ± 6.7

Seventeen compounds were also investigated for ability to condense with L-cysteine (Table III). Some derivatives, *e.g.* Im, were not sufficiently soluble for use in the procedure. Of the antitumor agents tested, Ia, IIa, and Ic were among the seven compounds possessing the highest degree of reactivity with the amino acid while Ih gave anomalous results. Based on this evidence, there is no precise correlation between biological and chemical activities but there appears to be some relationship between these effects.

REFERENCES

(1) J. R. Dimmock, P. J. Smith, I. M. Noble, and W. R. Pennekoek, *J. Pharm. Sci.*, **67**, 1536 (1978).

(2) J. R. Dimmock, C. B. Nyathi, and P. J. Smith, *ibid.*, **67**, 1543 (1978).

(3) N. W. Hamon, D. L. Bassenlowski, D. E. Wright, J. R. Dimmock, and L. M. Noble, *ibid.*, **67**, 1539 (1978).

(4) C. B. Nyathi, U. S. Gupta, and J. R. Dimmock, *ibid.*, **68**, 1383 (1979).

(5) J. R. Dimmock, N. W. Hamon, L. M. Noble, and D. E. Wright, *ibid.*, **68**, 1033 (1979).

(6) J. R. Dimmock, C. B. Nyathi, and P. J. Smith, *ibid.*, **68**, 1216 (1979).

(7) M. J. Kornet, *ibid.*, **68**, 350 (1979).

(8) I. H. Hall, K.-H. Lee, C. O. Starnes, S. A. Eigebaly, T. Ibuka, Y.-S. Wu, T. Kimura, and M. Haruna, *ibid.*, **67**, 1235 (1978).

(9) J. M. Cassady, S. R. Byrn, I. K. Stamos, S. M. Evans, and A. McKenzie, *J. Med. Chem.*, **21**, 815 (1978).

(10) K.-H. Lee, T. Ibuka, E.-C. Mar, and I. H. Hall, *ibid.*, **21**, 698 (1978).

(11) K.-H. Lee, E.-C. Mar, M. Okamoto, and I. H. Hall, *ibid.*, **21**, 819 (1978).
(12) J. W. Lown, K. C. Majumdar, A. I. Meyers, and A. Hecht, *Bioorg. Chem.*, **6**, 453 (1977).
(13) A. I. Meyers and C.-C. Shaw, *Tetrahedron Lett.*, **9**, 717 (1974).
(14) C. Fenselau, M.-N. N. Kan, S. S. Rao, A. Myles, O. M. Friedman, and M. Colvin, *Cancer Res.*, **37**, 2538 (1977).
(15) G. Peter and H.-J. Hohorst, *Cancer Chemother. Pharmacol.*, **3**, 181 (1979).
(16) S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, *Science*, **168**, 376 (1970).
(17) S. M. Kupchan, M. A. Eakin, and A. M. Thomas, *J. Med. Chem.*, **14**, 1147 (1971).
(18) F. Cramer and A. Vollmar, *Chem. Ber.*, **91**, 911 (1958).
(19) *Ibid.*, **91**, 919 (1958).
(20) V.-S. Li and L. A. Cates, *J. Heterocycl. Chem.*, **18**, 503 (1981).
(21) G. I. Derkach and A. V. Narbut, *J. Gen. Chem. USSR*, **36**, 330 (1966).
(22) G. I. Derkach and M. V. Kolotilo, *ibid.*, **36**, 85 (1966).
(23) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 655 (1954).
(24) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).

ACKNOWLEDGMENTS

This investigation was supported by Grant CA24970-02, from the National Cancer Institute, National Institutes of Health.

Presented in part at the Medicinal Chemistry and Pharmacognosy Section, 29th National Meeting, APhA Academy of Pharmaceutical Sciences, San Antonio, Tex., November, 1980.

Synthesis and Antimicrobial Activity of Triorganotin 5-Nitro-2-furoates

EUGENE J. KUPCHIK *x, MICHAEL A. PISANO ‡, STEPHEN M. WHALEN *, and JOSEPH LYNCH ‡

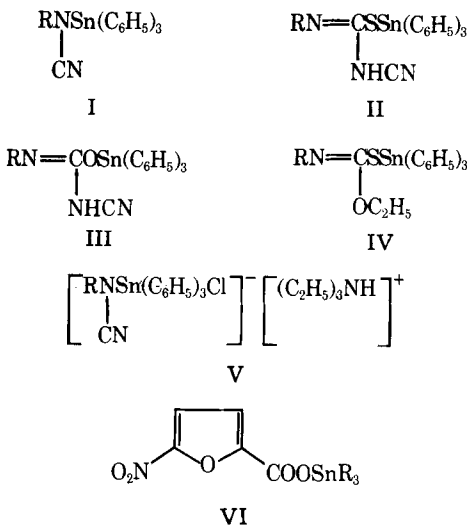
Received June 29, 1981, from the **Department of Chemistry and the ‡Department of Biological Sciences, St. John's University, New York, NY 11439.* Accepted for publication July 27, 1981.

Abstract □ Five triorganotin 5-nitro-2-furoates were synthesized by reacting 5-nitro-2-furoic acid with either the corresponding bis(triorganotin) oxide or the corresponding triorganotin hydroxide. The IR spectrum of each compound was obtained over the 4000–200-cm^{-1} range, and some of the bands were assigned. One compound, tri-*n*-butyltin 5-nitro-2-furoate, was an excellent antifungal agent, completely inhibiting the growth of six of ten test fungi at a concentration of 1 μg/ml. The new compounds were also investigated for antibacterial activity and were especially inhibitory toward Gram-positive species. Two of the compounds completely inhibited the Gram-negative bacterium *Escherichia coli* at a concentration of 100 μg/ml.

Keyphrases □ Organometallics—triorganotin compounds, synthesis, tested for antifungal and antibacterial activity □ Tin—triorganotin compounds, synthesis and evaluation as antifungal and antibacterial agents □ Antifungal agents, potential—triorganotin compounds, synthesis □ Antibacterials, potential—triorganotin compounds, synthesis

Many biocidal applications have been found or suggested for organotin compounds (1, 2). The specific organotin compounds currently used in agriculture were reviewed recently (3–5). Their use in agriculture as fungicides and pesticides is of special interest because they degrade to nontoxic inorganic compounds and, therefore, appear to pose little threat to the environment (6–10). Recently, a series of diorganotin dihalide complexes was shown to exhibit antitumor activity (11).

It was reported previously that *N*-substituted *N*-(triphenylstannyl)cyanamides (I) are better antifungal agents than *N*-substituted *N'*-cyano-*S*-(triphenylstannyl)isothioureas (II) and *N*-substituted *N'*-cyano-*O*-(triphenylstannyl)isoureas (III) (12). The I compounds were similar in activity to ethyl *N*-aryl-*S*-(triphenylstannyl)isothiocarbamates (IV). Triethylammonium (organocyanoamino)chlorotriphenylstannates (V), which are the triethylammonium chloride complexes of the I compounds, reportedly exhibit higher antifungal activity than the I compounds (12). Although all of the compounds mentioned inhibited Gram-positive bacteria, they showed little inhibitory activity toward Gram-negative bacteria. In this respect, they resemble numerous other organotin compounds (13–16). One purpose of the present study was to synthesize some organotin compounds that might inhibit both Gram-positive and Gram-negative bacteria. Since 5-nitro-2-substituted furans are known to inhibit both Gram-positive and Gram-negative bacteria (17–19), the antibacterial activity of some triorganotin 5-nitro-2-furoates (VI) was studied. The antifungal activity of these



0022-3549/82/0300-0311$01.00/0