REFERENCES

(1) J. H. Welsh, *Ann. Rev. Pharmacol.*, **4**, 293(1964).

(2) B. W. Halstead, "Poisonous and Venomous Marine Animals of the World," vol. 1, U.S. Government Printing Office, Washington, D.C., 1965, p. 297.

(3) C. E. Lane, *Ann. Rev. Pharmacol.*, **8**, 409(1968).

(4) F. E. Russell, *Clin. Pharmcol. Ther.*, **8**, 849(1968).

(5) M. H. Baslow, *Ann. Rev. Pharmacol.*, **11**, 447(1971).

(6) J. H. Phillips, *Nature*, **178**, 932(1956).

(7) C. E. Lane and E. Dodge, *Biol. Bull.*, **115**, 219(1958).

(8) R. Blanquet, *Comp. Biochem. Physiol.*, **25**, 893(1968).

(9) N. E. Rice and W. A. Powell, *Biol. Bull.*, **139**, 180(1970).

(10) M. H. Goodwin and M. Telford, *ibid.*, **140**, 389(1971).

(11) J. H. Barnes, in "Animal Toxins," F. E. Russell and P. R. Saunders, Eds., Pergamon, Oxford, England, 1967, p. 115.

(12) J. W. Burnett, J. H. Stone, L. H. Pierce, D. G. Cargo, E. C. Layne, and J. S. Sutton, *J. Invest. Dermatol.*, **51**, 330(1968).

(13) A. G. Gornall, C. J. Bardawill, and M. M. David, *J. Biol. Chem.*, **177**, 751(1949).

(14) J. Litchfield and J. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99(1949).

(15) G. Rosenfeld, E. M. A. Kelen, and F. Nudel, *Mem. Inst. Butantan*, **30**, 117(1960–1962).

(16) H. D. Crone and T. E. B. Keen, *Toxicon*, **7**, 79(1969).

(17) E. H. Baxter and A. G. M. Marr, *ibid.*, **7**, 195(1969).

(18) T. R. Norton and M. Kashiwagi, *J. Pharm. Sci.*, **61**, 1814(1972).

(19) I. J. Goldstein, C. E. Hollerman, and J. M. Merrick, *Biochim. Biophys. Acta*, **97**, 68(1965).

(20) A. E. Clarke and M. A. Denborough, *Biochem. J.*, **121**, 811(1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 4, 1974, from *Pharma-Research Canada Limited, Pointe Claire, Quebec H9R 1G8, Canada.*

Accepted for publication April 10, 1974.

The author is grateful to Dr. John B. Lewis and Dr. Finn Sander of the Bellairs Research Institute, McGill University, St. James, Barbados, for the collection and identification of the anemones, to Dr. G. Possanza and Mr. A. Bauen for toxicity assays, and to Dr. J. T. Oliver for his advice in relation to the hemolysis studies.

Phosphorus–Nitrogen Compounds XVI: Phosphoramantadine Derivatives

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Abstract □ Nine derivatives containing adamantyl moieties were synthesized and screened for antileukemic properties. Five 1-*N*-adamantylphosphoramidate esters, phenyl *N*-1-adamantylphosphoramidochloridate, phenyl P-1-aziridinyl-*N*-1-adamantylphosphonamidate, and *N,N'*-diadamantyl-*N''*-bis(2-chloroethyl)phosphoric triamide were inactive. P,P-Bis(1-aziridinyl)-*N*-1-adamantylphosphinic amide, however, showed good activity when tested against L-1210 lymphoid and P-388 lymphocytic leukemia, giving %T/C values of 225 (20 mg/kg) and 244 (10 mg/kg), respectively.

Keyphrases □ Phosphoramantadine derivatives—synthesized and screened for antileukemic properties □ Phosphorus–nitrogen compounds—synthesis and screening of phosphoramantadine derivatives for antileukemic properties □ Amantadines, phosphorylated—synthesis and screening for antileukemic properties □ Anticancer agents, potential—synthesis and screening of phosphoramantadine derivatives □ Antileukemic agents, potential—synthesis and screening of phosphoramantadine derivatives

Amantadine (1-aminoadamantane, I) has been shown to inhibit the penetration of Rous Sarcoma virus in chick embryo cells (1) and to decrease mitosis and DNA synthesis in sea urchin ova (2), chick embryo fibroblasts, and Hela cell cultures (3). It also displayed activity *in vitro* against angiosarcoma and pancreatic sarcoma (4). The *N*-methyl, *N*-acetyl, and *N*-formyl derivatives of amantadine also gave an effect in the latter tumor system (4). Other compounds containing the adamantyl[1] moiety which have shown antineoplastic activity include those classified as thiosemicarbazones (5) and pyrimidines (6). Also, the insertion of an adamantyl grouping into cytarabine resulted in an immunosuppressant effect twice that of the parent molecule (7).

This paper reports the synthesis (Schemes I and II and Table I) and anticancer screening results (Table II) of nine phosphorylated amantadines. New ester derivatives (II–V) were synthesized by the phosphonate–carbon tetrachloride–amine method, while VII and XI were prepared by amidation of VI and IX, respectively. Compound VIII was synthesized by the reaction of I and bis(2-chloroethyl)phosphoramidic dichloride.

EXPERIMENTAL

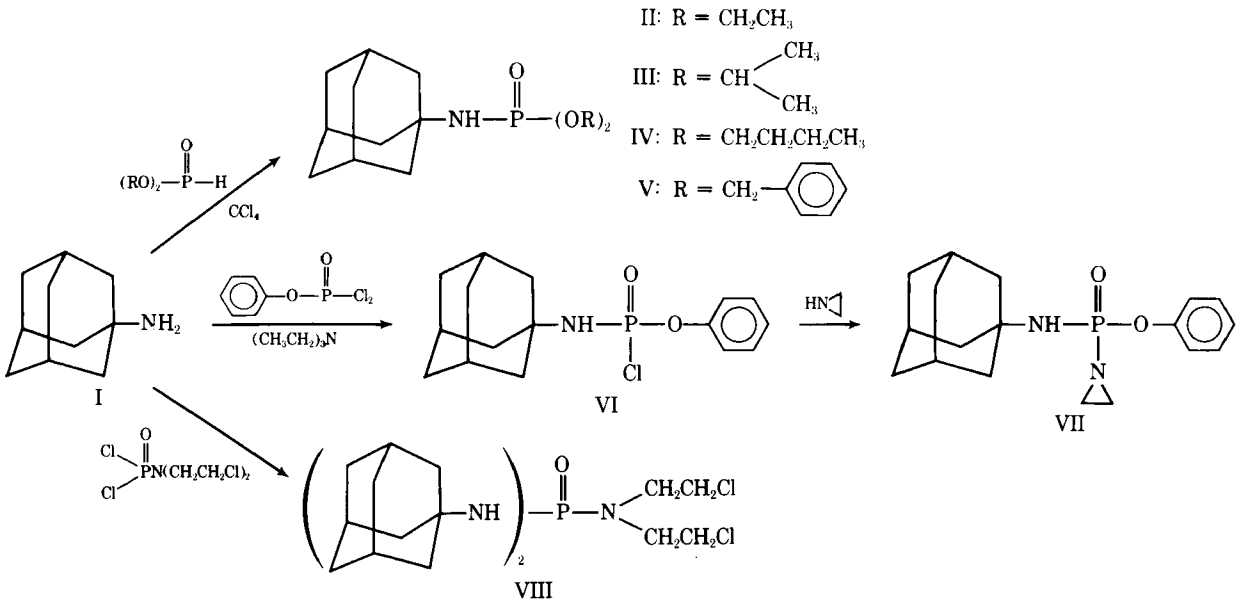
Phosphoramantadine Diesters (II–V)—These compounds were synthesized by the reaction of I, carbon tetrachloride, and the appropriate phosphonate as previously reported for the diphenyl ester (8).

Di-*o*-chlorophenyl *N*-1-Adamantylphosphoramidate (X)—This derivative was prepared from IX, triethylamine, and *o*-chlorophenol according to a previously described procedure (8).

Phenyl *N*-1-Adamantylphosphoramidochloridate (VI)—To a stirred solution of 7.5 g (0.05 mole) of I and 5.0 g (0.05 mole) of triethylamine in 50 ml of anhydrous ether was added 10.5 g (0.05 mole) of phenyl phosphorodichloridate at 30° over 45 min. After remaining overnight, the reaction mixture was filtered and the residue was washed free of chloride with water. Recrystallization from ethanol gave the pure product.

Phenyl P-1-Aziridinyl-*N*-1-adamantylphosphonamidate (VII)—A solution of 3.3 g (0.01 mole) of VI and 1.3 g (0.03 mole) of aziridine in 50 ml of dioxane was refluxed for 4 hr and then left

[1] This name for the radical, rather than the Chemical Abstracts nomenclature of tricyclo(3,3,1,1^{3,7})dec-1-yl, is used in this paper.



Scheme I

overnight at room temperature. The dioxane solution was decanted from the water-soluble oil and treated with water to yield the crystalline product.

P,P-Bis(1-aziridinyl)-*N*-1-adamantylphosphonic Amide (XI)—A solution of 3.9 g (0.09 mole) of aziridine in 50 ml of anhydrous ether was placed in a two-necked, round-bottom flask equipped with a dropping funnel and a condenser fitted with a drying tube. A solution of IX in 75 ml of ether was added dropwise with magnetic stirring at a rate sufficient to maintain reflux conditions. After remaining overnight, the precipitate was collected and washed free of chloride with water. Recrystallization of residue from acetone yielded the white crystalline product.

***N,N'*-Diadamantyl-*N''*-bis(2-chloroethyl)phosphoric Triamide (VIII)**—To a solution of 13 g (0.05 mole) of bis(2-chloroethyl)phosphoramidic dichloride (9) in 60 ml of chloroform was added dropwise a solution of 15.1 g (0.1 mole) of I in 300 ml of chloroform, with stirring and cooling to 0–5°. The reaction mixture was warmed to room temperature and refluxed for 2 hr. The chloroform was removed by spin evaporation, the residue was washed with water, and the remaining solid was dissolved in hot ethanol. The product precipitated in the form of white needles when the ethanol solution was cooled.

RESULTS AND DISCUSSION

The substitution of an adamantyl moiety for that of an aziridinyl results in a pronounced change in physical properties. Triethylenephosphoramide, for example, is extremely soluble in water, with a melting point of 41° (10), whereas XI has low water and high lipid solubility and melts at 177–178°. This increase in lipid solubility due to the introduction of an adamantyl fragment in any of the derivatives is expected to facilitate passage of the agents from the blood into the central nervous system (CNS). The penetration of the blood–brain barrier is achieved by only a few antineoplastic compounds such as 1,3-bis(2-chloroethyl)-1-nitrosourea. Leukemic cells in the CNS, which occur in about 80% of pa-

Table I—Phosphoramantadines

Compound	Melting Point[a]	Formula		Analysis[b], % Calc.	Found	NMR Spectra[c,d] δ, ppm	Signal	Protons
II	94.5–96.5°	$C_{14}H_{26}NO_3P$	C	58.52	58.67	3.95	m	4
			H	9.12	9.13	1.30	t	6
			N	4.87	4.94			
III	68–71°	$C_{16}H_{30}NO_3P$	C	60.93	60.86	4.54	m	2
			H	9.59	9.59	1.32	d	12
			N	4.44	4.50			
IV	36–39°[e]	$C_{18}H_{34}NO_3P$	C	62.95	62.50	3.98	m	4
			H	9.98	9.77	0.95	m	10[f]
			N	4.08	3.99			
V	96–97.5°	$C_{24}H_{30}NO_3P$	C	70.06	69.87	7.30	s	13
			H	7.35	7.41	5.08	d	4
			N	3.40	3.44			
VI	135–137°	$C_{16}H_{21}ClNO_2P$	Cl	10.88	10.78	7.30	s	5
			N	4.30	4.25			
VII	114–115°	$C_{18}H_{25}N_2O_2P$	C	65.25	65.33	7.25	s	5
			H	7.54	7.67	2.18	d	4
VIII	178–180°	$C_{24}H_{40}Cl_2N_3OP$	Cl	14.51	14.01	3.18 to	m	8
			N	8.60	8.50	3.82		
X	159–161.5°	$C_{22}H_{24}Cl_2NO_3P$	Cl	15.68	15.78	7.75	m	8
			N	3.10	3.13			
XI	177–178°	$C_{14}H_{24}N_3OP$	C	59.77	59.44	2.20	d	8
			H	8.60	8.58			
			N	14.94	14.87			

[a] Performed on a Fisher-Johns apparatus and are corrected. [b] Performed by Atlantic Microlab, Inc., Atlanta, Ga. [c] Taken on a Varian T-60 spectrometer using deuterated chloroform as the solvent and tetramethylsilane as the internal reference. Singlet, doublet, triplet, and multiplet splitting is abbreviated s, d, t, and m, respectively. [d] All products gave the characteristic three NMR signals for the adamantyl grouping which integrated for 3 (δ 2.00–2.04), 6 (δ 1.80–1.90), and 6 (δ 1.60–1.68) protons. [e] Bp 156–159°/0.2 mm. [f] The signal for the remaining four protons occurs at about δ 1.20–1.60 and overlaps with a portion of the signal from the adamantyl protons.

Table II—Antileukemic (L-1210 Lymphoid) Activity[a]

Compound	Dose[b], mg/kg	Toxicity Day[c] Survivors	T/C, %
II	400[d]	5/6	90
	200[d]		98
	100[d]		93
III	400[d]		100
	200[d]		96
	100[d]		95
IV	400[d]		96
	200[d]	5/6	92
	100[d]		100
V	300[e]		95
	150[e]		90
	75[e]		97
VI	75[e]	0/6	—
	50[e]	5/6	—
	25[e]		100
VII	260[f]		90
	130[f]		98
	65[f]		93
VIII	400[f]	5/6	98
	200[f]		94
	100[f]		105
X	400[e]		95
	200[e]		101
	100[e]		101
XI	80[d]	5/6	—
	40[d]		—
	20[d]		225
	20[e]	5/6	—
	13.3[d]		152
	10[e]		145
	9[d]		138
	6[d]		125
	5[e]		132
	4[d]		107
	10[e,g]		244
	6.6[e,g]		211
	4.4[e,g]		183

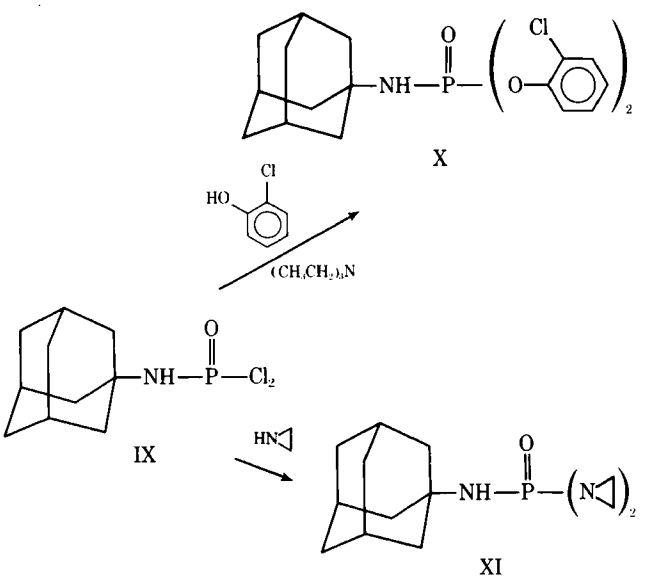
[a] Performed by the screening contractors of the National Cancer Institute, Bethesda, Md. [b] All initial injections were given at Day 1. [c] Number of survivors of total number of animals started on test (recorded on Day 5 in survival systems as a measure of drug toxicity); six out of six survivors unless otherwise indicated. [d] Three injections were administered at 4-day intervals. [e] Nine injections were administered on consecutive days. [f] One injection. [g] P-388 lymphocytic leukemia.

tients (11), can metastasize even though the disease is controlled peripherally, and the eradication of these cells is an important aspect in the chemotherapy of the disease (12).

Similar reasoning is applied to the series II–V[2] but with regard to the ester portion. As the carbon content of the ester grouping increases, so does lipid solubility and these changes in the hydrophilic–lipophilic balance are expected to produce differences in their *in vivo* distribution. The anticipated anticancer effect in these types of compounds is based on that shown by amantadine and/or adamantylphosphoramidic acids, both of which are possible hydrolytic products of the parent structures; the latter compounds are related to acylated amantadine with proven activity. Four derivatives possess moieties, in addition to the adamantyl, with demonstrated cytotoxic properties. Two of the groupings, the bis(2-chloroethyl)amino (in VIII) and the aziridinyl (in VII and XI), are well recognized for these activities.

Anticancer screening (Table II) indicates that a good antileukemic effect is produced by XI. The highest %T/C value was 225 (20 mg/kg), while acute toxicity was not noted until a fourfold dose was reached. Against lymphocytic P-388 in more limited testing, XI gave a %T/C value of 244 (10 mg). The other compounds have not, as yet, given indication of beneficial effect in this tumor system. As anticipated, the phosphoramidochloridate VI was the most toxic compound. The lack of activity by the aziridinyl derivative VII may be attributable to its operating as a monofunctional

[2] Compound II was independently synthesized and reported by V. D. Warner, D. B. Mirth, A. S. Dey, S. S. Turesky, and B. Soloway, *J. Med. Chem.*, **16**, 1185(1973).



Scheme II

alkylator. It has been established (14) that tumor systems vary in their sensitivity to these two chemical types. On this basis, however, it is difficult to rationalize the absence of activity in VIII, a nitrogen mustard derivative.

REFERENCES

(1) N. Oker-Blom and L. Andersen, *Eur. J. Cancer*, **2**, 9(1966).

(2) P. Soupart, R. A. Rinaldi, and V. M. Pickel, *J. Reprod. Fert.*, **20**, 349(1969).

(3) V. Balode and R. A. Gibadulin, *Latv. PSR Zinat. Akad. Vestis*, **6**, 104(1969); through *Chem. Abstr.*, **71**, 69093r(1969).

(4) V. N. Kolmykova, *Vop. Onkol.*, **14**, 89(1968); through *Chem. Abstr.*, **69**, 42580u(1968).

(5) B. Prescott, G. Lones, C. Peacock, and G. Caldes, *Antimicrob. Ag. Chemother.*, **1969**, 275.

(6) J. P. Jonak, S. F. Sakrequewski, L. H. Mead, and M. T. Hakals, *J. Med. Chem.*, **13**, 1170(1970); J. P. Jonak, S. F. Sakreqewski, and L. H. Lawrence, *ibid.*, **14**, 408(1971).

(7) M. E. Greig and A. J. Gibbons, *Arch. Int. Pharmacodyn. Ther.*, **182**, 364(1969).

(8) L. A. Cates, R. L. Gallio, and M. B. Cramer, *J. Pharm. Sci.*, **62**, 1719(1973).

(9) O. M. Friedman, E. Boger, V. Grubliauskas, and H. Sommer, *J. Med. Chem.*, **6**, 50(1963).

(10) "The Merck Index," 7th ed., Merck and Co., Inc., Rahway, N.J., 1960.

(11) "Research Report: Progress Against Leukemia," DHEW (NIH) Pub. 73-367, 1972, p. 10.

(12) C. Hansch, N. Smith, R. Engle, and H. Wood, *Cancer Chemother. Rep.*, **56**, 443(1972).

(13) J. H. Billman, F. Koehler, and R. May, *J. Pharm. Sci.*, **58**, 767(1969).

(14) R. J. Cushley and H. G. Mautner, *Tetrahedron*, **26**, 2151(1970), and references cited therein.

(15) T. A. Connors, *Cancer Res.*, **29**, 2443(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 28, 1973, from the *Department of Medicinal Chemistry, College of Pharmacy, University of Houston, Houston, TX 77004*

Accepted for publication April 29, 1974.

Presented to the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, San Diego meeting, November 1973.

Supported in part by Grant E-297 from the Robert A. Welch Foundation, Houston, Tex., and Grant CA-13123 from the National Cancer Institute, U.S. Public Health Service, Bethesda, MD 20014

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