Phosphorus–Nitrogen Compounds VII. Urethan Derivatives

By L. A. CATES and J. A. NELSON

A series of phosphorylated urethans was synthesized for antitumor screening. Results indicate that the P(O)NHC(O)OEt moiety does not contribute directly to an oncolytic effect against lymphoid leukemia.

BIS(1-AZIRIDINYL)PHOSPHINYL CARBAMATES have been extensively investigated as potential anticancer drugs (1–4); these derivatives being synthesized specifically to incorporate two biologically active groups into a single molecule, *i.e.*, the so-called "dual antagonist." Papanastassiou and Bardos (2) prepared a phosphorylated urethan lacking an aziridine group to support their premise that for a dual antagonist "each synergistic component should be present in the molecule in such form (chemical linkage) that it can exert its biological activity." This "open-chain" analog, *N,N,N′,N′*-tetraethyl-*N″*-carbethoxyphosphoramide, was inactive in Walker carcinosarcoma 256 but equal to urethan against adenocarcinoma 755 in mice. The difficulty these investigators encountered in the synthesis of this compound involved ammonolysis of the labile ester group and separation of the desired carbamate from the corresponding carbamide.

In view of the antileukemic activity of urethan it was considered advisable to prepare further *N*-phosphorylated derivatives (Table I) for testing against this tumor system. None of the phosphorylated urethans (compounds I–V, VII, and IX, Table II) exhibited significant oncolytic properties. These results indicate that previously reported (1) superior activities of bis(1-aziridinyl)phosphinyl carbamates over nitrogen mustards against leukemia may be due to the effect of the carbamate portion on some other factor, such as distribution, absorption, or elimination.[1]

It has been reported that urethan is *N*-hydroxylated *in vivo* and the resulting *N*-hydroxyurethan may be the active metabolite which is responsible for its radio-mimetic effects (5). Also, Skipper *et al.* (6, 7) observed reduced antileukemic activity in *N*-alkyl derivatives of urethan. It is possible, therefore, that *N*-substitution, such as phosphorylation, may interfere with hydroxylation, and thus with the oncolytic activity, of urethan.

Previous studies (8, 9) have indicated that direct phosphorylation of physiologically active amines results in a reduction of activity and toxicity, whereas separation of phosphorus and nitrogen atoms by a carbamoyl group has afforded derivatives with some degree of antitumor activity (10). VIII represents a ureido separation of these atoms and a comparison of its activity with that of VII may provide a basis for the study of related derivatives. A future paper in this series describes the synthesis of phosphoric triamides incorporating urethan and methoxyurea moieties.

The phosphinylcarbamates herein reported were prepared *via* the reaction between dichlorophosphinylurethan and various amines or ammonia. Ammonolysis of carbamate to carbamide was shown, by means of elemental microanalyses and infrared absorption in the 1660 cm.$^{-1}$ (ureas) instead of the 1700–1735 cm.$^{-1}$ range (urethanes) (11), to occur in only one case (IX). The low yield of VI may be accounted for on this basis although no corresponding carbamide was isolated. A high yield of III was obtained despite the use of a strong base which would expectedly result in ammonolysis. VII and VIII were prepared in good yield by condensing phosphinylidynetris isocyanate with ethanol or ethyl carbazate, respectively.

Compounds II–V and IX were incidentally screened for antimalarial activity.[2] None of these derivatives extended survival time of mice infected with lethal doses of *Plasmodium berghei*.

EXPERIMENTAL

Ethyl Bis(diaminophosphinyl) Carbamates[3] (I–VI)—Dichlorophosphinylurethan (2) (0.1 mole) in toluene or benzene (100 ml.) was added to aniline, *p*-toluidine, cyclohexylamine, 2-aminopyridine, or phenylhydrazine (0.4 mole) in toluene or benzene (100 ml.) with stirring and <50°. The reaction mixtures were allowed to remain overnight and filtered, and the residues were washed with water and recrystallized once or twice from the appropriate solvent. In the case of II, the reaction mixture filtrate was spin-evaporated to yield a dark oil which was dissolved in dilute NaOH, treated with charcoal, filtered, and acidified with concentrated HCl. Recrystallization of the resulting white precipitate from ether gave the product. I and IV were similarly dissolved in alkali and precipitated with acid as a purification step. VI was prepared by passing dry ammonia into an ethereal solution of dichlorophosphinylurethan (0.2 mole) for 5 min. with stirring and isolating the product from the precipitate.

Triethyl Phosphenylidynetris Carbamate (VII) and Triethyl 3, 3′, 3″-[phosphinylidynetris (iminocarbonyl)] tricarbazate (VIII)—Ethanol or ethyl carbazate (0.1 mole) in acetonitrile (50 ml.) was added to phosphinylidynetris isocyanate (0.03 mole) (phosphoryl isocyanate, Alfa Inorganics, Inc.) in ether or acetonitrile (200 ml.) with stirring and 5–10°. After remaining overnight, the precipitates were collected, washed with ether, and dried *in vacuo*. Neither product showed absorption in the 2250–2275 cm.$^{-1}$ region (isocyanates).

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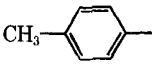
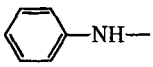
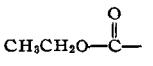
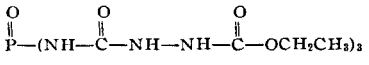
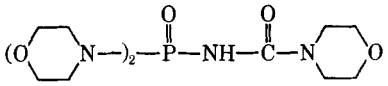
[1] Since the submission of this paper, it has been reported that the replacement of a urethan moiety in bis(2,2-dimethylaziridinyl) phosphinyl carbamate by an ethoxy group gives a much more effective antitumor agent. See Chmielewicz, Z. F., Bardos, T. J., Munson, A., Babbitt, H. I., and Ambrus, J. L., *J. Pharm Sci.*, **56**, 1179(1967).

[2] Testing results furnished by Walter Reed Army Medical Center, Washington, D. C.

[3] Also termed *N*-ethoxycarbonyl-*N′,N″*-disubstituted phosphoric triamides or phosphoramides.

TABLE I—PHOSPHORYLATED URETHANS

Compd.	R	M.p.,[a] °C.	Recrystn.[b] Solvent	Yield, %	ν cm.⁻¹ (C=O)[c]	Formula	Anal., %[d] Calcd.	Found
I		193–196	Et	47	1700	$C_{15}H_{18}N_3O_3P$	C, 56.5 H, 5.7 N, 13.2	C, 56.1 H, 5.8 N, 13.0
II		174–176	E	40	1700	$C_{17}H_{22}N_3O_3P$	C, 58.8 H, 6.4 N, 12.1	C, 58.3 H, 6.4 N, 12.0
III		161–163	Et	81	1700	$C_{15}H_{30}N_3O_3P$	C, 54.4 H, 9.1 N 12.7	C, 54.4[e] H, 9.2[e] N, 12.6[e]
IV		170–175	Et	20	1710	$C_{13}H_{16}N_5O_3P$	C, 48.6 H, 5.0 N, 21.8	C, 48.7 H, 5.2 N, 22.0
V		184–186	Et	70	1700	$C_{15}H_{20}N_5O_3P$	C, 51.6 H, 5.8 N, 20.0	C, 51.5 H, 5.7 N, 19.8
VI	H—	156–158	A	7	1710	$C_8H_{10}N_3O_3P$	C, 21.6 H, 6.0 N, 25.1	C, 21.6 H, 5.9 N, 24.7
VII		145	...	75	1700	$C_9H_{18}N_3O_7P$	C, 34.7 H, 5.8 N, 13.5	C, 34.7 H, 5.9 N, 13.4
Misc.								
VIII		175–177	...	72	1670–1750	$C_{12}H_{24}N_9O_{10}P$	C, 29.7 H, 5.0 N, 26.0	C, 29.6 H, 5.0 N, 25.7
IX		192–195	Et	30	1640	$C_{13}H_{25}N_4O_5P$	C, 44.8 H, 7.2 N, 16.1	C, 44.8 H, 7.4 N, 16.0

[a] Determined on a Fisher-Johns apparatus and are uncorrected. [b] Et, ethanol; E, ether; A, acetone. [c] Obtained with a Beckman IR-8 spectrophotometer using a Nujol mull. All compounds had infrared spectra in agreement with their assigned structures. [d] Determined by Coleman C—H and N analyzers. [e] Performed by Schwarzkopf Analytical Laboratories, Woodside, N. Y.

TABLE II—ANTICANCER SCREENING[a]

Compd.	Test System	Dose, mg./Kg.[d]	T/C × 100
I	LE[b]	400	104
II	LE	400	98
III	LE	200	90
	WM[c]	150[e]	72
IV	LE	400	101
V	LE	400	89
	WM	200	72
VII	LE	400	93
IX	LE	400	107

[a] Data from CCNSC. [b] Lymphoid leukemia L-1210. [c] Walker 256 (intramuscular). [d] Highest dose permitting all test animals to survive. [e] Only dose used; permitted 4/6 survivors.

N-(Dimorpholinophosphinyl)-4-morpholinecarboxamide (IX)—This compound was isolated during an attempted synthesis of the corresponding carbamate using the method described for I–V.

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Keyphrases

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