Synthesis of Hexahydroazepinyl-bis(aziridinyl)phosphine Oxide, Sulfide, and Related Compounds for Cancer Therapy[1]

K. C. TSOU AND KARL HOEGERLE

Central Research Laboratory, The Borden Chemical Company, A Division of The Borden Company, Philadelphia 24, Pennsylvania

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Hexahydroazepinyl-bis(aziridinyl)phosphine oxide (VII), and hexahydroazepinyl-bis(aziridinyl)phosphine sulfide (IX) have been synthesized. Related compounds with variation in number of ethylenimine and hexamethylenimine rings have been made in order to compare antitumor activity. While these compounds are analogs of TEPA and ThioTEPA, it was found that the introduction of the hexamethylenimine ring reduced the toxicity appreciably. The possibility that the 7-membered ring can be opened under biological conditions and act as a mild alkylating agent has been considered. Compound VII and IX are active against animal tumors in the Walker 256 carcinoma and in the Dunning leukemia system. Infrared spectra of all compounds show absorption in the P–N band region 13.50–14.40 μ.

Our interest in the synthesis of hexamethylenimine derivatives of phosphine oxides and sulfides began a few years ago while investigating the amidase hydrolysis of normal and neoplastic tissue on substrates of the N-acyl aziridines. The high reactivity of the ethylenimine ring system makes it extremely difficult to obtain reliable animal screening data through its use. In order to understand alkylating ability better under biological conditions, we were led to consider other groups that might react in ring opening in a manner similar to ethylenimine and act as alkylating agents.

Hexamethylenimine, normally a stable compound, can undergo ring opening under rigorous conditions.[2,3] It was believed reasonable to expect that introduction of this ring system into a known alkylating compound could impart additional mild alkylating potency. Since tris(aziridinyl)phosphine oxide and its thio analog have been shown to be useful drugs, it was of interest for us to learn the effect of introducing the hexamethylenimine ring into these drugs. Furthermore, if toxicity of the drug were in part due to the hydrolysis product, the lower toxicity of hexamethylenimine (approximate lethal oral dose for rats equals 450 mg./kg.) as compared to that of ethylenimine (15 mg./kg.)[4] would be expected to impart to the drug a significant reduction in toxicity. Preliminary clinical results on hexahydroazepinyl-bis(aziridinyl)phosphine sulfide (IX) have been reported elsewhere recently.[5] The present paper reports the synthesis, preliminary screening and attempted correlation of structure and biological activity of such compounds.

The preparation of hexahydroazepinyl dichlorophosphine oxide (I) proceeded with relative ease by adding the hexamethylenimine to phosphorus oxychloride in the presence of a strong organic tertiary amine such as triethylamine. By reversing the addition procedure, bis(hexahydroazepinyl)chlorophosphine oxide (II) was prepared. In order to prepare the tris-(hexahydroazepinyl)phosphine oxide (III) in good yield, it was necessary to use an excess of hexamethylenimine. The reaction of the third hexamethylenimine molecule was extremely slow, due probably to the steric hindrance of the chlorine atom in II. For similar reasons, hexahydroazepinyl-bis(aziridinyl)phosphine oxide (VII) could be prepared from (I) with relative ease, while the synthesis of bis-(hexahydroazepinyl)aziridinylphosphine oxide (VIII) from II was difficult.

Hexahydroazepinyl dichlorophosphine sulfide (IV) and bis(hexahydroazepinyl)chlorophosphine sulfide (VI) were prepared with thiophosphoryl chloride in place of phosphorus oxychloride. The conversion of these chloro intermediates to the corresponding sulfide derivatives, hexahydroazepinyl-bis(aziridinyl)phosphine sulfide and bis(hexahydroazepinyl)-aziridinylphosphine sulfide (X) was easier than were similar conversions in the phosphine oxide series. Tris(hexahydroazepinyl)phosphine sulfide (XI) was only obtained in the presence of an excess of hexamethylenimine. In order to avoid violent polymerization, sodium hydroxide pellets were used during all distillations of the amine-substituted phosphine oxides or sulfides. The physical and analytical data of the compounds are summarized in Table I.

The phosphine sulfides IX and X were screened against Dunning Leukemia (Table II). While these data are preliminary in nature, they do illustrate again the striking difference in toxicity between the compounds with two ethylenimine rings and one ethylenimine ring, respectively. The effectiveness of IX in this screening system is confirmed. The change of one ethylenimine ring to one hexamethylenimine ring in IX gives compound X which is inactive in the dose range tested. Because of the limited sample available, a toxic level was not established. The oxygen analog of X (compound VIII) shows minimal activity in the dose range tested. The bis-hexahydroazepinyl compound III was also tested and was found to be inactive again in the dose range tested. In both cases, no toxic level was reached. Thus the drastic reduction n toxicity from the introduction of the hexamethylenimine ring is illustrated. Compound VII was screened in the Walker 256 tumor system and found to be very active (Table III). The therapeutic index of this compound has been determined to be 6–10, based on a probit plot from the available data. In order to compare the activity of this drug with related analogs, comparative data are given in Table IV. The rela-

(1) This work has been supported by Research Grant CY-2530 from The National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

(2) A. Miller, *Ber.*, **61**, 568 (1928).

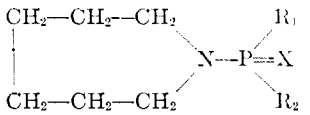
(3) H. Friedrich, German Patent 1,037,126 (1958).

(4) J. P. Danehy and D. J. Pflaum, *Ind. Eng. Chem.*, **30**, 778 (1938).

(5) L. E. Goodman, K. C. Tsou, S. P. Kramer, A. Ulfohn, K. Hoegerle, S. D. Galey, D. Bakal, T. Weinberg and A. M. Seligman, *Proc. Am. Assoc. Cancer Res.*, **3**, 229 (1961).

TABLE I

PHYSICAL AND ANALYTICAL DATA OF HEXAMETHYLENIMINE SUBSTITUTED COMPOUNDS



No.	Compound X	R_1	R_2	M.p., °C.[d]	B.p., °C. (mm.)[d]	Refractive index n_D	Formula	Analyses, %[a] Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen[c] Calcd.	Nitrogen[c] Found
I	O	Cl	Cl	...	89–92 (0.3)	1.5032^{24}	$C_6H_{12}Cl_2NOP$	33.35	33.21	5.60	5.70	6.48	6.50
II	O	Hx[b]	Cl	...	162–3 (0.15)	1.5170^{26}	$C_{12}H_{24}ClN_2OP$	...	...	...	...	10.05	10.30[e]
III	O	Hx	Hx	60–70	199–202 (0.15)	...	$C_{18}H_{36}N_3OP$	63.61	63.52	10.63	10.60	12.31	12.21[f]
IV	S	Cl	Cl	...	65–67 (0.02)	1.5560	$C_6H_{12}Cl_2NPS$	31.05	31.12	5.21	5.10	6.03	6.10[g]
V	S	Hx	Cl	29.5–30	...	...	$C_{12}H_{24}ClN_2PS$	48.88	49.02	8.21	8.47	9.50	9.47[h]
VI	S	Hx	Hx	104–105	...	...	$C_{18}H_{36}N_3PS$	60.46	60.50	10.15	10.21	11.75	12.04[i]
VII	O	E[c]	E	...	96.8 (0.025)	1.5064^{21}	$C_{10}H_{20}N_3OP$	52.39	52.28	8.79	8.83	18.33	18.27[j]
VIII	O	Hx	E	...	135–136 (0.05)	1.5113^{22}	$C_{14}H_{28}N_3OP$	58.93	58.93	9.89	9.92	14.75	14.86[k]
IX	S	E	E	5–6	117 (0.5)	1.5429^{31}	$C_{10}H_{20}N_3PS$	48.96	49.63	8.22	8.20	17.13	17.13[l]
X	S	Hx	E	−10	...	...	$C_{14}H_{28}N_3PS$	55.78	55.79	9.37	9.41	13.94	13.96[m]
XI	S	E	Cl	128–130.5	...	...	$C_8H_{16}ClN_2PS$	40.25	40.76	6.76	6.72	11.74	11.57

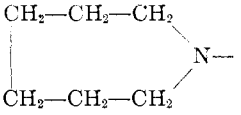
[a] Microanalyses by Dr. S. M. Nagy, Microchemical Laboratory, Belmont, Mass. [b] Hx is Hexahydroazipinyl  [c] E is Aziridinyl  [d] All melting points and boiling points are corrected. [e] Calcd.: P, 11.11. Found: P, 10.70. [f] Calcd.: P, 9.07. Found: P, 9.07. [g] Calcd.: P, 13.35; S, 13.81. Found: P, 13.19; S, 13.86. [h] Calcd.: S, 10.51; Cl, 12.03. Found: S, 10.62; Cl, 12.34. [i] Calcd.: S, 8.97. Found: S, 8.95. [j] Calcd.: P, 13.51. Found: P, 13.75. [k] Calcd.: P, 10.85. Found: P, 10.93. [l] Calcd.: S, 13.43. Found: S, 13.31. [m] Calcd.: S, 10.64. Found: S, 10.31.

TABLE II

EVALUATION OF SOME HEXAHYDROAZEPINYL SUBSTITUTED PHOSPHINE OXIDES AND SULFIDES IN DUNNING LEUKEMIA SYSTEM

Compound	Dose mg./kg.	Vehicle (route)	Survivors	Weight change T/C[a] (g.)	Survival Time T/C (days)	Percentage
IX	100	CMC (I.P.)	0/6	−/44.0	5.0/14.0	35
	50		0/6	−/44.0	5.0/14.0	35
	25		0/6	−/44.0	6.5/14.0	46
	12.5		3/6	22.0/44.0	30.0/14.0	214
	12.5	CMC (I.P.)	2/6	−25.0/40.0	7.0/14.0	50
	6.25		6/6	33.0/40.0	30.0/14.0	214
	3.12		6/6	26.0/40.0	25.5/14.0	182
	1.56		6/6	40.0/40.0	16.0/14.0	125
X	100.0	Oil (S.C.)	3/3	37/33	14.7/14.3	103
	25.0		3/3	17/23	14.0/14.3	98
	5.0		3/3	21/33	14.7/14.3	103
VIII	250.0	Oil (S.C.)	3/3	7/23	18.0/13.7	132
	100.0		3/3	20/23	14.3/13.7	104
	25.0		3/3	32/23	14.3/13.7	104
	10.0		3/3	36/23	13.7/13.7	100
	5.0		3/3	22/23	15.7/13.7	114
III	100.0	Oil (S.C.)	3/3	19/23	13.3/14.3	93
	25.0		3/3	28/23	14.3/14.3	100
	5.0		3/3	24/23	17.0/14.3	119

[a] T/C = Tumor/Control.

tively low toxicity of compound VII and its good therapeutic effectiveness as compared to ThioTEPA and TEPA warrant further extensive study.

The infrared spectra of the compounds prepared in this series provide useful information leading to the determination of the structure of compounds and were used to advantage in preparing highly purified samples for animal screening and clinical evaluation. All the ethylenimine derivatives contain a characteristic 3.25 μ band. The hexamethylenimine ring is characterized by the 3.42 and 3.50 μ $-CH_2-$ stretching bands. The interaction of C–N vibration bands in the 8.0–10.0 μ region is interfered with by the P=O or P=S stretching vibrations and definite assignment has to await more data. Another useful band lies in the P–N region. All compounds prepared in this series have a characteristic band in the 13.75–14.40 μ region. This has been labeled by Bellamy[6] as a doubtful region for P–N stretching vibration because of the absence of any absorption of bis(dimethylamino)-fluorophosphine oxide in this region. The bis(hexahydroazepinyl)chlorophosphine oxide, however, does have an absorption band at 14.15 μ. The tris-dimethylaminophosphine oxide, on the other hand, has been found by us to have a characteristic P–N band at 13.50 μ. It therefore seems to us that the absence of absorption in the case cited by Bellamy[6] is due to the symmetry of the molecule and is an exception to the general rule, and this region can be used safely in the characterization of X=P(<)–N< groups.

(6) L. J. Bellamy "The Infrared Spectra of Complex Molecules," Methuen and Co. Ltd., London, 1956, p. 265.

TABLE IV

COMPARISON OF DRUG EVALUATION OF VII AGAINST WALKER CARCINOMA 256 WITH RELATED ANALOGS

Agent	Host	Dosage schedule (days)	Route	Observation period days	MTD (LD_{10}) (mg./kg.)	Dosage schedule (days)	Days of tumor assessment	MED (T/C 0.10) (mg./kg.)	Therapeutic index LD_{10} T/C (0.10)	Maximum effectiveness T/C at LD_{10} %
A-139[a]	H. Rat	qd 1–5	IP	1–21	0.98	qd 1–5	28	0.24	4	2
TEPA[a]	H. Rat	qd 1–5	IP	1–21	4.3	qd 1–5	28	0.52	8	4
ThioTEPA[a]	H. Rat	qd 1–5	IP	1–21	3.0	qd 1–5	28	0.53	6	1
OPSPA[a]	H. Rat	qd 1–5	IP	1–21	9.6	qd 1–5	28	3.3	3	5
KC-36[b,c]	H. Rat	qd 1–5	IP	1–10	12.5	qd 1–5	14	2.0	6	*ca.* 10

[a] Data from H. E. Skipper and L. H. Schmidt, *Cancer Chemotherapy Reports*, **17**, 70 (1962), A Manual on Quantitative Drug Evaluation in Experimental Tumor Systems. [b] Data obtained through the Cancer Chemotherapy National Service Center and carried out by the Southern Research Institute and should be considered as preliminary. Opinions expressed in the discussion are those of the authors and not of CCNSC. [c] Clinical code-name for VII. Exploratory study is being carried out by the Moss Hospital of Albert Einstein Medical Center under Drs. S. Levick and I. Woldow, Philadelphia, Pennsylvania.

TABLE III

EVALUATION OF HEXAHYDROAZEPINYL-BIS(AZIRIDINYL)PHOSPHINE OXIDE (VII) IN WALKER 256 TUMOR SYSTEM

Compound	Dose mg./kg.	Survivors	Weight change T/C[a]	Tumor weight T/C	Percentage
VII	12.5	6/6	37/66	0/6.3	0
	6.25	6/6	50/66	0/6.3	0
	3.12	6/6	56/66	0/6.3	0
	1.56	6/6	64/66	4.0/6.3	63
	2.34	6/6	47/48	0.1/7.1	1
	1.57	6/6	41/48	0.3/7.1	4
	0.78	6/6	50/48	5.3/7.1	74
	0.39	6/6	43/48	4.8/7.1	67
	41.0	0/6	—	—	—
	20.0	0/6	—	—	—
	10.0	6/6	17/43	0.0/5.5	0
	5.0	6/6	34/43	0.0/5.5	0
	3.12	6/6	55/61	0.0/4.6	0
	2.34	6/6	42/61	0.0/4.6	0
	1.56	6/6	59/61	0.0/4.6	0

[a] T/C = Tumor/Control.

The alkylating potency measurement was carried out by a modification of the method of Epstein and coworkers[7] which is similar to that used by us for the hemisulfur mustard derivatives.[8] Hexamethylenimine forms a reddish color whereas ethylenimine forms a blue color, thus indicating the possible existence of *in vitro* alkylating activity of hexamethylenimine. Quantitative procedure, however, has not been possible because of the instability of the dye formed. Various attempts of *in vivo* demonstration of the alkylating activity have not been successful because of the poor color produced under the conditions used. The screening results do indicate that several of the compounds synthesized in this series are active against animal tumors and are potential candidates for clinical evaluation in cancer chemotherapy. The possible role of hexamethylenimine ring as a mild alkylating group remains speculation.

Experimental

Hexahydroazepinyl-dichlorophosphine Oxide (I).—To a solution of $POCl_3$ (30.6 g., 0.2 mole) in dry benzene (400 ml.) a solution of hexamethyleneimine (19.83 g., 0.2 mole) and triethylamine (20.5 g., 0.22 mole) in dry benzene (100 ml.) was added. The reaction mixture was stirred vigorously without external cooling. After 40 min. the addition was complete, the mixture was heated to 40–50° and then stirred overnight at room temperature. The precipitated triethylammonium chloride (m.p. 255°) was filtered and the filtrate concentrated under reduced pressure on the water bath. The residue (44.6 g.) was distilled twice to give a clear main fraction of 36.8 g. of I.

Bis(hexahydroazepinyl)chlorophosphine Oxide (II).—Hexamethylenimine (61.5 g., 0.61 mole) and triethylamine (61.7 g., 0.61 mole) dissolved in 400 ml. of benzene reacted with $POCl_3$ (30.7 g., 0.2 mole) in 100 ml. of benzene, at room temperature. The addition of the reagents was complete in 1 hr. The reaction mixture was stirred overnight, then heated to reflux for 2 hr. Usual working up of the reaction mixture including extraction of the benzene solution with 2 *N* NaOH solution gave 56 g. of crude oil which after two distillations, gave pure II (40.2 g.).

Tris(hexahydroazepinyl)phosphine Oxide (III).—To a stirred solution of 300 g. of hexamethylenimine in 300 ml. benzene was added 76.7 g. (0.5 mole) of $POCl_3$ dropwise. The reaction mixture was stirred overnight, then was filtered to give 156 g. of hexamethylenimine hydrochloride. The precipitate was washed with ether and the solvents were evaporated from the combined solution and washings. This residue was then stirred on a steam bath with another 350 ml. of hexamethylenimine for 24 hr. After addition of approximately 300 ml. of ether, the precipitate was filtered and washed with ether, giving 203.9 g. (equal to 100% of theor.) of hexamethylenimine hydrochloride. The filtrate was concentrated and the residual oil distilled in a high vacuum. At b.p. 199–202° (0.15–0.2 mm.) 32 g. of product was collected, which crystallized instantly to a soft mass, m.p. 60–70°.

Hexahydroazepinyl-bis(aziridinyl)phosphine Oxide (VII).—To the dichlorophosphine oxide (I) (36.6 g., 0.17 mole) dissolved in 400 ml. of dry benzene, a solution of ethylenimine (14.7 g., 0.34 mole) and triethylamine (34.5 g., 0.34 mole) in 100 ml. of dry benzene was added dropwise. The temperature rose to approximately 40°. The mixture was stirred overnight, filtered, the filtrate concentrated in vacuum and the residue (39 g.) subjected to vacuum distillation. Pure VII was collected at b.p. 96–98° (0.025 mm.); yield, 26.90 g., $n^{21}D$ 1.5064.

Bis(hexahydroazepinyl)-aziridinylphosphine Oxide (VIII).—To a mixture of 43.5 g. (0.34 mole) of triethylamine and 18.5 g. (0.43 mole) of ethylenimine was added 23.9 g. of II with good stirring. After standing overnight, the liquid phase was decanted and the residue taken up in ether. After removal of ether the residual oil was distilled under high vacuum to yield 16.8 g. of a pure fraction of VIII.

Phosphine Sulfides.—The preparation of the corresponding phosphine sulfide compounds was carried out by essentially the same procedure, using thiophosphoryl chloride in place of phosphorus oxychloride. The physical constants are listed in Table I.

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(7) J. Epstein, R. W. Rosenthal and R. J. Ess, *Anal. Chem.*, **27**, 1435 (1955).

(8) K. C. Tsou, C. F. Su, C. Saegebarth and U. Mirarchi, *J. Org. Chem.*, **26**, 4987 (1961).