H_2O and basified with 12 M NH_4OH to precipitate pale violet crystals of 17b. Recrystallization of the crude crystals from EtOH yielded 0.7 g of pure 17b as colorless crystals: mp 204°. Anal. ($C_{15}H_{21}NO$) C, H, N.

Similarly, compounds 17c–e were obtained from 16c–e in 40, 41, and 79% yield, respectively. 17c: mp 165° (from EtOH). Anal. ($C_{15}H_{21}NO$) C, H, N. 17d: mp 245–252° (from EtOH). Anal. ($C_{15}H_{21}NO$) C, H, N. 17e: mp 208–210° (from MeOH). Anal. ($C_{16}H_{23}NO$) C, H, N.

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Synthesis of Aziridinylallylaminophosphine Oxides and Sulfides as Potential Adjuvant Cancer Chemotherapeutic Agents

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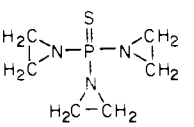
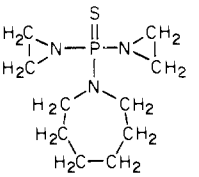
Received July 15, 1975

Bis(1-aziridinyl)(hexahydro-1*H*-azepin-1-yl)phosphine sulfide, an active anticancer agent with low hematopoietic toxicity in animals and man, was recommended several years ago for breast cancer adjuvant chemotherapy as an alternate drug to thiotepa. This hope had led to the syntheses of aziridinylallylaminophosphine oxides or sulfides (compounds I–XVII) in our laboratories. The resurgent interest in this area of cancer chemotherapy encouraged us to report our synthetic work as well as their evaluation as both anticancer agents and insect chemosterilants. Based on observed antitumor activity in animals, low chemosterilant activity in female species (insects and rats), and histochemical observation of tissue toxicity in rat testes but not in ovaries, these new agents are of potential interest to the breast cancer adjuvant chemotherapy program.

Bis(1-aziridinyl)(hexahydro-1*H*-azepin-1-yl)phosphine sulfide,[1] an active anticancer agent with low hematopoietic toxicity in animals and man, was recommended several years ago for breast cancer adjuvant chemotherapy as an improvement over thiotepa.[2] This work had provided impetus for us to search for other related drugs that could be useful for similar applications. Allylamino derivatives of some aziridinylphosphine oxides and sulfides were found to have activity in several animal tumor models.[3] Furthermore, the introduction of the diallylamino group does not lead to carcinogenicity. β-(1-Aziridinyl)diallylaminopropionamide was found to cause 100% lymphoma regression in rats without inducing subsequent mammary tumor development in the same rats.[4] These results suggested to us that it might be worthwhile to synthesize additional candidate agents in this area of cancer chemotherapy. The resurgent interest in breast cancer adjuvant chemotherapy with a nitrogen mustard analogue L-phenylalanine (L-PAM)[5] encouraged us to report the syntheses of these new phosphoramides, together with their biological evaluations.



tris(1-aziridinyl)phosphine sulfide ("thiotepa")



bis(1-aziridinyl)(hexahydro-1*H*-azepin-1-yl)-phosphine sulfide ("thiohexadepa")

Syntheses. The syntheses of these aziridinyl allylaminophosphine oxides and sulfides were carried out similar to our previous work on the azepinyl analogues and are shown in Scheme I. Essentially, diallylamine was allowed to react with either phosphorus oxychloride or thiophosphoryl chloride to yield the corresponding phosphochloridate or thiophosphochloridate. These intermediates were then used to couple with aziridine or methylaziridine under anhydrous conditions and in the presence of a base such as triethylamine. Further purification was often necessary and high-vacuum distillation over sodium hydroxide pellets was used to obtain polymer-free, analytically pure samples. The preparation

Scheme I

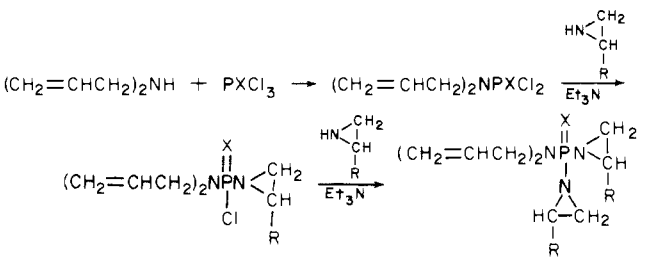


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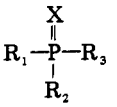
Table I. Physical Data of Some Phosphoramides



Compd	X	R_1	R_2	R_3	Yield, %	Bp (mm), °C	Refractive index (°C)	Mol formula	Analyses
I	O	DA[a]	Cl	Cl	50	87 (0.5)	1.4842 (20)	$C_6H_{10}Cl_2NOP$	C, H, N
II	S	DA	Cl	Cl	38	70-72 (0.05)	1.5379 (20)	$C_6H_{10}Cl_2NPS$	C, H, N
III	O	DA	E[b]	E	27	117 (0.4)	1.4948 (20)	$C_{10}H_{18}N_3OP$	C, H, N
IV	S	DA	E	E	18	110-115 (0.15)	1.5340 (20)	$C_{10}H_{18}N_3PS$	C, H, N
V	O	DA	ME[c]	ME	22	80-86 (0.4)	1.4890 (20)	$C_{12}H_{22}N_3OP$	C, H, N, S
VI	S	DA	ME	ME	20	92-94 (0.2)	1.5297 (20)	$C_{12}H_{22}N_3PS$	C, H, N
VII	O	DA	E	ME	16	97.5 (0.4)	1.4910 (20)	$C_{11}H_{20}N_3OP$	C, H, N
VIII	S	DA	E	ME	23	93-94 (0.15)	1.5307 (20)	$C_{11}H_{20}N_3PS$	C, H, N, S
IX	O	DA	DA	Cl	72	122 (0.3)	1.4914 (24)	$C_{12}H_{20}ClNOP$	Not analyzed
X	O	DA	DA	E	27	109-123 (0.25)	1.4967 (24)	$C_{14}H_{24}N_3OP$	C, H, N
XI	O	DA	DA	ME	18	82-92 (0.3)	1.4876 (24)	$C_{15}H_{26}N_3OP$	C, H, N
XII	O	PhA[d]	Cl	Cl	17	90-95 (0.025)	1.5500 (25)	$C_9H_{10}Cl_2NOP$	Unstable, not analyzed
XIII	O	PhA	E	E	20	54 (0.05)	1.5576 (26)	$C_{13}H_{18}N_3OP$	C, H, N
XIV	O	CA[e]	Cl	Cl	44	89-94 (0.1)	1.5020 (24)	$C_9H_{15}Cl_2NOP$	C, H, N, P, Cl
XV	S	CA	Cl	Cl	13	110.5-112.5 (0.35)	1.5485 (25)	$C_9H_{15}Cl_2NPS$	C, H, N, S, Cl
XVI	O	CA	ME	ME	37	111-115 (0.15)	1.4910 (25)	$C_{15}H_{27}N_3OP$	C, H, N, P
XVII	O	CA	E	E	88	120-124 (0.2)	1.5060 (24)	$C_{13}H_{23}N_3OP$	C, H, N, P

[a] DA = $(CH_2{=}CHCH_2)_2N$-. [b] E = c-CH_2CH_2N-. [c] ME = CH_3-c-$CHCH_2N$-. [d] APh = $CH_2{=}CHCH_2NPh$. [e] CA = -C_6H_{10}-$NCH_2CH{=}CH_2$.

of the mixed aziridinyl compounds was very difficult, and these derivatives could be isolated only in low yield. Preparation of phenylallylamine and cyclohexylallylamine derivatives was carried out in a similar manner. The physical constants of the compounds are listed in Table I.

Anticancer Screening and Structure–Activity Relationship. The results of antitumor tests were obtained through the generous cooperation of the Division of Cancer Treatment of the National Cancer Institute. Testing was done in Walker 256 (WA), leukemia 1210 (L1210), and, more recently, in P-388 leukemia (PSS) systems. Although it was not always possible to screen in all systems, sufficient data are now available from the last few years to enable us to make some comments on structure–activity relationships (Tables II and III). It was noted that in these compounds, the thio analogue was more toxic than the oxygen analogue. This result was not anticipated since it had been previously shown that the replacement of oxygen in tris(1-aziridinyl)phosphine oxide (Tepa) and bis(1-aziridinyl)-1-azepinylphosphine oxide (Hexadepa) by sulfur resulted in lower toxicity. This difference was related to the in vivo hydrolysis of thiotepa to Tepa[6] and presumably also in the azepinyl analogue. Hence, it might be that the P=S compound does not have to be converted to the P=O compound to be physiologically active. Replacement of aziridine in compound III with compound V significantly reduces the toxicity of the drug from $LD_{50} \sim 5$ mg/kg to $LD_{50} = 22$ mg/kg. However, because slightly larger doses were required to achieve a similar activity in the Walker system, there was no advantage in the therapeutic index (T.I. = 10 for III and T.I. = 9 for V). Introduction of S to the methylaziridine analogue VI again resulted in slightly increasing the toxicity by the S derivative. Replacement of one aziridine in compound III with methylaziridine (VII) reduced the toxicity only slightly and the difference is very likely insignificant. Compound VII showed a significant improvement of therapeutic index in both Walker and leukemia 1210 [T.I. = 13 (WA) and T.I. = 25 (L1210)]. This result may support the design of alkylating agents that have a different reactive half-life in the same molecule. A dose–response study carried out recently showed that this compound could be given at low doses repeatedly to achieve improved prolongation of survival in leukemia PSS systems. For instance, in the PSS system at 0.6 mg/kg with nine injections, T/C = 189; at 1.65 mg/kg with three injections, T/C = 159. Again, the introduction of S into this compound was not advantageous, since the compound became slightly more toxic and less effective.

Attention was then turned to the use of two diallylamine groups and one aziridinyl group (X) or one methylaziridinyl group (XI). As mentioned earlier, diallylamino-β-aziridinopropionamide was tested in lymphoma-8 [4] and showed complete regression with no delayed carcinogenic activity. Both of these compounds had low toxicity ($LD_{50} = 75$ mg/kg). The monoaziridinyl compound X had very good activity and even showed activity in Walker resistant to cytoxan with a T.I. of 30. The methylaziridinyl compound XI was similarly active and had a T.I. = 125 in the WA system (Table III), the highest T.I. achieved in this series. Since compound X is also active in the L1210 system, we would like to recommend further study with X for combination chemotherapy, especially for adjuvant chemotherapy. Its activity in Walker resistant to cytoxan (Table II) may be of interest in human breast cancer therapy, since cytoxan has been a conventional drug for breast cancer patients who have postoperative recurrence. Both III and VII had long survival animals in the screening data in leukemia PSS and Walker intramuscular systems.

Another unexpected structure–activity relationship was found when we substituted the diallylamine group in compound III with $PhNCH_2CH{=}CH_2$ (XIII). The phenyl group must render the incipient ethylenimonium formation difficult. The drastic lowering of toxicity to $LD_{50} \sim 300$ mg/kg was a surprising finding and perhaps could be best rationalized by such a mechanism as we proposed for the allylamino derivatives.[3] This compound was not tested further, as the dose would have to be much higher than a practically feasible level. Conversion of the phenyl group to the cyclohexyl group restores the basicity of the N atom, but no further testing was done, as no initial improvement in T.I. was seen. In the subsequent paragraph, it can also be seen that the chemosterilant activity did not justify further work.

Even though sterility is not a subject considered in the design of anticancer agents, at the time this work was

Table II. Anticancer Screening of Some Allylaminophosphoramides

Compd	Tumor	Dose, mg/kg	Survivors	Wt change (T − C)[a]	Tumor wt (T/C)[b]	%[c]
III	WA[d]	2.00[g]	4/6	−44.0	0/5.7	0
		1.00	5/6	−26.0	0/5.7	0
		0.50	6/6	−6.0	0/5.7	0
		0.25	6/6	−12.0	0/5.7	0
		0.125	6/6	−1.0	0.1/6.6	1
		0.062	6/6	−5.0	0.5/6.6	7
		0.031	6/6	0	1.6/6.6	24
	WC[e]	2.0[g]	4/6	−2.0	1.0/10.3	9
		1.0	6/6	−13.0	4.2/10.3	40
		0.50	6/6	−13.0	6.0/10.3	58
		0.25	6/6	0	4.1/6.8	60
		0.12	6/6	−14.0	6.7/6.8	98
V	WA	6.25[g]	6/6	−28	0.5/15.3	3
		3.12	6/6	−15	0.1/15.3	0
		1.56	6/6	−22	0.8/15.3	5
		0.78	6/6	−8	3.8/15.3	24
	LE[f]	5.00[h]	6/6	−3.5	11.0/8.6[k]	127
		3.00	6/6	−2.5	14.8/8.6	172
		1.80	6/6	−2.1	12.3/8.6	143
		1.00	6/6	−1.1	10.7/8.6	124
IV	WA	0.30[g]	6/6	0	9.5/9.2	
VI	WA	3.0[g]	6/6	−7.0	0.8/5.7	14
VII	WA	1.00[g]	6/6	−9.0	0.2/8.1	2
		1.90	6/6	−13	0/15.3	0
		0.95	6/6	−8	0/15.3	0
		0.47	6/6	0	0.8/15.3	5
		0.23	6/6	−7.0	4.3/15.3	30
	LE[f]	3.00[i]	6/6	−1.9	10.5/8.2[k]	128
		1.50	6/6	−2.3	10.6/8.2	128
		0.75	6/6	−1.4	10.6/8.2	129
		0.37	6/6	−0.7	9.6/8.2	117
VIII	WA	0.40[g]	6/6	−4.0	7.1/9.2	77
	LE	2.50[i]	6/6	−5.4	9.8/8.5	115
X	WA	12.0[g]	6/6	−1.0	0/5.7	0
	WC	25.0[g]	6/6	−28	3.4/6.5	52
		12.0	6/6	−4	4.3/6.5	66
		6.00	6/6	−7.0	4.6/6.5	70
		3.00	6/6	−11.0	5.5/6.5	84
	LE	11.0[i]	6/6	−4.9	15.7/8.8[k]	178
		7.00	6/6	−4.2	15.3/8.8	173
		5.00	6/6	−4.4	13.7/8.8	155
		3.00	6/6	−4.2	13.3/8.8	151
XI	WA	6.25[g]	6/6	−28.0	0.5/15.3	3
		3.12	6/6	−15.0	0.1/15.3	1
		1.56	6/6	−22.0	0.8/15.3	2
		0.78	6/6	−8.0	3.8/15.3	4
		0.47	6/6	−14.0	0.8/15.3	2
		0.23	6/6	−3.0	4.6/15.3	33
XIII	LE	400.0[j]	6/6	−2.1	10.5/10.9[k]	
		200.0	6/6	−0.2	10.5/10.9	
		100.0	6/6	+1.3	10.5/10.9	

[a] For survival test systems, average weight change of test group minus average weight change of control animals in grams; for tumor inhibition systems, average net weight change of test group minus average net weight change of control animals in grams.[10] [b] Test (T), measure of response (treated animals); control (C), measure of tumor progression (untreated animals).[10] [c] Percentage: ratio of test (T) evaluation to control (C) evaluation expressed as a percentage.[10] [d] Walker 256 carcinoma; solid tumor system; the smaller the average, the more active the compound. [e] Walker 256, resistant to cytoxan; solid tumor. [f] Leukemia 1210; ascites tumor system; the larger the percentage, the more active the compound. [g] One injection per day for 5 days. [h] One injection per day for 9 days. [i] One injection per day for 15 days. [j] One injection. [k] Increase in survival time.

carried out there were data already suggesting that thiotepa might be beneficial for breast adjuvant chemotherapy for those in the premenopausal group. These data could now be found in a recent review by Tormey.[9] An interesting study related to sterility was therefore initiated in our laboratories some time ago. Our approaches were to find the chemosterilant activities of these compounds in the insect sterilization program and to carry out some studies of mechanism and sterility in rats. These results are summarized in Table IV.

The chemosterilant program of the U.S. Agricultural Research Service is a unique program in that it provides data on susceptibility of male and female insects to chemical sterilants. These data may provide useful leads to sort out differences among these compounds before animal work is started. When the compounds were screened in male and female flies, *Musca domestic*, compounds III and IV were highly active chemosterilants in the male but much less active in the female (Table IV). The activities were similar to those of the previously known aziridine derivatives.[7] The results indicated that the bisaziridines were more active than the monoaziridines and at least one aziridinyl group is necessary for chemosterilant activity.

A preliminary animal study[8] (see Experimental Section) showed that both compounds III and bis(1-aziridinyl)-

Table III. Comparison of Toxicity and Therapeutic Index (LD_{50}/ED_{10}) in the Walker System

Compd	LD_{50} dose, mg/kg	ED_{10},[a] mg/kg	Therapeutic index
III	5	0.5	10[b]
IV	2	N.D.[g]	
V	22	~3.5	9[c]
VI	7	~0.7	~10[d]
VII	7	0.52	13
VIII	4	~0.4	10
X	75	~2.5	30
XI	75	0.62	125[e]
XIII	>300	*f*	

[a] ED_{10} = 10% weight reduction in effective dose for WA; 10% lifetime increase; data are extropolative values. [b] In Walker resistant to cytoxan (WC), T.I. = 2.5. [c] In leukemia system, T.I. = 10. [d] In L1210, T.I. = 25. [e] Only a single dose level of 12.0 mg/kg was tested. No tumor was observed at sacrifice and no toxic death. [f] Toxicity too low to be tested for anticancer agent. [g] Not determined.

Table IV. Male House Fly Sterilizing Activity of Some Aziridinyldiallylaminophosphine Oxides and Sulfides

Compd	Lowest dosage causing complete sterility in male house flies[a]	
	% in sugar	% in fly food[b]
III	0.05	0.1
IV	0.05	0.05
V	0.5	0.25
VI	1.0	1.0
VII	0.25	0.1
VIII	0.5	0.25
XIII	No activity in sugar at 1%	
XVI	Not effective as a male sterilant[c]	
XVII	1.0	1.0

[a] Compared with Tepa, which is an effective chemosterilant which causes complete sterility at 0.05% in sugar with little or no mortality. For convenience, screening was also done in male and female mixed species; a higher dose was always needed. The ineffectiveness of these agents for female flies at low dose levels was tested in the past with other analogues; see also A. B. Borkovec, C. W. Woods, and R. T. Brown, *J. Med. Chem.*, 9, 522 (1966). [b] Composition of fly food: mixture of dry milk, sugar, and dry eggs (6:6:1). [c] It does produce 82% sterility in mixed sexes at 1% concentration in sugar.

1-azepinylphosphine oxide are good male chemosterilants in rats. A histochemical basis for these results was also found in an evaluation of compound III in male rats where the secondary spermatocytes, but not the mature spermatocytes, were affected. In female rats, no effect on ovaries was noted. Further data are necessary to determine inhibitory dose levels if optimal conditions for sterilization are of interest. The design and evaluation of these new phosphine oxides and sulfides for breast cancer adjuvant chemotherapy and the consideration of their chemosterilizing activity are certainly justified in this series.

Experimental Section

Microanalyses were run by the late Dr. S. M. Nagy, M.I.T., or at Galbraith Laboratories, Knoxville, Tenn. All physical data are summarized in Table I.

Diallylphosphoramidic Dichloride (I). The preparation of this compound was similar to that reported previously using diallylamine and phosphorus oxychloride.[1]

Diallylphosphoramidothioic Dichloride (II). To a solution of 16.9 g (0.1 mol) of thiophosphoryl chloride in 75 ml of dry benzene was added 9.7 g (0.1 mol) of diallylamine and 12.1 g (0.12 mol) of triethylamine in 50 ml of dry benzene; 50 mg of hydroquinone was added to retard polymerization. After the mixture was stirred overnight at room temperature, the hydrochloride was removed by filtration and the benzene was removed on a rotary evaporator. Vacuum distillation under reduced pressure in a nitrogen atmosphere gave 8.8 g (38%) of the desired product, bp 102–105° (2.0 mm).

***N,N,N',N'*-Tetraallylphosphorodiamidic Chloride (IX).** To 15.35 g (0.1 mol) of phosphorus oxychloride in 200 ml of dry benzene 19.4 g (0.2 mol) of diallylamine and 20.2 g (0.2 mol) of triethylamine in 150 ml of dry benzene were added at room temperature with stirring. The mixture was allowed to stir overnight. The hydrochloride was filtered off under suction in a nitrogen atmosphere in a glove bag and washed with dry benzene. After removal of the solvent on the rotary evaporator, the residue was treated with 20 ml of dry ether, filtered from a small amount (1.3 g) of diallylamine hydrochloride, evaporated, and distilled in vacuo to give 18.1 g (72%) of product, bp 61–122° (0.3 mm).

Allylcyclohexylphosphoramidic Dichloride (XIV). To 34.5 g (0.226 mol) of phosphorus oxychloride, dissolved in 300 ml of dry benzene, were added 15.7 g (0.113 mol) of allylcyclohexylamine and 11.5 g (0.113 mol) of triethylamine (distilled over sodium) in 100 ml of dry benzene with stirring at room temperature. An immediate precipitate of Et_3NHCl was observed; the temperature in the flask rose to 40 °C. After 2 h, addition was completed and the mixture was left to stir overnight. Triethylammonium chloride (15.0 g, 96%) was filtered off in a glove bag under N_2 and the remaining brown liquid was evaporated on the rotary condenser. Distillation yielded 12.8 g (44%) of product, bp 110.5–128 °C (0.15 mm). The product was distilled again for analysis: bp 89–94 °C (0.1 mm); $n^{24}D$ 1.5020.

Allylphenylphosphoramidic Dichloride (VII). A procedure similar to the preceding was used to prepare this intermediate in 16.6% yield: bp 90–95 °C (0.025 mm). The product was not very stable and was used immediately in the subsequent reaction.

Allylcyclohexylthioicphosphoramidic Dichloride (XV). To 33.9 g (0.2 mol) of freshly distilled thiophosphoryl chloride and 50 mg of hydroquinone in 100 ml of dry benzene were added 11.28 g (0.1 mol) of diazabicyclooctane and 13.98 g (0.1 mol) of allylcyclohexylamine in 100 ml of dry benzene at a temperature between 50 and 60 °C. The mixture was kept at 70 °C for 3 h and then allowed to stir at room temperature overnight. The solid diazabicyclooctane hydrochloride (14.6 g, 100%) was filtered off under N_2 in a glove bag and the benzene was removed on the rotary condenser. Distillation of the residue yielded 4.4 g (16%) of the desired product, bp 106–113° (0.1 mm).

General Procedure for the Preparation of the Substituted Aminophosphine Oxides and Sulfides. 1. To 10 mmol of phosphoramidic dichloride dissolved in 50 ml of dry benzene were added 20 mmol of triethylamine and 20 mmol of aziridine or 2-methylaziridine in 50 ml of dry benzene at room temperature. The mixture was allowed to stir overnight, the hydrochloride filtered off under suction, and the solvent removed on a rotary evaporator. The residue was distilled in vacuo.

2. **Mixed Compounds VII and VIII.** To 10 mmol of phosphoramidic dichloride dissolved in 50 ml of dry benzene, 10 mmol of 2-methylaziridine and 10 mmol of triethylamine in 20 ml of dry benzene were added with stirring at room temperature. After the addition was completed, 10 mmol of aziridine and 10 mmol of triethylamine in 20 ml of dry benzene were added to the reaction mixture which was then allowed to stir overnight. The hydrochloride was removed by filtration, the benzene evaporated on the rotary condenser, and the residue distilled under reduced pressure.

3. **Bis(diallylamino) Compounds X and XI.** To 10 mmol of phosphorodiamidic chloride dissolved in 50 ml of dry benzene 10 mmol of aziridine or 2-methylaziridine and 10 mmol of triethylamine in 20 ml of dry benzene were added at 50°. After the addition was completed, the mixture was stirred overnight at room temperature, the precipitated hydrochloride filtered under suction, and the solvent removed on the rotary evaporator. The residue, consisting of product and starting phosphorodiamidic chloride, was purified by fractional distillation.

House Fly Screening. Screening of house flies was carried out at the U.S. Department of Agriculture Insect Affecting Man Research Laboratory, Gainesville, Fla. The detailed protocol can be seen in ref 7.

Antifertility Action Study. The antifertility action study in rats was done with III (LD_{50} = 5 mg/kg) and bis(1-

aziridinyl)-1-azepinylphosphine oxide (hexadepa) (LD_{50} = 22 mg/kg). Twenty-four rats of Wistar–Furth strain were used in each of these groups, six each of male and female rats were retained as controls, and six male or female rats were injected with 1 mg/kg of III daily for 5 days and 5 mg/kg of hexadepa for 5 days. They were then mated with noninjected male or female rats (six in each group) in separate cages, fed with the same food. The total number of litters was counted after 12 weeks. The male injected group yielded a total of six (for III) and zero (for hexadepa). Both of these numbers are lower than the normal control group of 19. While the difference was obvious, no effort was made to determine either the optimum sterilizing dose or the reversibility of such treatment.

Histochemical Study. The histochemical study of the effect of III and hexadepa was carried out at the same dose level in Wistar–Furth rats and was tested for 1-, 3-, and 24-h periods. The testes or ovary was obtained when the animal was sacrificed and frozen sections were put on a cryostat. Tissues were fixed with 95% alcohol for 10 min. Acridine orange was used as a fluorescent stain for DNA (green) and RNA (red). No difference was noted between the treated and control groups. Only in the 24-h group could one find a decrease in the visible number of secondary spermatocytes but not in the mature spermatocytes. No effect on ovaries was noticed. *Corpus luteus* effect was attributed to the usual estrus cycle of female rats. This result suggested to us that the damage to testes also might be reversible and further extensive study should be necessary.

Acknowledgment. We thank Dr. W. Springer and Mrs. M. Kapral for the antifertility and part of the histochemical studies, the NIH for sponsoring this work with Grant No. CA 07339, and the U.S. Department of Agriculture for contract 12-14-10C-9172-33. This work also benefited from the advice and encouragement of the late Dr. R. G. Ravdin, a member of the breast adjuvant chemotherapy group.

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Synthesis and Properties of 2-*S*-[2'-(*N*,*N*-Dialkylamino)ethyl]thio-1,3,2-dioxaphosphorinane 2-Oxide and of the Corresponding Quaternary Derivatives as Potential Nontoxic Antiglaucoma Agents

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A new series of cyclic organophosphorus esters, 2-*S*-[2'-(*N*,*N*-dialkylamino)ethyl]thio-1,3,2-dioxaphosphorinane 2-oxide and their quaternary derivatives, was synthesized and studied as potential antiglaucoma agents. These compounds inhibit acetylcholinesterase (E.C. 3.1.1.7) at a bimolecular rate constant (k_i) in the range of 10^3–10^4 M^{-1} min^{-1}. Values of the affinity (K) and phosphorylation (k') rate constants for this enzyme indicate that k' is responsible for the relatively low values of k_i as compared with similar data for the open-chain analogues, *O*,*O*-diethyl phosphorothiolates (10^6 M^{-1} min^{-1}). The mammalian toxicity of the new compounds in terms of acute LD_{50} values in mice is 1–3 × 10^3 less than that of phospholine, an open-chain analogue. In an initial clinical trial, one member of the new series (alkyl = C_2H_5) caused a significant decrease of intraocular pressure in aphakic glaucoma, while phospholine proved to be ineffective.

Anticholinesterase agents are used as topical agents for the treatment of ophthalmological conditions such as glaucoma.[1] For example, carbamates and organophosphates have found considerable use in the therapy of primary glaucoma (narrow and wide angle) or secondary glaucoma (aphakic glaucoma). The severe ocular and general side effects which follow the treatment with powerful AcChE inhibitors such as DFP or phospholine[2,3] have limited their application in the relief of intraocular pressure.

Recently it has been shown that AcChE (electric eel) inhibited with 1,3,2-dioxaphosphorinane 2-oxide derivatives (I, X = F, Cl, *p*-nitrophenyl) undergoes spontaneous reactivation with $t_{1/2} \simeq 12$ min at pH 7.0.[4] This conceivably contributes to the very low mammalian toxicity of these compounds (>100 mg/kg sc in mice).

Since it has been established that phosphorothiolates with leaving groups of the type $(alkyl)_2NCH_2CH_2S$– are excellent inhibitors of AcChE,[5] it seemed rational to investigate compounds of structure I, but where X is a thiocholine analogue. This approach dwells on the assumption that the proposed structure (I) may prove more reactive toward the enzyme than the cyclic analogue, where X = halogen, but at the same time, the rate of spontaneous reactivation should not be affected. Compounds abiding by these criteria would constitute a new class of relatively safe AcChE inhibitors for drug therapy of glaucoma or conditions where cholinergic function is impaired (e.g., myasthenia gravis).

The present report deals with the syntheses and some biochemical and toxicological properties of new cyclic phosphorothiolates I as compared to the open-chain analogues II.

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