Pyrimidines. V. Some Higher 5-Substituted 2,4,6-Trichloropyrimidines[1]

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In earlier work on ring-polychlorinated pyrimidines, we reported[2] that a series of 5-substituted 2,4,6-trichloropyrimidines showed confirmed anticancer activity in the KB cell culture test system. It should also be pointed out that none of these compounds demonstrated *in vivo* activity in the usual tumor systems; however, 5-propyl-2,4,6-trichloropyrimidine did show a sufficient degree of cytotoxicity to warrant further evaluation in other *in vivo* tumor systems.[3]

Since this compound was the last member of the series tested, it was considered worthwhile to make additional members for evaluation. The compounds were prepared by condensing the appropriate malonic ester with urea by means of sodium ethoxide to form barbituric acids (I) which, in turn, were treated with phosphorus oxychloride in the presence of dimethylaniline to yield the desired 5-substituted 2,4,6-trichloropyrimidines (II). These methods are all found in the literature,[2,4,5] and the specific compounds are listed in Tables I and II.

Of the 22 compounds prepared, the following barbituric acids were previously described: Ia,[6] Ib,[5,6] Ic,[7] Id,[8] Ie,[6] If,[9] Ij,[10] and Ik.[11] Compound Ih[12] was mentioned but not characterized. Only IIb[5] and IIk[11] of the trichloropyrimidines were described; IIa[12] and IIh[12] were reported but not fully characterized. The pertinent data on the 5-substituted 2,4,6-trichloropyrimidines are summarized in Table I.

All of the barbituric acids and trichloropyrimidines were screened by the Cancer Chemotherapy National Service Center[13] against four animal tumors each, lymphoid leukemia L1210, lymphosarcoma P1789 or Sarcoma 180, Dunning ascites leukemia, Adenocarcinoma 755 or Lewis lung carcinoma, Walker 256 carcinosarcoma (intramuscular), and KB cell culture. These data are contained in Table II and indicate that the compounds are inactive against the tumors studied.

With regard to the effect of increasing the chain length of the substituent in the 5 position of the 2,4,6-trichloropyrimidines, it can be seen that the cytotoxicity of the compounds in the KB cell culture system reached a maximum when the side chain was propyl[2] and diminished with increasing chain length. Furthermore, branching of the side chains of this family of compounds yields less toxic products than the corresponding, straight-chain, parent compounds.

It should also be noted that in our previous paper,[2] compounds were considered active with greater ED_{50} values than some of the compounds in our present report. This is due to a recent change in protocol in which the base line for activity has been lowered.[14]

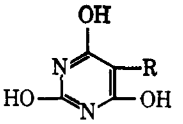
It was reported by Gershon and Parmegiani[15] that ring-polychlorinated pyrimidines possessed antifungal vapor effects, and in the 2,4,6-trichloropyrimidine series, the vapors of 2,4,6-trichloropyrimidine and of the 5-

TABLE I

5-SUBSTITUTED 2,4,6-TRICHLOROPYRIMIDINES

Compd II	Yield, %	Bp, °C (mm)	n_D (°C)	Formula	Calcd, % C	H	Cl	N	Found, % C	H	Cl	N
a[a]	54	83 (0.3)[b]	1.5368 (23.5)	$C_8H_9Cl_3N_2$	40.11	3.79	44.40	11.69	40.18	3.82	44.17	11.96
c	60	95 (0.25)	1.5271 (23)	$C_9H_{11}Cl_3N_2$	42.63	4.37	41.94	11.05	42.56	4.51	41.57	10.83
d	39	81 (0.2)	1.5346 (23)	$C_9H_{11}Cl_3N_2$	42.63	4.37	41.94	11.05	42.50	4.45	41.83	11.14
e	16	89 (0.3)	1.5293 (24.5)	$C_9H_{11}Cl_3N_2$	42.63	4.37	41.94	11.05	42.32	4.24	42.23	11.48
f	32	107 (0.3)	1.5237 (23)	$C_{10}H_{13}Cl_3N_2$	44.88	4.90	39.75	10.47	44.75	5.00	39.75	10.44
g	45	120 (0.35)	1.5219 (22.5)	$C_{11}H_{15}Cl_3N_2$	46.91	5.37	37.77	9.95	46.76	5.29	38.09	9.75
h[a]	52	127 (0.2)[c]	1.5176 (24)	$C_{12}H_{17}Cl_3N_2$	48.75	5.80	35.98	9.48	48.45	5.94	36.07	9.23
i	26	141 (0.25)	1.5156 (23)	$C_{13}H_{19}Cl_3N_2$	50.42	6.18	34.35	9.05	50.07	6.38	34.09	9.00
j	62	96–98 (3.0)[d]		$C_7H_5Cl_3N_2$	37.62	2.25	46.59	12.53	37.45	2.21	47.28	12.98

[a] Mentioned in ref 12, not characterized. [b] Lit.[12] bp 84–89.5° (0.1 mm), n^{25}_D 1.5375. [c] Lit.[12] bp 139–143° (0.5–0.55 mm), n^{25}_D 1.5150–1.5178. [d] Mp 38–39°.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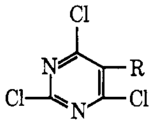
II

a, R = $CH_3(CH_2)_2CH_2$
b, R = $CH_3CHCH_2CH_3$
c, R = $CH_3(CH_2)_3CH_2$
d, R = $CH_3(CH_2)_2CHCH_3$
e, R = $(CH_3)_2CHCH_2CH_2$
f, R = $CH_3(CH_3)_4CH_2$
g, R = $CH_3(CH_2)_5CH_2$
h, R = $CH_3(CH_2)_6CH_2$
i, R = $CH_3(CH_2)_7CH_2$
j, R = $CH_2{=}CHCH_2$
k, R = $C_6H_5CH_2$

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[1] This investigation was supported, in part, by the Cancer Chemotherapy National Service Center, National Institutes of Health, under Contract No. PH 43-63-579.

[2] H. Gershon, R. Braun, A. Scala, and R. Rodin, *J. Med. Chem.*, **7**, 808 (1964).

[3] M. M. Macdonald, Cancer Chemotherapy National Service Center, personal communication.

[4] O. Gerngross, *Ber.*, **38**, 3394 (1905).

[5] A. W. Dox, *J. Am. Chem. Soc.*, **53**, 1559 (1931).

[6] E. H. Volwiler, *J. Am. Chem. Soc.*, **47**, 2236 (1925).

[7] A. W. Dox and E. G. Jones, *ibid.*, **50**, 2033 (1928).

[8] W. Wenner, Jubilee Volume Dedicated to Emil Christoph Barell Frederick Reinhardt, Ltd. Co., Basel, 1946, p 435.

[9] A. W. Dox, *J. Am. Chem. Soc.*, **46**, 1707 (1924).

[10] T. B. Johnson and A. J. Hill, *Am. Chem. J.*, **46**, 537 (1911).

[11] H. Kast, *Ber.*, **45**, 3124 (1912).

[12] G. F. Deebel and P. C. Hamm, U. S. Patent 2,879,150 (1959).

[13] (a) The details of the screening procedures can be found in *Cancer Chemotherapy Rept.*, **1**, 42 (1959); (b) J. Leiter, B. J. Abbott, and S. A. Schepartz, *Cancer Res.*, **25**, 1779 (1965).

[14] N. H. Greenberg, Cancer Chemotherapy National Service Center, personal communication. Whereas in ref 13b, a compound was considered KB active when $ED_{50} \leq 4$ μg/ml, the revised standard is $ED_{50} \leq 1$ μg/ml. On this basis, four compounds of ref 2 are still considered active: 5-methyl-, 5-bromomethyl-, 5-propyl-, and 5-phenyl-2,4,6-trichloropyrimidine.

[15] (a) H. Gershon and R. Parmegiani, *Appl. Microbiol.*, **11**, 78 (1963); (b) H. Gershon and R. Parmegiani, *Trans. N. Y. Acad. Sci.*, **25**, 638 (1963); (c) H. Gershon, U. S. Patent 3,227,612 (1966).

TABLE II

SUMMARY OF ANTICANCER SCREENING DATA AGAINST FOUR TUMORS[a,b]

	L1210		SP or S180			DA, Ca755, LL, or WM			KB	
Compd	NTL,[c] mg/kg	T/C,[d] %	NTL, mg/kg		T/C, %	NTL, mg/kg		T/C, %	Slope	ED_{50}, µg/ml
Ia	75	114	95	S	56	75	C	65		
Ib	170	103				153	C	113		
Ic	400	97	400	P	83	200	D	86		99
Id	400	92	400	P	100	100	D	90		99
Ie	125	96	500	S	60	500	L	60		100
If	200	98	400	P	85	100	D	92		99
Ig	400	97	400	P	94	200	D	86		100
Ih	400	98	400	P	70	200	D	109	−0.43	76
Ii	400	92	400	P	78	200	D	95		100
Ij	200	97	250	S	94					100
Ik	400	109	400	P	95	200	D	90		100
IIa	50	105	62.5	S	56	25	WM	100	−0.33	1.0
						12.5	WM	114	−0.20	0.49
						6.25	WM	113	−0.99	5.8
									−0.95	3.0
IIb	100	95	125	S	65	100	WM	91	−1.1	2.4
						50	WM	105	−0.97	6.6
						25	WM	91		
						12.5	WM	95		
IIc	20	98	25	S	99	20	WM	82	−0.65	2.6
						10	WM	77	−1.5	3.7
						5		72		9.9
						2.5		81		
IId	20	93	25	S	66	20		96	−0.55	5.2
						10		94	−0.97	12
						5		78		
						2.5		63		
IIe	100	96	125	S	66	50		54	−0.72	2.8
						25		107	−0.86	5.7
						12.5		123		
IIf	40	91	50	S	57	40		46	−0.53	5.4
						20		70		19
						10		94		
						5		106		
IIg	20	98	25	S	116	20		54	−0.85	19
								10	91	
						5		85		
						2.5		80		
IIh	20	96	25	S	92	20		62	−0.82	24
						10		97		
						5		104		
						2.5		107		
IIi	20	94	25	S	75	20		77	−0.39	62
						10		92		
IIj	100	104	125	S	115	25		121		1.0
						12.5		92	−0.50	0.66
									−1.3	3.8
									−1.5	2.4
IIk	400	96	500	S	66	200		62	−0.	2.9
						100		65	−0.51	3.9
						50		85	43	
						25		91		

[a] We are indebted to Dr. Harry B. Wood, Jr., Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md. 20014, for making these data available to us. [b] L1210 = lymphoid leukemia, S180 = Sarcoma 180, 8P = lymphosarcoma P1789, Ca755 = Adenocarcinoma 755, DA = Dunning ascites leukemia, LL = Lewis lung carcinoma, WM = Walker 256 carcinoma (intramuscular), and KB = KB cell culture. [c] NTL = maximum nontoxic level. [d] T/C = treated tumor/control tumor.

methyl derivative were sporicidal. The vapors of the 5-ethyl, 5-propyl, and 5-isopropyl derivatives were sporistatic. Since additional members of this series became available, it was of interest to determine the effect of increasing chain length on the antifungal activity of such compounds. Compounds IIa–k were screened for antifungal vapor effects against three fungi, *Aspergillus niger*, *Trichoderma viride*, and *Aspergillus oryzae* according to published methods.[15a] The results of these tests, as summarized in Table III, show that none of the compounds was fungicidal, and only 5-allyl-2,4,6-trichloropyrimidine (IIj) was markedly fungistatic. As the chain length increased from three to four carbon atoms, the fungistatic activity of the vapors fell off: an increase of the chain length to five carbon atoms caused a complete loss of antifungal vapor activity.

TABLE III

EFFECT OF VAPORS OF 5-SUBSTITUTED 2,4,6-TRICHLOROPYRIMIDINES ON FUNGAL SPORES INCORPORATED IN SABOURAUD DEXTROSE AGAR AT 28° AFTER 5 DAYS

Compd II	Spores from *A. niger*	*T. viride*	*A. oryzae*
a	50[a]	10	+
b	+	10	+
c	+	+	+
d	+	+	+
e	+	+	+
f	+	+	+
g	+	+	+
h	+	+	+
i	+	+	+
j	S	S	S
k	+	+	+

[a] Symbols: a number = approximate per cent of area showing no growth, + = no inhibition, S = sporistatic.

Experimental Section[16]

5-Nonylbarbituric Acid (Ii).—To a solution prepared by dissolving 23 g (1 g-atom) of sodium in 1000 ml of anhydrous ethanol were added 34.5 g (0.57 mole) of dry urea in 300 ml of anhydrous ethanol and 165 g (0.57 mole) of diethyl nonylmalonate.[16] The mixture was heated under reflux with agitation for 2.5 hr. Excess alcohol was removed by flash evaporation, and the residue was dissolved in water and acidified to pH 1–2 with concentrated HCl. The compound was collected by filtration, washed free of salts with water, and dried at 70° overnight. The yield of product was 103 g (71%), mp 195–201°. An analytical sample was crystallized from methanol; mp 208–209°.

Anal. Calcd for $C_{13}H_{22}N_2O_3$: C, 61.39; H, 8.72; N, 11.02. Found: C, 61.25; H, 8.72; N, 11.28.

5-Octylbarbituric acid (Ih) was prepared in the same manner as Ii in 55% yield, mp 211–213° (from methanol).

Anal. Calcd for $C_{12}H_{20}N_2O_3$: C, 59.98; H, 8.39; N, 11.66. Found: C, 60.00; H, 8.31; N, 11.63.

5-Heptylbarbituric acid (Ig) was prepared in 51% yield in the same manner as Ii; mp 207–209° (from methanol).

Anal. Calcd for $C_{11}H_{18}N_2O_3$: C, 58.39; H, 8.02; N, 12.38. Found: C, 58.52; H, 7.87; N, 12.62.

5-Nonyl-2,4,6-trichloropyrimidine (IIi).—A mixture of 50 g (0.18 mole) of Ii, 500 ml of $POCl_3$, and 50 ml of dimethylaniline was heated under reflux with agitation for 2 hr. Most of the $POCl_3$ was removed by flash evaporation, and the residue was decomposed by pouring it onto 1.5 l. of ice flakes. The chloropyrimidine was extracted with ether. The extract was washed with water and dried over Na_2SO_4, and the ether was flash evaporated. The residue was distilled to yield 15 g of product (27%), bp 147–152° (0.45 mm). The analytical sample boiled at 141° (0.25 mm).

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(16) Melting points were taken in a Mel-Temp melting point apparatus and are uncorrected. Ultraviolet data were obtained with a Perkin-Elmer Spectracord 4000A. The synthetic procedures are general, and the malonic esters were commercially available except where the alkyl groups were hexyl, heptyl, octyl, and nonyl. These were prepared by the method of B. Rothstein, *Bull. Soc. Chim. France*, **2**, 80 (1935).

Selenophosphorus Compounds as Powerful Cholinesterase Inhibitors

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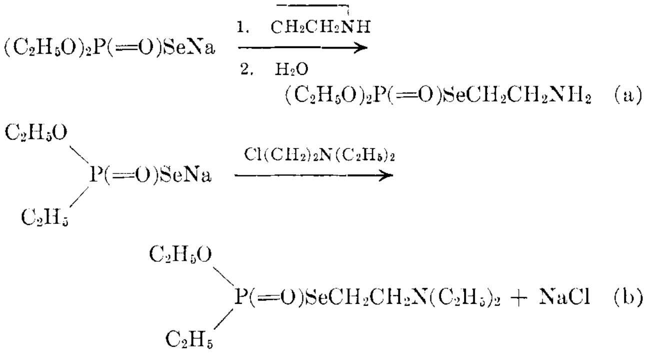
Certain organophosphorus compounds containing sulfur have been known for a long time to be powerful inhibitors of cholinesterase.[1] Very little is known, however, about the chemical properties and toxicity of the corresponding selenium compounds.

The present paper describes some methods which have been found convenient for the preparation of certain types of compounds containing the PSeC group. The synthetic routes adopted consist of reactions of phosphoro- or phosphonoselenoates with (a) aziridine or its derivatives, or (b) aminoalkylhalogenides, as exemplified below. In principle, these reactions are analogous to the ones used for phosphorothioates.[2]



$$(C_2H_5O)_2P({=}O)SeNa \xrightarrow[2.\ H_2O]{1.\ \overline{CH_2CH_2NH}} (C_2H_5O)_2P({=}O)SeCH_2CH_2NH_2 \quad (a)$$

$$(C_2H_5O)(C_2H_5)P({=}O)SeNa \xrightarrow{Cl(CH_2)_2N(C_2H_5)_2} (C_2H_5O)(C_2H_5)P({=}O)SeCH_2CH_2N(C_2H_5)_2 + NaCl \quad (b)$$

Reaction a proceeds almost immediately at room temperature. Reaction b is slower, but is best carried out at room temperature. The isolated compounds are less stable than the corresponding sulfur compounds. The intermediates especially are rapidly attacked by oxygen from the air and become discolored due to the formation of elemental selenium.

The purity of the compounds was checked by gas chromatography,[3] and their structure was confirmed by infrared and nmr spectra.[4] The shift in the nmr spectra of CH_2OP is about 6.0 ppm, whereas that of CH_2SeP is about 7.0 ppm in this type of compounds.[5] The infrared spectra of the prepared compounds are practically identical with the ones obtained from the corresponding sulfur analogs.

The selenium derivatives reported belong to the most toxic phosphorus compounds known having LD_{50} values ranging from 0.02 to 0.06 mg/kg when injected subcutaneously in mice, *i.e.*, they are more toxic than the corresponding sulfur analogs. The toxicity is due to the ability of these compounds to inhibit cholinesterase, as shown in tests with human erythrocyte enzyme. The pI_{50} values[6] were in the range 6.8–9.7 as shown in Table I.

Experimental Section

Sodium O,O-diethyl phosphoroselenoate was prepared according to Foss.[7]

Anal. Calcd for $C_4H_{10}NaO_3PSe$: C, 20.1; H, 4.23; P, 13.0. Found: C, 19.8; H, 4.20; P, 12.9.

Ethylphosphonoselenoic Dichloride.[8]—A mixture of 8 g of dry, powdered $AlCl_3$ and 65.5 g (0.5 mole) of ethylphosphonous di-

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(1) For a recent summary see E. Heilbronn-Wilkström, *Svensk Kem. Tidskr.*, **77**, 598 (1965).

(2) (a) S. Åkerfeldt, *Acta Chem. Scand.*, **16**, 1897 (1962); (b) *ibid.*, **17**, 329 (1963); (c) *Svensk Kem. Tidskr.*, **75**, 231 (1963).

(3) Aerograph 1520, SE 30 column at 162–170° with nitrogen as carrier gas (60 ml/min). FID detector.

(4) Varian A-60 A spectrophotometer; TMS as external reference.

(5) B. Östman, personal communication.

(6) pI_{50} = −log (molar concentration for 50% inhibition).

(7) O. Foss, *Acta Chem. Scand.*, **1**, 8 (1947).

(8) This preparation procedure is similar to the one used for the sulfur analogs as described by F. W. Hoffman, D. H. Wadsworth, and H. D. Weiss, *J. Am. Chem. Soc.*, **80**, 3945 (1958).