phatidylserine. Analysis of the fatty acid composition by glc showed the expected composition of an equal amount of stearic and oleic acid.

Anal. Calcd for $C_{42}H_{80}NO_{10}P$: C, 63.85; H, 10.21; N, 1.77; P, 3.92. Found: C, 64.04; H, 10.02; N, 1.83; P, 3.76.

Biological Results.—The phosphatidyl-(oleoyl stearoyl)-serine was suspended in buffered saline solution or solubilized with buffered sodium deoxycholate solution and tested in several tests of blood coagulation described earlier.[9,13] The results indicated that the material had the same quantitative activity as the phosphatidyl-(oleoyl stearoyl)-serine prepared earlier[9] by a different method, *i.e.*, weak to moderate acceleratory activity in simple suspensions, and anticoagulant activity when well solubilized. This activity was present although the material was racemic.

Acknowledgment.—Analysis of the fatty acids from the phosphatidylserine was kindly performed for us by S. F. Herb and Francis E. Luddy of the Eastern Regional Research Laboratory, U. S. Department of Agriculture, Wyndmoor, Pa., using the techniques developed by them. References to the publications describing their techniques can be found in our earlier paper.[9] We wish to thank Mrs. Peggy Moser for technical assistance.

(13) M. J. Silver, D. L. Turner, I. Rodalewicz, N. Giordano, R. Holburn S. F. Herb, and F. E. Luddy, *Thromb. Diath. Haemorrhag.*, **10**, 164 (1963).

1,3,2-Diazaphosphorine 2-Oxides. III.[1] Preparation and Biological Evaluation of Some 1,3-Bis(aralkyl)-2-halo-1,3,2-diazaphosphorine 2-Oxides and Related Compounds[2]

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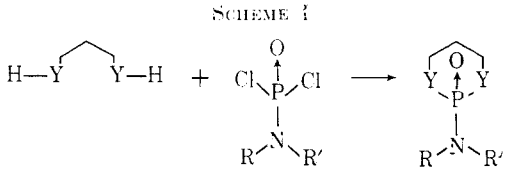
The discovery of 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide (I)[3] as a valuable antitumor drug has stimulated considerable interest in derivatives of phosphoric acid.[4] The standard procedure for the preparation of I and other closely related heterocyclic ring systems of the same general type has been by the reaction of diamines, diols, and amino alcohols with phosphoramidic dichloride according to Scheme I.[5]

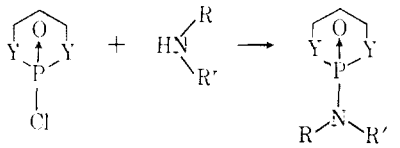
SCHEME I



Y = O, NH

Although Scheme I is in general quite satisfactory, it is limited in its scope due to the fact that phosphoramidic dichlorides cannot be prepared from amines containing other functional groups which are sensitive to phosphorus oxychloride. In order to overcome this difficulty, an alternate method for synthesizing I and related heterocyclic molecules would be to use chlorodiazaphosphorine 2-oxides (II), chlorodioxaphosphorinane 2-oxides (III), or chlorooxazaphosphorines (IV) and allow them to react with the appropriate amine. If the latter type chlorides were available, this would also afford the opportunity for the preparation of many new amides (Scheme II) that may be active antitumor agents.

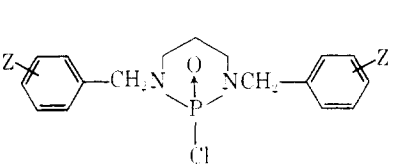
SCHEME II



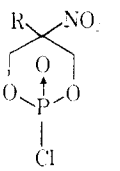
Y = O, NH

A review of the literature reveals little information concerning the preparation of chlorophosphorine 2-oxides. It was therefore decided that a study should be made to determine if a good general method could be developed for making such chlorides.

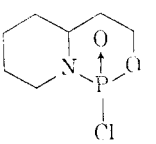
The present investigation reveals that the chlorophosphorine 2-oxides II and IV and the chlorophosphorinane III can be made in very satisfactory yields (Table I) by the general reaction shown in Scheme III.



IIa, Z = *p*-Cl
b, Z = *p*-OCH_3
c, Z = *p*-$N(CH_3)_2$

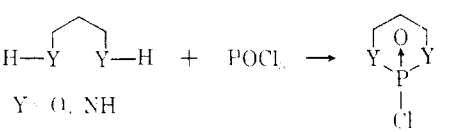


IIIa, R = CH
b, R = C_2H_5



IV

SCHEME III



Y = O, NH

Compounds of type II were the first to be synthesized in the hope of finding a more versatile route to the 1,3-bis(aralkyl)-2-(N-arylamino)-1,3,2-diazaphosphorine 2-oxides (VIII) which have been of interest as potential antitumor agents in this laboratory.[1] The original method used for making these latter compounds involved the reaction of a diamine (VI) with a phosphoramidic dichloride (VII), as shown below in Scheme IV in the equation labeled path A. Now that compounds of type II are available, it is anticipated that the same products VIII can be synthesized according to path B.

(1) Part II: J. H. Billman and J. L. Meisenheimer, *J. Med. Chem.*, **8**, 264 (1965).

(2) This investigation was supported by a Public Health Service Grant (CA-06448-03) from the National Institutes of Health, Public Health Service.

(3) Cytoxan®.

(4) H. Arnold, F. Bourseaux, and N. Brock, *Arzneimittel-Forsch.*, **11**, 146 (1961).

(5) H. Arnold and F. Bourseaux, *Angew. Chem.*, **70**, 539 (1938).

SCHEME IV

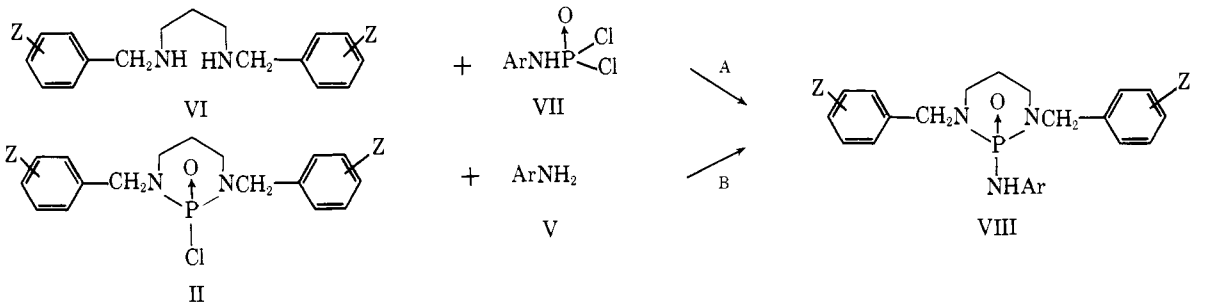


TABLE I

Compd	Yield (pure), %	Mp, °C	Formula	% N Calcd	% N Found	Infrared assignment, μ P→O	POC	PN
IIa	85.0	117–118	$C_{17}H_{18}Cl_3N_2OP$	6.95	7.01	7.62		13.60
IIb	55.7	151–151.5	$C_{19}H_{24}ClN_2O_3P$	7.10	7.02	7.79		13.92
IIc	42.0	155.5–157	$C_{21}H_{30}ClN_4OP$	13.31	13.19	7.75		13.75
IIIa	82.0	162–163	$C_4H_7ClNO_5P$	6.53	6.66	7.62	9.52	
IIIb	70.0	94–96	$C_5H_9ClNO_5P$	6.24	6.12	7.62	9.51	
IV	63.5	63–65	$C_7H_{13}ClNO_2P$	6.70	6.51	7.72	9.52	14.50

TABLE II

Compd	Test system[a]	Dose, mg/kg	Survivors	Wt change (T − C),[b] g	Tumor wt[c] (T/C)[b]	% T/C[b]	ED_{50}, μg/ml	Slope
IIa	KB						26	−1.10
	FV	400	2/10	−53	90/517			
	FV	200	9/10	−7	1369/1076	127		
	HE	500	6/6	−13	939/713	131		
	HE	400	0/6					
	HE	200	3/6	−18	467/672			
	HE	100	3/6	−28	352/725			
	SA	500	5/6	−4	1248/1102	113		
IIb	KB						1.0	
	KB						8.1	−0.58
	KB						7.2	−0.49
	KB						10	
	KB						28	
	KB						30	−1.19
	CA	200	10/10	7	1109/1042	106		
	LE	200	6/6	−1	8.3/9.2	90		
	SA	250	6/6	−19	1627/1394	116		
IIc	KB						3.1	−0.36
	KB						10	
	CA	200	9/10	2	1056/1042	101		
	LE	200	6/6	−1	8.5/9.2	92		
	SA	250	6/6	23	1088/1394	78		
III	KB						95	−0.73
	LE	200	6/6	−10	8.5/8.4	101		
	LL	100	5/6	3	676/584	115		
	SA	500	0/6					
	SA	125	6/6	−3	388/578	67		
IV	KB						29	−1.25
	LE	200	6/6	−7	8.5/9.0	94		
	LL	200	5/6	−4	504/584	86		
	SA	500	3/6	−6	705/916			
	SA	250	5/6	−25	560/578	96		

[a] Abbreviations are FV, Friend virus leukemia; HE, Hepatoma 129; SA, Sarcoma 180; LE, L1210 lymphoid leukemia; CA, Adenocarcinoma 775; LL, Lewis lung carcinoma; and KB, cell culture. [b] T stands for test animals, C for controls. [c] FV, HE, SA, LL, and CA are in milligrams and LE is in grams.

Compounds of type III were considered to be of interest as intermediates similar to type II with the exception that the heterocyclic nitrogen atoms are replaced by oxygen atoms. In addition, there is a nitro group in the 5 position which may be modified to expand the number of cancer-inhibiting moieties that could be incorporated into the molecule.

Compound IV would be desirable not only in its own right, but also as a valuable intermediate in the syntheses of potential antineoplastic agents closely related

to I. Interest in this particular heterocyclic ring system and already been displayed in another laboratory.[6] However, the chlorooxazaphosphorine 2-oxide IV has not been previously made. In addition to the above-mentioned interests, IV was synthesized so that a comparison might be made between it and types II and III with respect to animal toxicity, antineoplastic activity, as well as reactivity toward amines.

The rate of the reaction of all of the above compounds was qualitatively determined by observing the rate of formation of triethylamine hydrochloride during the reactions with piperidine in nonpolar solvents containing triethylamine. It was found that III and IV were comparable in rate and faster than compounds of type II.

The structure proof of compounds of types II and III and of IV was based on their nitrogen analysis, characteristic infrared data (Table I), and the nitrogen analysis of their piperidine derivatives. The structure of compounds of type II was further characterized by comparing the product formed when paths A and B in Scheme IV were investigated. The identical product VIII was obtained, as shown by mixture melting points and identical infrared curves.

Biological.—According to all the available data, which is presented in Table II, none of the compounds showed any appreciable *in vivo* activity according to the criteria established by the Cancer Chemotherapy National Service Center.[7] The systems used were Friend virus leukemia, Hepatoma 129, Sarcoma 180, L1210 lymphoid leukemia, Adenocarcinoma 775, and Lewis lung carcinoma. On the other hand, IIb and IIc showed appreciable activity in cell culture (KB) of human epidermoid carcinoma of the nasopharynx.

Experimental Section

2-Chloro-1,3-bis(aralkyl)-1,3,2-diazaphosphorine 2-Oxides (II).—The synthesis of these compounds is typified by the preparation of 2-chloro-1,3-bis(*p*-methoxybenzyl)-1,3,2-diazaphosphorine 2-oxide. An ether solution of 10 g (0.032 mole) of the desired diamine and 6.5 g (0.064 mole) of triethylamine was added dropwise to 4.86 g (0.032 mole) of $POCl_3$ in 200 ml of cold ether as the mixture was stirred. The triethylamine hydrochloride formed at once and, after stirring the mixture for 1 hr, the hydrochloride was removed by filtration. The ether was removed under reduced pressure and 11.9 g (95% crude) of white solid was obtained. After one recrystallization from acetonitrile, 7.0 g of white solid melting at 151–152° was collected; over-all yield 55.7%.

2-Chloro-5-alkyl-5-nitro-1,3,2-dioxaphosphorinane 2-oxide (III) was synthesized by a procedure related to that used by Lanham.[8] The synthesis is typified by the production of 2-chloro-5-methyl-5-nitro-1,3,2-dioxaphosphorinane 2-oxide (IIIa). 2-Methyl-2-nitro-1,3-propanediol (20 g, 0.148 mole) was placed in 33 g (0.200 mole) of $POCl_3$. The solid diol went into solution after heating at 70° overnight. The solution was allowed to cool and a solid formed at once. This solid was collected on a sintered-glass funnel and washed with 200 ml of CCl_4 followed by 200 ml of petroleum ether (bp 30–60°). After drying overnight under vacuum the product weighed 31 g (97% yield). Recrystallization was carried out in acetonitrile to give 26 g of pure sample melting at 162–163°, over-all yield 82.0%.

2-Chlorohexahydro-1H,3H-pyrido[1,2-c][1,3,2]oxazaphosphorine 2-Oxide (IV).—Phosphorus oxychloride (5.9 g, 0.039 mole) was dissolved in 100 ml of anhydrous diethyl ether. A mixture of 5.0 g (0.039 mole) of 2-(2-hydroxyethyl)piperidine and 7.9 g (0.078 mole) of triethylamine in 50 ml of diethyl ether was added dropwise to the stirring solution, which was kept at 5°. A precipitate of triethylamine hydrochloride formed at once and after all the amine solution was added, the mixture was stirred at room temperature for 2 hr. At the end of the this time, 5 g of amine hydrochloride was removed by filtration. The ether was removed under reduced pressure to yield 8.0 g of crude product melting at 62–65°. One recrystallization from a 5:1 mixture of ethyl acetate–petroleum ether gave 5 g (62.5%) of pure product melting at 63–65°.

(6) L. Molnar and T. Wagner-Jauregg, Swiss Patent 387,639 (1965).

(7) *Cancer Chemotherapy Rept.*, **25**, 1 (1962). A compound is active against Walker 256 if it has a therapeutic index TI ≥ 4, where TI = LD_{10}/ED_{50}. A compound is confirmed active in (a) KB cell culture if the average $ED_{50} \geq 4$ μg ml for results from two laboratories; (b) Sarcoma 180, Lewis lung carcinoma, and solid Friend virus leukemia if the average T/C ≥ 42% in three confirming tests; and (c) lymphoid leukemia L1210 if T/C ≥ 125% in a confirmation test.

(8) W. M. Lanham, U. S. Patent 2,892,862 (1959).

Synthesis of Potential Antineoplastic Agents. XVI. Cyano Derivatives of 1,2,3,4-Tetrahydroquinoxaline and Related Compounds[1a,b]

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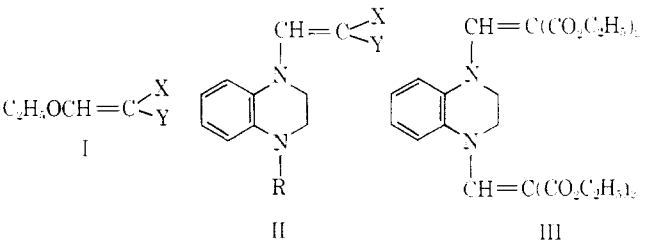
Received May 10, 1966

Cyano derivatives of both antimetabolites and alkylating agents have shown some promise as anticancer agents in preliminary studies.[2] This fact coupled with the observation[1a] that some derivatives of 1,2,3,4-tetrahydroquinoxaline showed activity in the KB line tissue culture screen prompted us to attempt to prepare derivatives of 1,2,3,4-tetrahydroquinoxaline containing a cyano group.

A convenient route to such compounds would appear to be the condensation[3,4] of compounds such as ethoxymethylenemalononitrile (I, X = Y = CN) with the tetrahydroquinoxaline. A series of model reactions were first run with I and 1,2,3,4-tetrahydroquinoline and 1,2,3,4-tetrahydroisoquinoline. The results are shown in Table I.



When these reactions were extended to 1,2,3,4-tetrahydroquinoxaline, it was found that the products obtained from the reactions with ethyl ethoxymethylenecyanoacetate (I, X = CN; Y = $CO_2C_2N_5$) and ethoxymethylenemalononitrile (I, X = Y = CN) were the monosubstituted derivatives II (R = H) (Table

(1) (a) Part XV: P. Schuyler, F. D. Popp, A. C. Noble, D. W. Alwan, and B. R. Masters, *J. Med. Chem.*, **9**, 704 (1966). (b) Supported in part by research grants from the American Cancer Society (T-177D) and from the National Cancer Institute, U. S. Public Health Service (CA 06606-03). Presented in part at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965. (c) This work has been abstracted from the M. S. Thesis of P. S.

(2) For leading references see W. J. Burke, J. E. Brown, C. Weatherbee, and D. H. Curtis, *J. Med. Chem.*, **7**, 670 (1964).

(3) E. A. Steck, *J. Org. Chem.*, **27**, 306 (1962).

(4) A. A. Santilli, W. F. Bruce, and T. S. Osdene, *J. Med. Chem.*, **7**, 68 (1964).