there was added 100 mg. of sodium iodide and the mixture was refluxed in a nitrogen atmosphere for 6 hr. The usual working up[7a] afforded 70 mg. of a brownish oil which crystallized from ether–hexane. The product was filtered over 10 g. of aluminum oxide (activity II–III) affording 42 mg. (86% yield) of 11-dehydroprogesterone (VIII),[8,11b,19,20] m.p. 164–168°, identified by a mixture melting point with an authentic sample and by the comparison of its infrared spectrum with that of authentic material.

Negative Attempts of Isomerization of 11β,12α-Dibromoprogesterone (VII). (a)—A quantity of 50 mg. of 11β,12α-dibromoprogesterone (VII) was subjected for 30 min. to fusion, according to the procedure of Barton and King.[21] Chromatography on silica gel of the resulting product afforded 18 mg. of a crystalline material, m.p. 98–103°; $[\alpha]^{23}D$ +98° (*c*, 1.000 in $CHCl_3$), containing only little bromine (4.8%); ν_{max}^{KBr} 3450 cm.$^{-1}$ (very broad, associated hydroxy band), 1719–1710 cm.$^{-1}$ (broad carbonyl absorption). The product was not further investigated.

(b)—A solution of 60 mg. of 11β,12α-dibromoprogesterone (VII) in 20 ml. of *o*-xylene was refluxed for 4 hr. with 0.1 ml. of concd. hydrochloric acid and 0.03 ml. of water. The usual working up yielded 62 mg. of crude starting material, m.p. 110–116°, which melted upon one recrystallization at 126–128° dec.; $[\alpha]^{23}D$ +138° (*c*, 1.000 in $CHCl_3$). Other attempts of isomerization met with no more success.

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Potential Carcinolytic Agents Related to Cyclophosphamide[1]

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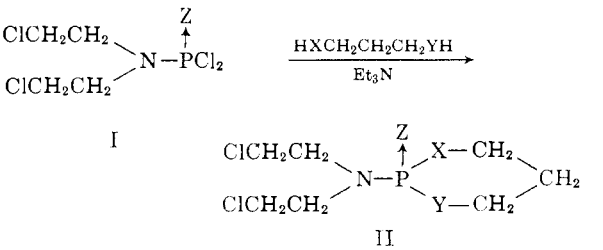
One of the early attempts at the development of enzyme-activated substrates for the much sought-after selectivity of drug action in cancer chemotherapy was the synthesis of N-phosphorylated nitrogen mustards.[3] These compounds, which are inactive precursors of cytotoxic mustards, could be activated by phosphamidases; and based on reports of abundance of these enzymes in some tumors it seemed possible for this "activation" to occur selectively in certain tumor cells. A number of phosphamide mustards in the original series[3] and in series synthesized subsequently[4,5] have shown remarkable selectivity of action in causing complete regression of many types of experimental tumors in animals with relatively little toxicity to the host.

In the case of cyclophosphamide IIa, the one compound tested clinically thus far, the results in humans are not nearly as dramatic as those in animals. Although this compound is not known to cause cures, it does produce beneficial effects in certain forms of human cancer and is one of the more effective drugs now in clinical use.

The mechanism of action of cyclophosphamide, which has been assumed to involve enzymatic "activation" by liberation of the bis(2-chloroethyl)amine (*nor*-nitrogen mustard) moiety, is still not clearly understood. In an attempt to gain insight into the nature of some structural features associated with the high activity of this compound and possibly to find related derivatives of even greater selectivity of action, we have synthesized a new series of derivatives II that are essentially isosteres of cyclophosphamide.

Compounds IIb and IIg were isolated as pure, crystalline products which were quite stable at low temperatures. In fact, the isosteres in which Z = O were all crystalline except IId which was prepared as a fairly pure but unstable oil. The isosteres in which Z = S were all oils; compound IIe was isolated in pure form, but was unstable even at low temperatures.



Ia, Z = O ((NSC-64119)[6]
Ib, Z = S (NSC-59505)

IIa, X, Z = O; Y = NH (NSC-26271)
IIb, X, Z = O; Y = S (NSC-65420)
IIc, Z = O; Y, Z = S (NSC-67105)
IId, X = NH; Y = S; Z = O (NSC-68118)
IIe, X = NH; Y, Z = S
IIf, X, Y = NH; Z = O
IIg, X, Y = S; Z = O (NSC-65422)
IIh, X, Y, Z = S

The remaining materials (IIe, IIf and IIh) were never obtained pure despite our intensive efforts. Attempts to purify the oily products by the usual techniques, including chromatography and molecular distillation at 10^{-6} mm. pressure, were all unsuccessful. Treatment with liberal amounts of activated carbon effected some improvement in purity as evidenced by analytical data.

After hydrolyzing cyclophosphamide with hydrochloric acid, one can account for about 85% of its *nor*-nitrogen mustard content by the γ-(4-nitrobenzyl)-pyridine (NBP) method.[7] However, when this method was used with the isosteres of cyclophosphamide and the intermediate phosphoramidic dichlorides (I) variable results (54% to 86% of *nor*-nitrogen mustard) were obtained depending on the nature of the isostere. Even when concentrated acid was used in an effort to inhibit destruction of the *nor*-nitrogen mustard during hydrolysis, the NBP method was not a useful analytical

(1) Cyclophosphamide: 2-[bis(2-chloroethyl)amino]-1,3,2-oxazaphosphorinane-2-oxide. This work was sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-4360.

(2) To whom inquiries should be addressed.

(3) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 655, 658 (1954).

(4) O. M. Friedman, E. Boger, V. Grubbiankas and H. Sommer, *J. Med. Chem.*, **6**, 50 (1963).

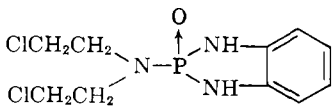
(5) H. Arnold and F. Bourseaux, *Angew. Chem.*, **70**, 539 (1958); H. Arnold, F. Bourseaux and M. Brock, *Arzneimittel-Forsch.*, **11**, 143 (1961).

(6) The NSC accession numbers were assigned by the Cancer Chemotherapy National Service Center.

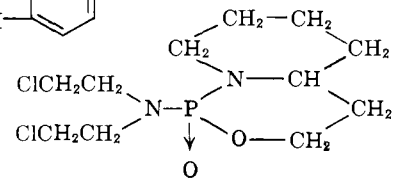
(7) O. M. Friedman and E. Boger, *Anal. Chem.*, **33**, 906 (1961).

tool for following the purification of these new isosteres.

Two analogs of cyclophosphamide have also been prepared and submitted for biological evaluation. One of them (III) was isolated as a stable, crystalline product; the other (IV) was an unstable oil which was not obtained analytically pure.



III (NSC-64909)



IV (NSC-68116)

Unsuccessful attempts were made to prepare similar analogs from catechol, *o*-aminobenzylamine, *o*-aminophenol, 1,3-dihydroxyacetone, 3-amino-2-(hydroxymethyl)-1,3-propanediol, semicarbazide and β-alanine. Each of these materials reacted readily with the phosphoramidic dichloride, Ia, as evidenced by the isolation of essentially the theoretical yields of triethylamine hydrochloride, but the products were not purified satisfactorily.

Preliminary screening data[8] revealed that IIc at 41 mg./kg./d. and IV at 200 mg./kg./d. cured all noninbred rats implanted with Walker 256 tumor fragments, but the therapeutic indices,[9] LD_{10}/ED_{90}, were approximately 1. Ib and IIb were active (100% inhibition of tumor growth at 100 and 50 mg./kg./d.) and the therapeutic indices of these compounds were approximately 2. The remaining compounds Ia, IId, IIg and III were inactive at their respective LD_{10} dose levels.

Experimental[10]

2-[Bis(2-chloroethyl)amino]-1,3,2-oxathiaphosphorinane-2-oxide (IIb).—Triethylamine (275 g., 2.7 moles) was added slowly to a solution of 70.5 g. (0.27 mole) of bis(2-chloroethyl)phosphoramidic dichloride (Ia)[3] in 500 ml. of chloroform; a solution of 25 g. (0.27 mole) of 3-mercapto-1-propanol in 100 ml. of chloroform was then added. After standing in darkness at room temperature for 7 days, the mixture was evaporated to dryness in a rotary vacuum evaporator. The residue was slurried with 500 ml. of benzene, filtered free of the amine hydrochloride (quantitative yield) and concentrated to dryness. The residue was dissolved in a small amount of benzene and the solution was decolorized and diluted with petroleum ether (30–60°) until slightly turbid and cooled. The solid obtained was recrystallized from the same solvents, yielding 17 g. (23%) of white crystals, m.p. 102–104°; ν_{max}^{KBr}: 2950, 1460, 1360, 1220, 1100, 985, 750 and 708 cm.$^{-1}$.

Anal. Calcd. for $C_7H_{14}Cl_2NO_2PS$: C, 30.23; H, 5.07; N, 5.04. Found: C, 30.44; H, 5.15; N, 5.26.

2-[Bis(2-chloroethyl)amino]-1,3,2-oxathiaphosphorinane-2-sulfide (IIc).—This compound was prepared by the same procedure used for IIb above substituting bis-(2-chloroethyl)phosphorothioamidic dichloride (Ib)[11] for the phosphoramidic dichloride. After the amine hydrochloride was removed, the crude product was isolated in excellent yield as an oil which could not be crystallized. It was chromatographed on Florisil, using benzene as an eluent. After evaporating the solvent in a rotary evaporator, the last traces of benzene were removed in a high vacuum, leaving a straw-yellow oil, $n^{25}D$ 1.5772. Even at low-temperature storage the compound decomposed slowly, depositing a solid material of different composition; $\nu_{max}^{CHCl_3}$: 3000, 1430, 1085, 1000, 975 and 910 cm.$^{-1}$.

Anal. Calcd. for $C_7H_{14}Cl_2NOPS_2$: C, 28.58; H, 4.80; Cl, 24.10; N, 4.76; P, 10.53. Found: C, 28.60; H, 4.95; Cl, 22.64; N, 4.38; P, 11.39.

2-[Bis(2-chloroethyl)amino]-1,3,2-azathiaphosphorinane-2-oxide (IId).—This compound was prepared in a manner similar to the above using only a fourfold excess of triethylamine in dioxane. The product was isolated as a light-yellow oil, $n^{25}D$ 1.5528, for which acceptable carbon and chlorine analyses could not be obtained. The material slowly became turbid when stored even at low temperatures due presumably to decomposition; $\nu_{max}^{CHCl_3}$: 3420, 3020, 1440, 1205, 1095, 975, and 910 cm.$^{-1}$.

Anal. Calcd. for $C_7H_{15}Cl_2N_2OPS$: C, 30.33; H, 5.46; Cl, 25.58; N, 10.11; S, 11.57. Found: C, 33.70; H, 5.72; Cl, 22.16; N, 9.95; S, 11.04.

2-[Bis(2-chloroethyl)amino]-1,3,2-dithiaphosphorinane-2-oxide (IIg).—The procedure described for IIb was used. A mixture of benzene and petroleum ether was used as the recrystallizing solvent, although recovery was poor. The yield of the white crystalline solid obtained was 7 g. (11%), m.p. 112–114°; $\nu_{max.}^{KBr}$: 2910, 1450, 1205, 1090, 975, 875, 765 and 700 cm.$^{-1}$.

Anal. Calcd. for $C_7H_{14}Cl_2NOPS_2$: C, 28.58; H, 4.80; Cl, 24.10; N, 4.76; P, 10.53. Found: C, 28.66; H, 5.03; Cl, 23.42; N, 4.85; P, 11.29.

2-[Bis(2-chloroethyl)amino]-1,3-dihydro-1,3,2-benzodiazaphosphole-2-oxide (III).—The procedure was that described for IId. The oil obtained after removing the triethylamine hydrochloride and solvent was dissolved in benzene, decolorized and then crystallized from the same solvent. The solid was dissolved in dioxane and benzene–ethyl ether, 1:1, was added until the solution was turbid. The cooled solution deposited 6.9 g. (50%) of white crystals, m.p. 162–164°; ν_{max}^{KBr}: 3200, 1620, 1500, 1400, 1280, 1190, 990 and 735 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{14}Cl_2N_3OP$: C, 40.83; H, 4.80; Cl, 24.11; N, 14.29. Found: C, 41.23; H, 4.91; Cl, 24.01; N, 14.16.

1-[Bis(2-chloroethyl)amino]hexahydro-1H,3H-pyrido[1,2-c]-[1,3,2]oxazaphosphorine-1oxide (IV).—This product was prepared in the manner described for IId. It was isolated as an uncrystallizable oil and was purified by successive treatments with decolorizing charcoal in benzene. The yellow oil, freed of solvent under high vacuum, amounted to 7.5 g., (60%), $n^{25}D$ 1.5150; $\nu_{max.}^{CHCl_3}$: 2960, 1440, 1220, 1090 and 995 cm.$^{-1}$.

Anal. Calcd. for $C_{11}H_{21}Cl_2N_2O_2P$: C, 41.92; H, 6.72; Cl, 22.50; N, 8.89. Found: C, 41.49; H, 6.58; Cl, 21.25; N, 7.82.

(8) The compounds are being evaluated by the Cancer Chemotherapy National Service Center, and complete data will be published in a future Cancer Chemotherapy Screening Data supplement to Cancer Research.

(9) Consult: H. E. Skipper and L. H. Schmidt, *Cancer Chemotherapy Reports*, **17**, 1 (1962).

(10) All starting materials and solvents were carefully purified before use. All reactions and most other manipulations were conducted in a nitrogen atmosphere. Melting points were corrected.

(11) Asta-Werke A-G Chemische Fabrik, British Patent 822,119 (1959).

Synthesis of Potential Antineoplastic Agents. IX. Some Cycloalkyl Mustards and Related Compounds[1-3]

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We have reported recently[5,6] the preparation of a number of Schiff bases from various amines and

(1) Part VIII, F. D. Popp, *J. Med. Pharm. Chem.*, **5**, 627 (1962).

(2) A portion of this material was presented before the Division of Medicinal Chemistry at the 142nd Meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1962.

(3) This work was supported in part by research grants from the American Cancer Society (T 177A) and from the National Cancer Institute, U.S.P.H.S. (CY 4814C2).

(4) To whom inquiries should be sent at Department of Chemistry, Clarkson College of Technology, Potsdam, N. Y.

(5) F. D. Popp, *J. Org. Chem.*, **26**, 1566 (1961).

(6) F. D. Popp and W. Kirsch, *J. Org. Chem.*, **26**, 3858 (1961).