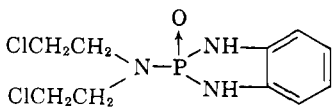
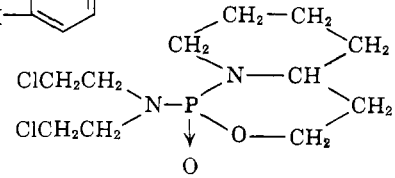
tool for following the purification of these new isosteres.

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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Received August 8, 1962

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).

TABLE I
CYCLOALKYL MUSTARDS

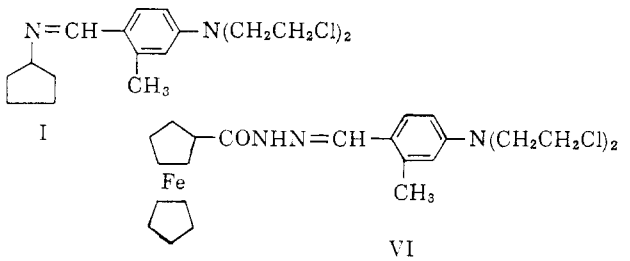
HCl

$$(CH_2)_{n-1}CH—Y—N(CH_2CH_2Cl)_2$$

n	Y	Formula	Yield,[a] %	M.p., °C.	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
3	CH_2	$C_8H_{16}Cl_3N$	37	86–88	40.88	6.93	6.02	41.25	7.15	6.15
4	CH_2	$C_9H_{18}Cl_3N$	40	101–102	43.83	7.36	5.68	44.11	7.66	5.36
5	CH_2	$C_{10}H_{20}Cl_3N$	70	139–140[b]	46.08	7.73	5.38	46.29	7.57	5.64
5	CH_2CH_2	$C_{11}H_{22}Cl_3N$	65	93–94	48.10	8.08	5.10	48.22	7.86	5.31
5	$CH_2CH_2CH_2$	$C_{12}H_{24}Cl_3N$	75	67–69			4.85			5.06
6	CH_2	$C_{11}H_{22}Cl_3N$	67	150–152[c]			5.10			5.36

[a] Over-all yield from commercially available acid or acid chloride. [b] Reported[9] m.p. 128–130°. [c] Reported[9] m.p. 145–147°.

mustard aldehydes. Among the most active compounds[7] in this series were those from various alicyclic amines. In fact, compound I is one of three Schiff bases from the series[5,6] currently being considered for clinical trial.[7] This fact coupled with the interest in 1-aminocyclopentanecarboxylic acid[8] has led us to investigate some simple cycloalkyl mustards in the hope of finding more active antineoplastic agents and to see whether activity still might center in the five-membered ring.



I

VI

A few compounds in this series with five, six, and seven membered rings had been prepared earlier[9] and some screening results have been reported.[10] We are presenting our results, however, since our synthetic sequence is more convenient. We have also prepared additional compounds in the series, and have additional screening results.[7]

The compounds shown in Table I were prepared from the commercially available acids or acid chlorides as shown in the sequence II to V. The amides (IV) were very hygroscopic and difficult to purify and the reduction to V was carried out using the crude material.

$$\underset{\text{II}}{R(CH_2)_xCO_2H} \xrightarrow{SOCl_2} \underset{\text{III}}{R(CH_2)_xCOCl} \xrightarrow{HN(CH_2CH_2Cl)_2} \underset{\text{IV}}{R(CH_2)_xCON(CH_2CH_2Cl)_2} \xrightarrow{LiAlH_4} \underset{\text{V}}{R(CH_2)_xCH_2N(CH_2CH_2Cl)_2.HCl}$$

$$R = (CH_2)_{n-1}CH-$$

No evidence for rearrangement of the mustard amides was noted. This sequence appears to be more convenient than the previous method[9] in which the appropriate amine was prepared and treated with ethylene oxide and thionyl chloride, or the appropriate chloro-compound was caused to react with diethanolamine and thionyl chloride.

In studies with a sarcoma in white rats previous workers[10] reported an increase in toxicity and decrease in activity with decrease in ring size from seven to five carbon atoms. The compounds reported here show, on a molar basis, a similar increase in toxicity but also an increase in activity with decrease in ring size from six to three carbons when given in single doses to Fisher rats with Dunning leukemia.[7] The best compound in this series was, however, less active, on a molar basis, than was nitrogen mustard itself. The crude amide (IV) from cyclopentanecarboxylic acid exhibited no toxicity and essentially no activity against the Dunning leukemia when given in doses of 250 mg./kg. as compared with toxicity at 25 mg./kg. and activity at 10 mg./kg. for the reduction product (V) of this amide.

In a further study of the effect of five membered rings ferrocenecarboxhydrazide was treated with tolualdehyde mustard[5] to give the ferrocene mustard VI. This compound was inactive and non-toxic against the Dunning leukemia.

Experimental

Preparation of Amides IV.—A cold solution of 0.1 mole of N,N-Bis-(β-chloroethyl)amine hydrochloride in a minimum volume of water was neutralized with cold 4 *N* sodium hydroxide. The free amine was extracted rapidly with benzene (3 × 50 ml.) and dried ($NaSO_4$). To the dried solution in an ice bath was added, over 30 min., 0.05 mole of acid chloride.[11] The mixture was then stirred for 1 hr. at 0°, 1 hr. at room temperature and finally refluxed for 1 hr. The cooled mixture was filtered and the filtrate concentrated to give the crude amide.[12]

Preparation of Mustards V.—A solution of 0.05 mole of the crude amide IV in ether (or ether with a minimum of methylene chloride) was added dropwise to a stirred mixture of 0.059 mole of lithium aluminum hydride in 100 ml. of anhydrous ether at room temperature. The mixture then was stirred for an additional 3 hr. at room temperature after which water was added dropwise. The mixture was filtered and the ether layer separated and washed with water and dried ($NaSO_4$). Passage of dried hydrogen chloride into the ether solution gave a white precipitate of the mustard hydrochloride V. This was recrystallized from ethyl acetate to give the compounds listed in Table I.

(7) Private communication from Drs. L. Rane and R. Jones, Jr., University of Miami.

(8) R. B. Ross, C. I. Noll, W. C. J. Ross, M. V. Nadkarni, B. H. Morrison, Jr., and H. W. Bond, *J. Med. Pharm. Chem.*, **3**, 1 (1961).

(9) S. I. Sergievskaya, K. V. Levshina, A. K. Chizkov, A. I. Gavrilova and A. I. Kravchenko, *Zhur. Obshchei Khim.*, **28**, 1839 (1958).

(10) A. I. Kravchenko, *Voprosyon Kol.*, **4**, 17 (1958).

(11) Obtained commercially or prepared by refluxing the acid with thionyl chloride.

(12) These crude amides were used directly in the next step. They could be crystallized only with difficulty and in these cases analytical results were consistent with 1–3 moles of water in each case.

4-[N,N-Bis(2-chloroethyl)amino]-2-methylbenzylidenehydrazide of Ferrocenecarboxylic Acid (VI).—A hot solution of 1.17 g. (0.0045 mole) of 4-[N,N-bis-(2-chloroethyl)amino]-2-methylbenzaldehyde in 10 ml. of absolute ethanol was added to 1 g. (0.0041 mole) of ferrocenecarboxhydrazide in 10 ml. of absolute ethanol and the mixture was refluxed for 30 min. and filtered hot. Cold filtration yielded 1.41 g. (71%), of VI, m.p. 195–198°. Recrystallization from absolute ethanol gave material, m.p. 196–197°.

Anal. Calcd. for $C_{23}H_{25}Cl_2FeN_3$: C, 56.81; H, 5.18; N, 8.64; Cl, 14.58. Found: C, 56.80; H, 5.25; N, 8.68; Cl, 14.62.

Acknowledgment.—We wish to acknowledge the technical assistance of Mr. J. Graff and several helpful discussions with Dr. Leo Rane regarding the screening results.

Potential Anticancer Agents.[1] LXXX. Alkylating Agents Related to Phenylalanine Mustard.[2] VI. Enantiomeric *meta*-Phenylalanine Mustards

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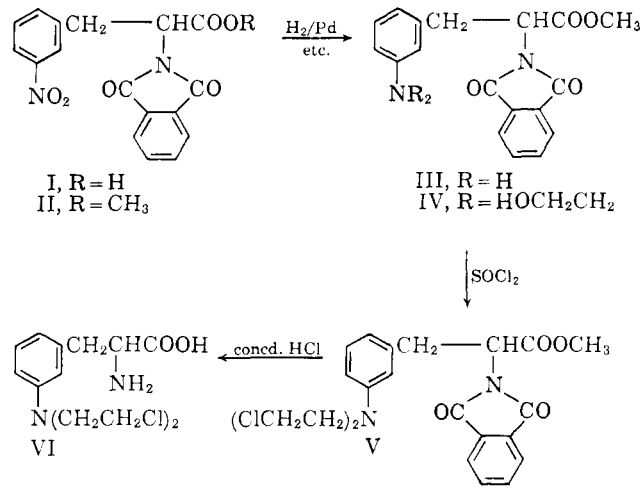
Preparation of the enantiomorphs of *m*-phenylalanine mustard (D- and L-VI) was prompted by the finding[3] that in the treatment of some transplanted mouse tumors the racemate DL-VI[4] was markedly superior to the *para*-mustard (L or DL); the L-form of the *para*-mustard, in turn, was several times more active than the D-*para*-mustard in certain tests.[5] If this optical selectivity should hold for the *meta*-mustards, then the L-isomer (L-VI) might be one of the most efficacious anticancer drugs of this highly interesting series.

Synthesis of D-VI and L-VI began with the chemical resolution of a precursor, N-phthalyl-*m*-nitrophenylalanine (DL-I), in the sequence[4] to the racemic mustard DL-VI, and was completed by that sequence. Optically pure brucine salts of both D-I and L-I were obtained from one treatment of DL-I with the alkaloid and were converted with methanolic hydrogen chloride directly to the methyl esters, D- and L-II. Intermediate regeneration with aqueous acid to the enantiomorphic acids D- and L-I was inconvenient and less efficient; attempted regeneration in mild base caused partial loss of the phthalyl group. Success in the conversion of IV to V was dependent on use of a carefully controlled excess (13–14%) of thionyl chloride in order to avoid tar formation. Because D-IV and L-IV (unlike racemic IV) could not be crystallized, the degree of purity from



ethylene oxide polymers was best estimated from the optical rotation of a given sample of sirup, and the amount to be used relative to thionyl chloride was verified on a small scale for each batch of D- or L-IV. Optimum yields of D- and L-V were 57–58%. Replacement of the hydroxyl groups in D-IV and L-IV by chlorine could also be accomplished with methanesulfonyl chloride in hot pyridine,[6] in lower yield of D-V and L-V but with no tendency toward darkening and tarring.

A crucial modification in the removal of blocking groups (V to VI) was use of concentrated hydrochloric acid at 95° rather than at reflux[4] (110–130°), otherwise yields of D-VI and L-VI varied from 10–0%. Under optimum conditions, over-all yields of D-VI and L-VI from the nitro esters D-II and L-II were 35% and 27%, respectively. Absence of racemization during the sequence could be expected from the work of Bergel[7] and of Luck[8] and was supported by observation of equal but opposite optical rotations for each enantiomeric pair; use of 12 *M* rather than 6 *M* hydrochloric acid in the final step apparently did not affect the optical center. The absolute configurations were inferred by comparison of optical rotations for the enantiomeric mustards VI and precursors (I, II, and III-hydrochlorides) with the rotations of the analogous *para*-isomers,[7] where starting materials of known absolute configuration were used; the rotations of D- and L-II also agreed very closely with those of N-phthalyl-D- and L-phenylalanine.[9]

Biological Results.—Preliminary studies[10] have been made in which the D- and L-*meta* mustards (D- and L-VI) were compared with the racemic form (DL-VI) and with L-*p*-phenylalanine mustard against four tumors in rats. The results are shown in Table I. The quantity ED_{90} is the dose which causes a 90% reduction in tumor weight, except with Dunning Leukemia where it is the dose causing 90% of the rats to be cured. These preliminary data certainly do not suggest any significant difference in antitumor activity or therapeutic index between the D- and L-forms of *m*-phenylalanine mustard.

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center.

(2) For the preceding paper on phenylalanine mustards, see R. H. Iwamoto, E. M. Acton, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 4743 (1961).

(3) M. O. Greene, B. R. Baker, and J. Greenberg, *Cancer Res.*, **20**, 1160 (1960); V. I. Trusheikina, *Voprosy Onkol.*, **6**, No. 10, 63 (1960).

(4) H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3103 (1959).

(5) F. Bergel and J. A. Stock, *Brit. Empire Cancer Campaign, Ann. Rep.*, **31**, 6 (1953).

(6) J. DeGraw and L. Goodman, *J. Org. Chem.*, **27**, 1395 (1962).

(7) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2409 (1954); F. Bergel, V. C. E. Burnop, and J. A. Stock, *ibid.*, 1223 (1955).

(8) H. E. Smith and J. M. Luck, *J. Org. Chem.*, **23**, 837 (1958).

(9) J. C. Sheehan, D. W. Chapman, and R. W. Roth, *J. Am. Chem. Soc.*, **74**, 3822 (1952).

(10) These studies were performed at the Christ Hospital Institute of Medical Research, Cincinnati 19, Ohio, under the direction of Dr. L. H. Schmidt, to whom we are indebted for the results.