Hexahydropyrimidines. VIII.[1] Synthesis of 2-Substituted 1,3-Bis{4-[N,N-bis(2-chloroethyl)amino]benzyl}hexahydropyrimidines as Transport Molecules for Tumor Inhibition[2]

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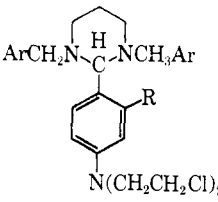
A new series of nitrogen mustard hexahydropyrimidines has been prepared by condensing N,N′-bis{4-[N,N-bis(2-chloroethyl)amino]benzyl}-1,3-diaminopropane (V) with various aldehydes and evaluated for antitumor activity against different test systems. Some of these hexahydropyrimidines were reported to be quite active against 5WC in test animals and in KB cell culture studies.

Since the discovery of nitrogen mustard as a therapeutic agent in the treatment of human malignancies,[3] a considerable number of aliphatic and aromatic analogs and related alkylating agents have been synthesized and evaluated as potential antitumor drugs. One of the principal disadvantages encountered in the use of these agents in chemotherapy is their general tendency to show cytotoxic effects on normal cells, thus prohibiting their prolonged therapeutic use.[4] Danielli[5,6] has suggested that specific selectivity of action on tumors might be achieved by designing the structure of a "nitrogen mustard" so as to take advantage of certain differences which may exist between neoplastic and normal cells. The concept of enzymic activation falls into this category and has already been investigated by Ross, *et al.*[6,7] For example, N,N-bis(2-chloroethyl)-*p*-phenylenediamine is an active antitumor agent against Walker carcinoma in rats but shows a high degree of toxicity. The introduction of electron-withdrawing groups, such as N-acetyl and N-benzoyl derivatives, results in a pronounced reduction in both toxicity and in the activity. Only the acetyl derivative retains a significant amount of activity. Since the tumors are known to possess an enzyme which can deacylate an acetamido group but not a benzamido group,[6] it is likely that the activity of the acetyl derivative is due to the liberation of a nitrogen mustard moiety *in vivo* by enzymic fission at the tumor site.

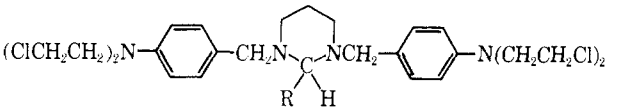
The antitumor activity of some of these aromatic nitrogen mustards suggests, therefore, that appropriately designed alkylating agents will be able to arrest and impair tumor cells by inactivating enzymes and other related macromolecular systems.

An approach, based on the above concept, has been exploited in this laboratory for the synthesis of potential antitumor agents. Previous studies of this series[1,8] were concerned with the preparation and structure–activity relationships of a series of 1,2,3-substituted hexahydropyrimidines of type I. Two of these compounds, 2-{4-[N,N-bis(2-chloroethyl)amino]phenyl}-1,3-bis(*p*-methoxybenzyl)hexahydropyrimidines (Ia) and 2-{4-[N,N-bis(2-chloroethyl)amino]-2-methylphenyl}-1,3-bis(*p*-methoxybenzyl)hexahydropyrimidines (Ib), exhibited reproducible antitumor activity against Walker carcinoma 256 in test animal (93 and 100% inhibition at 100 mg/kg, respectively). The interesting biological activity provided considerable evidence that properly oriented molecules of this type may possess specific tumorcidal properties against rapidly dividing cells without causing appreciable damage to the normal cells.



Ia, R = H
b, R = CH_3
Ar = *o*-methoxyphenyl or *p*-methoxyphenyl

The antitumor activity of the aforementioned compounds suggests that a study should be made of similar types of hexahydropyrimidines in which the nitrogen mustard moiety would be incorporated in the aromatic rings substituted in the 1 and 3 positions as in the following structure. Compounds of this type would be of



considerable interest for two reasons: (1) the hexahydropyrimidines would now contain two nitrogen mustard groupings, thus allowing for cross-linking at two different sites; and (2) the similarity of the diamine to N,N-bis(2-chloroethyl)-*p*-phenylenediamine[7] and *p*-[N,N-bis(2-chloroethyl)amino]benzylamine[9] which have already been shown to possess pronounced antitumor activity. Thus, these diamine intermediates might, in themselves, have some significant activity. The advantage of converting these diamines into pyrimidines is to provide a carrier which would transport the

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(2) This investigation was supported by a Public Health Service Research Grant No. CA-07227-02 from the National Cancer Institute.

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(4) C. T. Klopp and J. C. Bateman, *Advan. Cancer Res.*, **2**, 255 (1954); C. C. Stock, *ibid.*, **2**, 425 (1954).

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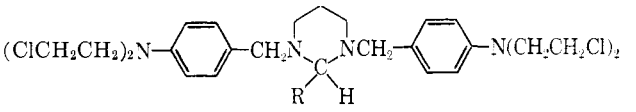
(7) W. C. J. Ross, G. P. Warwick, and J. J. Roberts, *J. Chem. Soc.*, 3110 (1955).

(8) J. H. Billman and J. L. Meisenheimer, *J. Med. Chem.*, **8**, 540 (1965); J. H. Billman and M. S. Khan, *ibid.*, **8**, 498 (1965); J. H. Billman and J. L. Meisenheimer, *ibid.*, **7**, 115 (1964); **6**, 682 (1963).

(9) F. Bergel, J. L. Everett, and W. C. J. Ross, *J. Chem. Soc.*, 3855 (1955).

TABLE I

2-SUBSTITUTED 1,3-BIS{4-[N,N-BIS(2-CHLOROETHYL)AMINO]BENZYL}HEXAHYDROPYRIMIDINES



No.	R	Reaction time, hr	Yield, % (pure)	Mp, °C (cor)	Formula	Analyses	Ir absorption bands for grouping N–C–N, cm^{-1}
1	C_6H_5[a]	5	64.2	112–113	$C_{32}H_{40}Cl_4N_4$	Cl, N	1085 (s), 1138, 1155 (sh), 1175 (s)
2	2-HOC_6H_4[b]	8	70.7	92–93	$C_{32}H_{40}Cl_4N_4O$	Cl, N	1075 (s), 1145 (m), 1175 (s)
3	2-HO-3-$(CH_3O)C_6H_3$[a]	7	61.4	140–141	$C_{33}H_{42}Cl_4N_4O_2$	Cl, N	1075 (s), 1145, 1155 (sh), 1175 (s)
4	2-HO-3,5-$Cl_2C_6H_2$[c]	4	70.3	135–136	$C_{32}H_{38}Cl_6N_4O$	Cl, N	1075 (s), 1143, 1153 (sh), 1180 (s)
5	2-HO-3-$(CH_3CH{=}CH)C_6H_3$[a]	6	70.4	105–106	$C_{35}H_{44}Cl_4N_4O$	Cl, N	1075 (s), 1140, 1155 (sh), 1180 (s)
6	2-HO-5-ClC_6H_3[d]	3	81.7	155–156	$C_{32}H_{39}Cl_5N_4O$	Cl, N	1080 (s), 1146 (m), 1180 (s)
7	2-HO-5-$NO_2C_6H_3$[a]	4	73.2	134–135	$C_{32}H_{39}Cl_5N_5O_3$	Cl, N	1090 (s), 1140, 1155 (sh), 1185 (s)
8	2-HO-5-BrC_6H_3[d]	3	97.6	172–173	$C_{32}H_{39}BrCl_4N_4O$	N	1080 (s), 1146 (m), 1180 (s)
9	4-$(CH_3)_2NC_6H_4$[c]	15	85.7	155–156	$C_{34}H_{45}Cl_4N_5$	Cl, N	1085 (s), 1135 (sh), 1175 (s)
10	4-C_6H_4CN[a]	10	72.6	87.5–88.5	$C_{33}H_{39}Cl_4N_5$	Cl, N	1080 (s), 1140, 1150 (sh), 1170 (s)
11	4-$CH_3OC_6H_4$[e]	17	70.0	103–104	$C_{33}H_{42}Cl_4N_4O$	Cl, N	1080 (s), 1140, 1155 (sh), 1175 (s)
12	4-FC_6H_4[e]	15	97.0	128–129	$C_{32}H_{39}Cl_4FN_4$	N	1080 (s), 1150 (sh), 1175 (s)
13	3-FC_6H_4[f]	17	70.4	105–106	$C_{32}H_{39}Cl_4FN_4$	N	1085 (s), 1140 (sh), 1175 (s)
14	2-FC_6H_4[a]	10	80.6	108–109	$C_{32}H_{39}Cl_4FN_4$	N	1089 (s), 1140, 1155 (sh), 1175 (s)
15	2-HO-3-CH_3-6-$HOCH_2$-4-C_5HN[f]	18	70.3	152–153	$C_{33}H_{43}Cl_4N_5O_2$	Cl, N	1075 (s), 1099 (w), 1136, 1136 (sh)
16	1,2,3,4-HO-4-$CO_2HC_4H_4$[g]	5	94.2	155–156	$C_{31}H_{44}Cl_4N_4O_6$	Cl, N	1080 (m), 1130 (w), 1180 (s), 1176 (s)

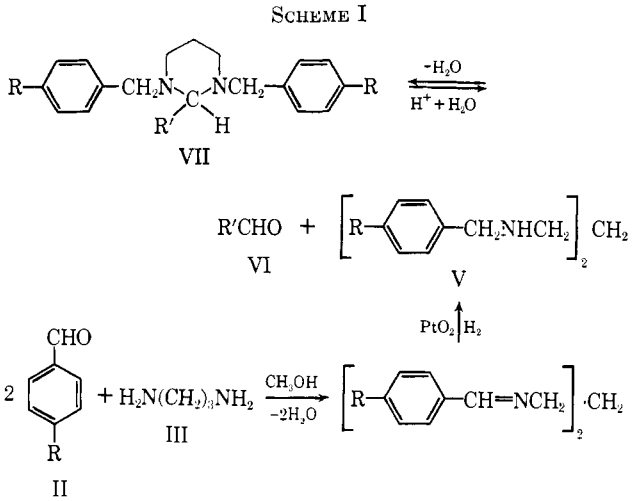
[a] Recrystallized from MeOH–MeCN (1:1). [b] Recrystallized from MeCN. [c] Recrystallized from MeOH–C_6H_6 (1:1). [d] Recrystallized from MeOH–C_6H_6 (2:1). [e] Recrystallized from absolute EtOH. [f] Recrystallized from absolute MeOH. [g] Analytical sample obtained by washing several times with MeOH and MeCN successively to constant melting point.

nitrogen mustard moiety as well as the aldehyde to the tumor site, where through chemical or enzymatic fission they can be released in high concentration to perform their intended function.

In order to prepare compounds of this type, it was decided that simple aldehyde derivatives of this diamine should be prepared first, because of the ease with which they can be made. As it turned out, derivatives of other diamines which have been prepared in this laboratory have shown significant antineoplastic activity. Consequently, a series of nitrogen mustard hexahydropyrimidines was synthesized in an attempt to obtain compounds with better therapeutic indices and to understand better their antitumor potentiality.

The hexahydropyrimidines reported in Table I were prepared by condensing the desired aldehydes with N,N′-bis{4-[N,N-bis(2-chloroethyl)amino]benzyl}-1,3-diaminopropane (V) in equimolar proportions as outlined in Scheme I. Generally, the condensation proceeded smoothly at room temperature, and in all cases solid product separated from the solution. Methanol was found to be a suitable solvent for the ring-closure reaction and provided crystalline hexahydropyrimidines in high yields. However, difficulties were experienced with formaldehyde and heptaldehyde, as these aldehydes failed to give solid derivatives with diamine V. Instead, a viscous oil was obtained from the reaction mixture, which could not be induced to crystallize. An attempted distillation of this material *in vacuo* resulted in the decomposition of the product.

SCHEME I



R = $N(CH_2CH_2Cl)_2$; R′ = phenyl or substituted phenyl

The diamine V was obtained in two steps from trimethylenediamine (III) and 4-[N,N-bis(2-chloroethyl)-amino]benzaldehyde (II). In the initial step, the diamine III was condensed with 2 moles of aldehyde II to give the di-Schiff base (IV), an oil which could not be characterized. The hydrogenation (PtO_2) of crude di-Schiff base IV furnished the desired diamine V, which was characterized as the dihydrochloride.

Bergmann, *et al.*,[10] state that the ir spectrum of the grouping N–C–N is characterized by three peaks occurring between 1080 and 1170 cm^{-1}. The ir spectra of these hexahydropyrimidines VII had similar absorption bands (Table I) between 1075 and 1175 cm^{-1}. These maxima presumably correspond to the C–N frequency. The shift in frequency is probably due to the presence of bulky substituents in the 1, 2, and 3 positions and to the strong hydrogen-bonding effects. It was noted that when there were electron-donating groups in position 2 of the phenyl ring substituted in position 2 of the pyrimidine, considerable shift to lower frequency was observed. On the other hand, when there were electron-withdrawing groups, absorption always fell within the reported region 1089–1170 cm^{-1}.

Biological Results.—The hexahydropyrimidines and the diamine dihydrochloride listed in Table I were

(10) E. D. Bergmann, E. Meeron, Y. Hirshberg, and S. Pinchas, *Rec. Trav. Chim.*, **71**, 200 (1952).

screened under the auspices of the Cancer Chemotherapy National Service Center for antitumor activity in doses up to 200 mg/kg/day and in cell culture tests. Toxicity tests were performed in rats by intraperitoneal daily injection in dose levels of 3–100 mg/kg. These animals were used in each of four dose levels and injections were continued for 5 days. All the animals survived for 10 days in the tests with each of the compounds except for 2, 3, 12, 14, 15, and 16, which were toxic at dose level of 100 mg/kg. Although the screening data are not available on all of the compounds included in Table I, the available test results indicate that the hexahydropyrimidines and the diamine had a moderate activity against Walker 256. All of the compounds had given a reproducible ED_{50} value of 0.17–0.0047 mg/ml in a KB human epidermoid carcinoma and were very active (by the criteria established by the CCNSC[11]) in cell culture tests. Compound **1** has ED_{50} 0.17, **2** 1.5; **6**, 0.0047; **7**, 0.31; and **8**, 0.03. Compound **9** shows a 5WC value of 4 at the 200-mg dose level.

Experimental Section

All melting points were taken on a Fisher-Johns melting point apparatus and are corrected. The elemental analyses were performed by Midwest Microlaboratories, Inc., Indianapolis, Ind., and Triangle Chemical Laboratories, Inc., Chapel Hill, N. C. Ir spectra were determined in KBr disks on a Beckman IR-10 spectrophotometer. Aldehydes used were either reagent grade or purified by distillation or recrystallization from appropriate solvents. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

N,N′-Bis{4-[N,N-bis(2-chloroethyl)amino]benzyl}-1,3-diaminopropane (V).—A solution of 4.92 g (0.02 mole) of 4-[N,N-bis(2-chloroethyl)amino]benzaldehyde[12] (II) in 50 ml of absolute MeOH was placed in a bottle, and to this solution 0.74 g (0.01 mole) of 1,3-diaminopropane (III) was added in one batch. The bottle was tightly stoppered and the mixture was shaken vigorously for 12 hr at room temperature. At the end of this period, the solvent was removed *in vacuo* to give 5.0 g (94.3%) of IV. After 5.3 g (0.01 mole) of crude V was dissolved in 100 ml of EtOH, it was then reduced, using 0.01 g of PtO_2, at room temperature in a low-pressure Parr hydrogenator with an initial pressure of 3.15 kg/cm². Approximately 30 min was required to complete the reduction. The catalyst was removed by filtration and the solvent was evaporated *in vacuo*. There was obtained 5.34 g (100%) of a light yellow oil. The oil (3.4 g, 94%) was converted to the dihydrochloride with dry ethereal HCl, mp 210–213°. Three crystallizations of this material from MeOH gave 3.2 g (88.5%) of an analytical sample, mp 215–216° dec. *Anal.* ($C_{25}H_{36}Cl_4N_4 \cdot 2HCl$) Cl, N.

2-Substituted 1,3-Bis{4-[N,N-bis(2-chloroethyl)amino]benzyl}-hexahydropyrimidines (Table I).—The 2-substituted derivatives were prepared in the following manner. A 5.34-g (0.01 mole) sample of N,N′-bis{4-[N,N-bis(2-chloroethyl)amino]benzyl}-1,3-diaminopropane (V) was dissolved in 60 ml of MeOH in a 250-ml hydrogenation bottle. To this solution was added 1.22 g (0.01 mole) of salicylaldehyde in 25 ml of MeOH. The bottle was well stoppered and the mixture was shaken vigorously for 8 hr at room temperature. The crystalline solid which separated from the solution was filtered to yield 4.0 g of product. The filtrate from the reaction mixture was reduced to approximately one-third of the original volume and allowed to stand overnight in the refrigerator. The crystalline solid was filtered to give an additional 1 g of the product, over-all crude yield 5.0 g (78.1%), mp 88–91°. Three crystallizations from MeCN gave 4.5 g (70.7%) of pure sample, mp 92–93°. *Anal.* ($C_{32}H_{40}Cl_4N_4O$) Cl, N.

Acknowledgment.—The authors acknowledge Dr. H. B. Wood of the Cancer Chemotherapy National Service Center for his cooperation in making the screening data available. They also wish to thank Union Carbide Chemical Co. for supplying the 1,3-diaminopropane used in this research.

(11) A compound is confirmed active in KB cell culture if the average $ED_{50} \leq 4$ mg/ml for results from two laboratories. A compound is active against 5WC if its T/C value is 25 or less.

(12) R. H. Wiley and G. Irick, *J. Org. Chem.*, **26**, 593 (1961).