Hexahydropyrimidines. III.[1] A Study of 2-Substituted 1,3-Bis(*p*-methoxybenzyl)hexahydropyrimidines and 2-Substituted 1,3-Bis(*p*-chlorobenzyl)hexahydropyrimidines as Transport Molecules for Tumor Inhibition[2]

JOHN H. BILLMAN AND JOHN L. MEISENHEIMER[3]

Department of Chemistry, Indiana University, Bloomington, Indiana

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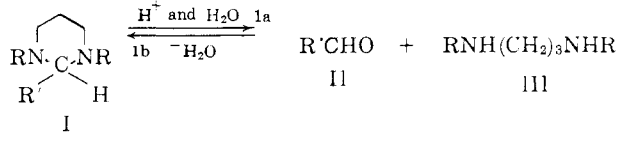
A series of 2-substituted 1,3-bis(*p*-methoxybenzyl)hexahydropyrimidines and 2-substituted 1,3-bis(*p*-chlorobenzyl)hexahydropyrimidines have been prepared and tested for antitumor activity. Some of the aldehydes which were used in the preparation of these hexahydropyrimidines were reported to cause tumor regression in animals. Since the hexahydropyrimidines can be hydrolyzed under mild acidic conditions to regenerate the aldehyde, it was felt that this class of compounds might be effective in transporting the aldehyde to a tumor site. These compounds were not active in rodent screens. However, three of these compounds displayed activity in tissue culture studies.

In the summary, "An Index of Tumor Chemotherapy,"[4] are listed the results of the testing of a number of aldehydes for antitumor activity. Several of these were reported to exhibit a slight amount of activity. A few, including heptaldehyde, phenanthrene-9-aldehyde, and phloroglucinaldehyde, apparently effected occasional regressions. Since aldehydes are so susceptible to oxidation, and react with so many other functional groups, there is a good possibility that little, if any, of the free aldehyde ever reaches the tumor site. It was, therefore, of interest to see if some of these aldehydes could be made more tumor inhibiting by converting them into a potential aldehyde from which the aldehyde might be liberated at the tumor site.

Hexahydropyrimidines I are one class of compounds which may be thought of as potential aldehydes, since these compounds readily hydrolyze under mild acid conditions to liberate the free aldehyde.[1a] In addition, there is also the possibility of enzymatic cleavage. If these reactions should take place preferentially in tumor cells, then one might expect that the activity of an aldehyde would be enhanced if incorporated into compounds such as I.

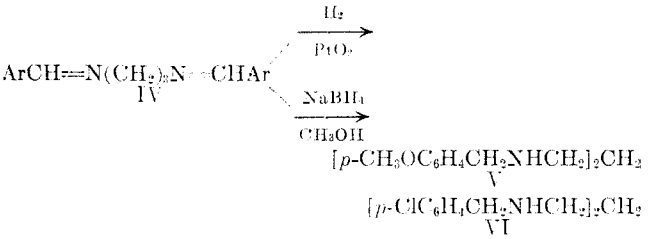


I II III

R and R′ = H, alkyl, aryl, or aralkyl

Evidence is available which indicates that one might expect some increased hydrolysis rate in tumors. Measurements have been made of the pH of human and experimental animal tumors and these were found to be generally of a slightly lower pH than that found in normal tissues.[5]

The hexahydropyrimidines reported in Tables I and II were synthesized by condensing an aldehyde (II) with a secondary 1,3-diamine (III) in absolute ethanol.[1b] The diamines used were N,N′-bis(*p*-methoxybenzyl)-1,3-diaminopropane (V) and N,N′-bis(*p*-chlorobenzyl)-1,3-diaminopropane (VI). These diamines were chosen because they are of low toxicity and because the electronegativity difference between the chloro and methoxy groups should effect some difference in the ease of liberation of the aldehyde from the corresponding hexahydropyrimidines. The diamines were prepared by reduction of the corresponding benzylidene di-Schiff base IV. Hydrogenation of IVa, using platinum oxide catalyst, furnished diamine V. Diamine VI was obtained by reducing compound IVb with sodium borohydride in methanol.[6]



IV a. Ar = *p*-$CH_3OC_6H_4$
IV b. Ar = *p*-ClC_6H_4

The di-Schiff bases were prepared by reacting a 2 molar ratio of the required aromatic aldehyde with one mole of 1,3-diaminopropane. Compound IVa was prepared from *p*-anisaldehyde and compound IVb from *p*-chlorobenzaldehyde.

An attempt to obtain the hexahydropyrimidine from phloroglucinaldehyde and N,N′-bis(*p*-methoxybenzyl)-1,3-diaminopropane gave a dark, resinous material.

Biological Results.—These compounds have been submitted to the Cancer Chemotherapy National Service Center for antitumor testing. Most of the screening has been against Sarcoma 180, Carcinoma 755, Leukemia 1210, and a cell culture system. The tested compounds have displayed no significant activity against any of these *in vivo* test systems. However, the first three compounds listed in Table I have given reproducible ED_{50} values below the 4 mg./ml. level in the tissue culture tests. The occurrence of this type of

(1) (a) J. H. Billman and L. C. Dorman, *J. Org. Chem.*, **27**, 2419 (1962); (b) J. H. Billman and L. C. Dorman, *J. Pharm. Sci.*, **51**, 1071 (1962).

(2) This investigation was supported in part by a Public Health Service Fellowship (GF-13,650) from the Division of General Medical Sciences, National Institutes of Health, Public Health Service.

(3) National Institutes of Health Fellow, 1961-1963. Taken from the Ph.D. thesis of J. L. M., Indiana University, 1963.

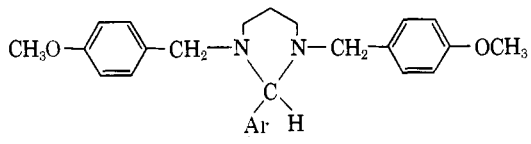
(4) "An Index of Tumor Chemotherapy," by H. M. Dyer, National Cancer Institute, National Institutes of Health, March, 1949.

(5) H. Kahler and W. B. Robertson, *J. Natl. Cancer Inst.*, **3**, 495 (1943).

(6) J. H. Billman and A. C. Diesing, *J. Org. Chem.*, **22**, 1068 (1957).

TABLE I

2-SUBSTITUTED
1,3-BIS(*p*-METHOXYBENZYL)HEXAHYDROPYRIMIDINES

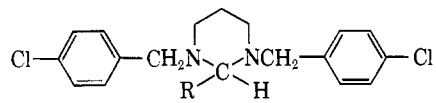


Ar	Yield, % (pure)	M.p., °C. cor.	Nitrogen, % Calcd.	Found
2,4-Dichlorophenyl	47	101.5–102.5	5.94	6.08
Phenyl	50	88.5–89.5	6.97	7.19
o-Ethoxyphenyl	48	97–98	6.24	6.25
p-Chlorophenyl	45	89–90.5	6.42	6.30
o-Methoxyphenyl[a]	72	83.5–85	6.48	6.53
2,4-Dihydroxyphenyl[b]	50	166–166.5	6.45	6.57
2-Hydroxy-5-nitrophenyl[c]	83	153.5–155	9.07	8.87
o-Chlorophenyl[d]	48	80–81	6.42	6.20
o-Hydroxyphenyl	82	155–156	6.70	6.71
p-Dimethylaminophenyl	59	124.5–125	9.43	9.37
p-Methoxyphenyl	65	85.5–86.5	6.48	6.49
2-Hydroxy-5-chlorophenyl	59	90–91	6.18	6.23
p-Nitrophenyl[e]	54	152.5–154	9.39	9.44
m-Nitrophenyl[c]	23	129–130	9.39	9.42
9-Phenanthryl[e]	39	162–163.5	5.50	5.57

[a] Mol. wt.: Calcd., 433. Found, 441 (Mechrolab osmometer). [b] Recrystallized from acetone–acetonitrile. [c] Recrystallized from absolute ethanol–acetone. [d] Recrystallized from methanol–acetonitrile. [e] Recrystallized from absolute ethanol–acetonitrile.

TABLE II

2-SUBSTITUTED
1,3-BIS(*p*-CHLOROBENZYL)HEXAHYDROPYRIMIDINES



R	Yield, % (pure)	M.p., °C. cor.	Nitrogen, % Calcd.	Found
o-Hydroxyphenyl	63	193.5–194	6.56	6.56
Hexyl	44[a]	Oil[b]	6.67	6.25[a] 6.44[b]
p-Chlorophenyl	42	112–112.5	6.28	6.23

[a] Undistilled. [b] Distilled, short path, 200° (0.1 mm.), $n^{25}D$ 1.5525.

activity in three of these compounds may be significant. In addition, these hexahydropyrimidines have been found to be relatively nontoxic.

Due to the positive results of the tissue culture tests, and the previously stated requirement for a pH lower in the tumor cells than in normal cells, arrangements have been made to retest certain of these compounds under conditions wherein the pH value at the tumor site will be reduced. It is felt that some slight increase in tumor acidity might evoke *in vivo* activity for these compounds. In addition to this approach, some related hexahydropyrimidines have been prepared from diamines and aldehydes which contain the nitrogen mustard moiety.

Experimental

Aldehydes.—Either a reagent grade of aldehyde was used or it was purified by distillation or recrystallization from an appropriate solvent.

N,N′-Bis(*p*-methoxybenzylidene)-1,3-diaminopropane (IVa).—To a refluxing solution of 600.0 g. (4.40 moles) of *p*-anisaldehyde in 300 ml. of absolute ethanol was added 163.0 g. (2.26 moles) of 1,3-diaminopropane during a 2-hr. period. After an additional 10 min. of refluxing, the solution was transferred to a large beaker. The crystals obtained by scratching were collected and washed twice with ethanol; yield, 540 g., 79%, m.p. 78–79°. Recrystallization of 110 g. of this material from absolute ethanol provided 103 g. of crystals, m.p. 79.0–79.5°, which was used to prepare V. The analytical sample was obtained by three additional recrystallizations from ethanol, m.p. 79.0–79.5°.

Anal. Calcd. for $C_{19}H_{22}N_2O_2$: N, 9.03. Found: N, 8.75.

N,N′-Bis(*p*-chlorobenzylidene)-1,3-diaminopropane (IVb).—This compound was prepared in a manner similar to IVa from 911 g. (6.47 moles) of *p*-chlorobenzaldehyde and 239.8 g. (3.23 moles) of 1,3-diaminopropane giving 822 g. (80%) of crude material, m.p. 61.5–66.5°. Two recrystallizations from ethanol provided 452 g. (44%) of colorless crystals, m.p. 66.0–67.0°.

Anal. Calcd. for $C_{17}H_{16}Cl_2N_2$: N, 8.78. Found: N, 8.82.

N,N′-Bis(*p*-methoxybenzyl)-1,3-diaminopropane (V).—Using 0.9 g. of platinum oxide catalyst, 60.0 g. (0.19 mole) of N,N′-bis-(*p*-methoxbenzylidene)-1,3-diaminopropane (IVa) in 200 ml. of absolute ethanol was reduced at room temperature in a Parr hydrogenator at 2–3 atm. pressure over a 2-hr. period. The catalyst was removed by filtration and the solvent was distilled from the filtrate at 25 mm. with the flask in an 80° oil bath. There was obtained 60.9 g. (100%) of a light yellow oil. A 10.0 g. sample of this oil in benzene was converted to 12.1 g. (99%) of the dihydrochloride with dry hydrogen chloride. This material was recrystallized twice from a water–acetone mixture to give 8.8 g. of solid which sublimed at 225° (*ca.* 0.2 mm.).

Anal. Calcd. for $C_{19}H_{26}N_2O_2 \cdot 2HCl$: N, 7.24; Cl, 18.31. Found: N, 7.40; Cl, 18.46.

N,N′-Bis(*p*-chlorobenzyl)-1,3-diaminopropane (VI).—A mixture of 100 g. (0.313 mole) of N,N′-bis(*p*-chlorobenzylidene)-1,3-diaminopropane (IVb) and 1 l. of methanol was cooled in an ice-water bath. As the mixture was stirred, 50.0 g. (1.33 moles) of solid sodium borohydride were added in small portions over a 2-hr. period. The solution was stirred an additional hour and then brought to reflux for 30 min. The solution was filtered while hot and then one-half of the solvent was distilled from the filtrate at atmospheric pressure. When the mixture had cooled to room temperature, a solution of 100.0 g. of sodium hydroxide in 900 ml. of water was added. The mixture was shaken briefly and then divided into two approximately equal parts, each part was extracted four times with 50-ml. portions of diethyl ether. The ether extracts were combined and dried over sodium hydroxide pellets. The solution was filtered and the ether was removed with a rotary evaporator. There was obtained 91.5 g. (90.4%) of a colorless oil, $n^{25}D$ 1.5715. An estimate of the purity of this compound can be made through its condensation with salicylaldehyde to form 2-(*o*-hydroxyphenyl)-1,3-bis(*p*-chlorobenzyl)hexahydropyrimidine. A 1.63-g. (0.005 mole) sample of the diamine oil was dissolved in 10 ml. of absolute ethanol and to this was added 0.67 g. (0.0055 mole) of salicylaldehyde, in 10 ml. of absolute ethanol. The solution was heated on a steam bath for 5 min. and then cooled in the refrigerator. There was obtained 2.10 g. (98.6%) of the hexahydropyrimidine.

2-Substituted 1,3-Bis(*p*-methoxybenzyl)hexahydropyrimidines (Table I).—The 2-phenyl derivative was prepared in the following manner. A solution of 6.76 g. (0.064 mole) of benzaldehyde in 25 ml. of absolute ethanol was slowly added to a stirred solution of 20.0 g. (0.064 mole) of diamine V in 25 ml. of absolute ethanol. The mixture was refluxed for 1 hr. (15 min. is sufficient) and then filtered while hot. The solution was allowed to cool slowly to room temperature and was then placed in the refrigerator overnight. The crystalline solid which separated was collected and washed with ethanol; yield, 21.4 g. (83.5%). Two recrystallizations from 50-ml. portions of absolute ethanol provided 1.9 g. (50.4%) of colorless crystals, m.p. 88.5–89.5°.

Anal. Calcd. for $C_{26}H_{30}N_2O_2$: N, 6.97. Found: N, 7.19.

2-Substituted 1,3-Bis(*p*-chlorobenzyl)hexahydropyrimidines (Table II).—These compounds were prepared by a procedure as in the previous section, from aldehydes and N,N′-bis(*p*-chlorobenzyl)-1,3-diaminopropane.

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