methylpiperidinomethyl)indole; 3-(3-methylpiperidinomethyl)indole; 1-(4-methylpiperidinomethyl)-benzimidazole; 1-(3-methylpiperidinomethyl)benzotriazole; 1-(4-methylpiperidinomethyl)benzotriazole; and 1-(3-methylpiperidinomethyl)-1*H*-indazole. It is interesting to note that in all cases, with one exception, the benzazoles which produce the highest locomotor activity are methylpiperidino derivatives. Death usually occurs coupled with excess secretions, respiratory failure, and cyanosis. Occasionally convulsions are observed.

REFERENCES

(1) Powell, C. E., Swanson, E. E., and Chen, K. K., *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 399(1955).
(2) Bertaccini, G., and Zamboni, P., *Arch. Intern. Pharmacodyn.*, **133**, 138(1961).
(3) Walshe, J. M., DeCarli, L., and Davidson, C. S., *Clin. Sci.*, **17**, 11(1958).
(4) Dubnik, B., Leeson, G. A., and Phillips, G. E., *J. Neurochem.*, **9**, 299(1962).
(5) Kuhn, H., and Stein, O., *Ber.*, **70**, 567(1937).
(6) Okuda, T., *Takugaku Zasshi*, **80**, 205(1960).
(7) Hellmann, H., and Teichmann, K., *Ber.*, **91**, 2432 (1958).
(8) Henry, D. W., and Leete, E., *J. Am. Chem. Soc.*, **79**, 5254(1957).
(9) Bachman, G. B., and Heisey, L. V., *ibid.*, **68**, 2496 (1946).
(10) Snyder, H. R., Thompson, C. B., and Hinman, R. L., *ibid.*, **74**, 2009 (1952).
(11) Kochetkov, N. K., and Dudykina, N. V., *Zhur. Obshchei. Khim.*, **31**, 201(1961).
(12) Pozharskii, F. T., Kazanbieva, M. A., and Tertov, B. A., *ibid.*, **34**, 10, 3367(1964).
(13) Reichert, B., "Die Mannich-Reaktion," Springer Verlag, Berlin, January, 1959, p. 93.
(14) Albert, A., "Heterocyclic Chemistry," Essential Books, Fair Lawn, N. J., 1959, pp. 143, 158–159, 343–345.

Keyphrases

Mannich bases—centrally acting
Benzazole derivatives—Mannich reaction synthesis
Pharmacological screening—benzazoles

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

By JOHN H. BILLMAN and M. SAMI KHAN

A series of nitrogen mustard hexahydropyrimidines has been prepared by reacting *N*,*N*-bis{2-methyl-4-[*N*,*N*-bis(2-chloroethyl)amino]benzyl}1,3-diaminopropane with various aldehydes and evaluated for antitumor activity against various test systems. Some of the aldehydes used herein for the preparation of hexahydropyrimidines were reported to cause temporary tumor regression in test animals. The majority of compounds were screened against Walker 256 in rats and KB cell culture.

IT IS WELL KNOWN that a majority of tumors contain cells with a lower pH than cells in normal tissues. It has been shown also by Fitch *et al.* (1) that the administration of glucose to tumor-bearing animals can produce an even lower pH value for the tumor cells. Since hexahydropyrimidines are one class of compounds which hydrolyze readily *in vitro* under mild acidic conditions, it is likely that these compounds could be selectively hydrolyzed by the tumor cells to liberate active aldehydes as well as diamines which in themselves might act as antitumor agents. Thus it seems reasonable to expect that properly designed hexahydropyrimidines might act as carrier molecules to direct the nitrogen mustard grouping or other active neoplastic agents into the cellular metabolism.

In an earlier publication (2) the authors have reported the synthesis and the antitumor activity of a number of hexahydropyrimidines containing two aromatic nitrogen mustard groupings in the N-1 and N-3 positions. The primary screening results indicate that all of these compounds were nontoxic at high dose levels and were very active in KB cell culture. (0.1–0.0047 mg./ml.). Against Walker 256 a somewhat moderate activity was observed. In view of these encouraging results, it was of interest to study the effect of the substitution of methyl groups in the 2-positions of the benzene rings containing the nitrogen mustard grouping in order to obtain compounds with better therapeutic indexes and establish more fully their antitumor potentiality. Previous test results have indicated that a methyl group in the 2-position of aldehydes

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TABLE I—2-SUBSTITUTED-1,3-BIS{2-METHYL-4-[*N,N*-BIS(2-CHLOROETHYL)AMINO]BENZYL}HEXAHYDROPYRIMIDINES

Compd.	R	Reaction Time, hr.	Yield (Pure), %	M.p., °C.	Formula	%Cl Calcd.	%Cl Found	%N Calcd.	%N Found	IR Absorption Bands for Grouping N—C—N, cm.$^{-1}$
1	Phenyl[a]	4	70	128–129	$C_{34}H_{46}Cl_4N_4$	21.79	21.62	8.61	8.88	1080,1160(s),1115,1135(shoulder)
2	2-Hydroxyphenyl[b]	4	60	132–133	$C_{34}H_{46}Cl_4N_4O$	21.27	21.41	8.40	8.64	1070,1140,1160(s),1115(shoulder)
3	2-Hydroxy-5-chlorophenyl[c]	6	71.3	164.5–165.5	$C_{34}H_{44}Cl_6N_4O$	25.29	24.98	7.99	7.92	1070(s),1115,1140(shoulder),1175(w)
4	2-Hydroxy-5-bromophenyl[d]	5	71.6	159–160	$C_{34}H_{44}BrCl_4N_4O$			7.51	7.38	1065(s),1130(shoulder),1170(m)
5	2-Hydroxy-3,5-dichlorophenyl[d]	10	74	150–151	$C_{34}H_{42}Cl_6N_4O$	28.92	28.64	7.61	7.82	1065,1175(s),1120,1135(shoulder)
6	2-Hydroxy-3-allylphenyl[e]	5	64.2	137–138	$C_{37}H_{48}Cl_4N_4O$	20.07	20.20	7.92	7.90	1065(s),1115,1135(shoulder),1155(w)
7	2-Hydroxy-3-methoxyphenyl[f]	12	66.9	87–88	$C_{35}H_{48}Cl_4N_4O_2$	20.37	20.10	8.18	8.02	1065(s),1120,1140(w),1165(m)
8	4-Dimethylaminophenyl[g]	18	79.7	119–120	$C_{36}H_{52}Cl_4N_6$	20.44	20.66	10.09	9.80	1075,1165(s),1120,1125(shoulder)
9	4-Methoxyphenyl[a]	10	85.4	132–133	$C_{35}H_{46}Cl_4N_4O$	20.83	21.26	8.23	8.22	1080,1165(s),1115(shoulder),1135(m)
10	4-Cyanophenyl[c]	18	77.6	154–155	$C_{36}H_{46}Cl_4N_6$	20.99	21.26	10.36	10.56	1080,1160(s),1115,1125(shoulder)
11	4-Fluorophenyl[c]	18	56.7	88–89	$C_{34}H_{44}Cl_4FN_4$			8.37	8.69	1080,1150(s),1120,1135(shoulder)
12	3-Fluorophenyl[b]	18	89.2	125–126	$C_{34}H_{44}Cl_4FN_4$			8.37	8.26	1080(s),1120,1135(shoulder),1175(m)
13	2-Fluorophenyl[c]	10	59.7	119–120	$C_{34}H_{44}Cl_4FN_4$			8.37	8.46	1080(s),1110(shoulder),1130(w),1160(m)

[a] Recrystallized from acetonitrile–methanol (2:1). [b] Recrystallized from acetonitrile. [c] Recrystallized from acetonitrile–methanol (1:1). [d] Recrystallized from acetonitrile–benzene (2:1). [e] Recrystallized from ethanol-benzene (1:1). [f] Recrystallized from acetonitrile. [g] Recrystallized from acetonitrile–ethanol (1:1).

used in the formation of their hexahydropyrimidine rings has enhanced the molecular activity.

The synthesis of the *N,N'*-bis{2-methyl-4-[*N,N*-bis(2-chloroethyl)amino]benzyl}1,3-diaminopropane (IV) reported in Table I was conveniently accomplished according to Scheme I.

The structure of these hexahydropyrimidines was proven by elemental analysis and IR spectra. Bergmann *et al.* (3) state that the IR spectrum of the grouping N—C—N is characterized by three peaks occurring between 1,089 and 1,170 cm.$^{-1}$. The IR spectra of these hexahydropyrimidines VI (Table I) in KBr disks had three similar major peaks between 1,070 and 1,175 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. When there were electron-withdrawing groups, on the other hand, absorption always fell within the reported region 1,089–1170 cm.$^{-1}$.

BIOLOGICAL RESULTS

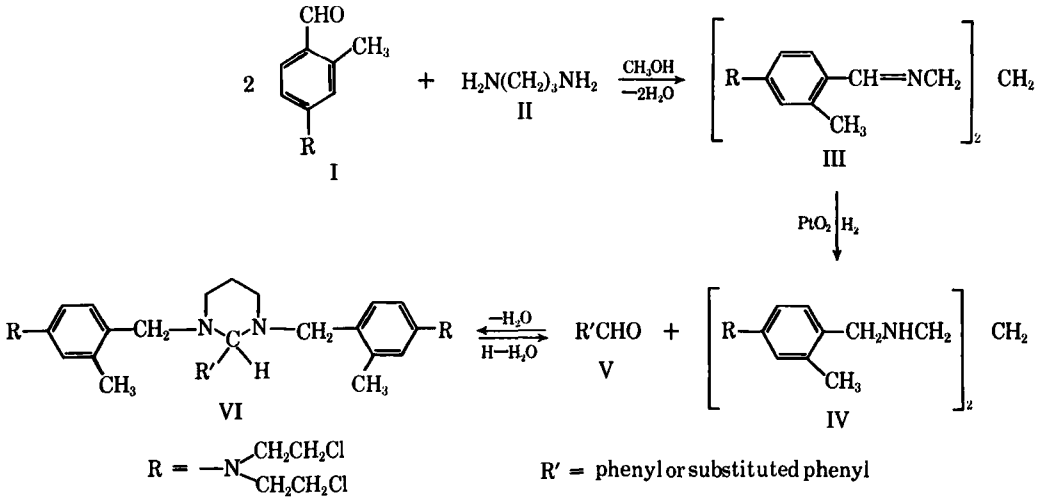
The nitrogen mustard hexahydropyrimidines (Table I) and the diaminedihydrochloride IV, which was reacted with the aldehydes, were submitted to the Cancer Chemotherapy National Service Center (CCNSC) for cytotoxic studies in tissue culture and animal testing for antitumor activity.

Although complete screening results have not been received for all of the compounds, the available figures indicate the hexahydropyrimidines and the diamine have a moderate activity against Walker 256. At a dose level of 50 mg./kg., Compound 1 produced 50% inhibition, Compound 4, 34% inhibition, Compound 10, 79% inhibition, Compound 11, 67% inhibition, Compound 12, 84% inhibition, and Compound 13, 48% inhibition against Walker 256. At a dose level of 15 mg./kg., Compound 8 gave 86% inhibition against Walker 256. Tissue culture activity had been received for only Compounds 1 and 2, which have given reproducible ED_{50} value of 0.19–0.97 mg./ml. in a KB human epidermoid carcinoma according to the criteria established by CCNSC.

EXPERIMENTAL[1]

N,N' - Bis{2 - methyl - 4 - [*N,N* - bis(2 - chloroethyl)amino]benzyl}1,3-diaminopropane (IV)—A solution of 5.2 g. (0.02 mole) of 4-[*N,N*-bis(2-chloroethyl)amino]-*o*-tolualdehyde (I) (4) in 60 ml. absolute methanol was transferred to a 250-ml. hydrogenation bottle and to this solution 0.74 g. (0.01 mole) of 1,3-diaminopropane (II) was added in one batch. The bottle was immediately wel

[1] All melting points were taken on a Fisher-John melting point apparatus and were corrected. The elemental analyses were performed by Midwest Microlaboratories, Inc., Indianapolis, In., and Triangle Chemical Laboratories, Inc., Chapel Hill, N. C. The IR spectra were determined in KBr disks on a Beckman IR-10 spectrophotometer. All evaporations, unless stated otherwise, were conducted in a rotary flask evaporator at water pressure. Aldehydes used were either reagent grade or purified by distillation or recrystallization from appropriate solvents.



Scheme I

stoppered and the mixture was allowed to shake vigorously for 18 hr., at room temperature. At the end of this period, the methanol was removed *in vacuo* to give 5.3 g. (95%) of desired di-Schiff base (III), a yellow oil which could not be induced to crystallize. The crude di-Schiff base, 5.3 g. (0.009 mole) in 200 ml. of absolute ethanol, was then reduced, using 0.1 g. of PtO_2 catalyst, at room temperature in a low-pressure Parr hydrogenator with an initial pressure of 3.15 kg./cm.^{2}. Approximately 40 min. were 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. A 4.0-g. sample of this oil in 200 ml. of dry ether was converted to 4.4 g. (97.3%) of the dihydrochloride with dry HCl, m.p. 215–218° (dec.). Three crystallizations from hot methanol gave 4.2 g. (92.9%) of an analytical sample, m.p. 219–220° (dec.).

Anal.—Calcd. for $C_{27}H_{40}Cl_4N_4 \cdot 2HCl$: Cl, 33.48; N, 8.81. Found: Cl, 33.26; N, 8.67.

2 - Substituted - 1,3 - bis{2 - methyl - 4 - [N,N-bis(2 - chloroethyl)amino]benzyl} hexahydropyrimidines (Table I)—The 2-substituted derivatives were prepared in the following manner. A 5.62-g. (0.01 mole) sample of *N,N'*-bis{2-methyl-4-[*N,N* - bis(2 - chloroethyl)amino]benzyl}1,3 - diaminopropane (IV) was dissolved in 60 ml. of absolute methanol and transferred to a 250-ml. hydrogenation bottle. To the solution was added 1.06 g. (0.01 mole) of benzaldehyde in 20 ml. of absolute methanol. The bottle was well stoppered and the mixture was shaken vigorously for 4 hr. at room temperature. The crystalline solid which separated from the solution was filtered to give 4.1 g. of desired product. The filtrate obtained from the reaction mixture was reduced to 1/3 of its original volume and allowed to stand overnight in a refrigerator. The crystalline solid was filtered to give an additional 0.7 g. of the product. The overall crude yield was 4.8 g. (73.8%), m.p. 126–129°. Three crystallizations from acetonitrile–methanol (2:1) provided 4.55 g. (70%) of pure sample, m.p. 128–129°.

Anal.—Calcd. for $C_{34}H_{44}Cl_4N_4$: Cl, 21.79; N, 8.61. Found: Cl, 21.62; N, 8.88.

REFERENCES

(1) Fitch, R. H., and Voegtlin, G., *U. S. Public Health Serv. Bull.*, **164**, 15(1935).
(2) Billman, J. H., and Khan, M. S., *J. Med. Chem.*, **11**, 312(1968).
(3) Bergmann, E. D., Meeron, E., Hirshberg, Y., and Pinchas, S., *Rec. Trav. Chim.*, **71**, 200(1952).
(4) Popp, F. D., *J. Org. Chem.*, **26**, 1566(1961).

Keyphrases

Hexahydropyrimidines—synthesis
Antitumor activity—hexahydropyrimidines
IR spectrophotometry—structure