were added to cooled solutions of 2.24 g. (26.7 mmoles) of trioxymethylene and 0.02 g. of potassium hydroxide in 18 ml. of methanol, to which these amines had first been added: (a) 3.70 g. of cyclohexylamine, (b) 4.10 g. of benzylamine, (c) 4.52 g. of phenethylamine, (d) 4.03 g. of 2-aminomethylpyridine, (e) 4.03 g. of 3-aminomethylpyridine (each 37.4 mmoles), or to a mixture of (f) 4.5 g. (37.4 mmoles) of 25% aqueous methylamine, 6.08 g. (75.0 mmoles) of 38% aqueous formaldehyde and 40 ml. of methanol. The methylamine reactions were refluxed for 90 min. and all other reactions for 30 min. After cooling, the reaction mixtures were poured into water, extracted with methylene chloride, the extracts concentrated to dryness and the residues passed in benzene over 20 g. of alumina (Woelm basic, activity II). After concentrating, the eluted material was dissolved in ether and treated with a slight excess of dry hydrochloric acid in ether. The hydrochlorides were collected by filtration, washed well with ether and added to ice cold dilute aqueous sodium hydroxide. Rapid extraction with ether and concentration gave amorphous white powders which were used for biological testing.

Anal. Calcd. for stilbestrol and (a) $C_{34}H_{46}N_2O_2$: N, 5.44. Found: N, 5.22. (b) $C_{26}H_{38}N_2O_2$: N, 5.28. Found: N, 5.00. (c) (gummy material, discarded); (d) $C_{34}H_{36}N_4O_2$: N, 10.52. Found: N, 9.78. (e) $C_{34}H_{36}N_4O_2$: N, 10.52. Found: N, 8.61. (f) $C_{24}H_{36}N_2O_2$: N, 7.40. Found: N, 6.21.

Anal. Calcd. for hexestrol and (a) $C_{34}H_{48}N_2O_2$: N, 5.42. Found: N, 5.24. (b) $C_{26}H_{40}N_2O_2$: N, 5.26. Found: N, 5.33. (c) $C_{38}H_{44}N_2O_2$: N, 5.00. Found: N, 4.56. (d) $C_{34}H_{38}N_4O_2$: N, 10.48. Found: N, 10.07. (e) $C_{34}H_{38}N_4O_2$: N, 10.48. Found: N, 8.63. (f) $C_{24}H_{32}N_2O_2$: N, 7.36. Found: N, 5.44.

Antineoplastic Agents. IV. Mannich Base Nitrogen Mustards (Part A)[1,2,3]

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Among the various types of alkylating agents which have received clinical trial the nitrogen mustards appear to offer the most promise

(1) See, G. R. Pettit, M. V. Kalnins, T. L. Liu, E. G. Thomas, and K. Parent, *J. Org. Chem.*, **26**, 2563 (1961), for the preceding contribution.

(2) Abstracted in part from the Master of Science Thesis submitted by J. A. Settepani to the Graduate School, University of Maine, June, 1961.

(3) This investigation was aided by Grants No. T-79A and T-79B from the American Cancer Society.

as cancer chemotherapeutic agents.[4] Interest in developing useful routes to nitrogen mustard derivatives of certain natural products has led us to study bis(2-chloroethyl)amine in Mannich reactions involving several acetophenones and cyclohexanone.

Only two examples of a Mannich reaction involving bis(2-chloroethyl)amine and a ketone appear to have been described. Both acetone[5] and more recently 3,4,5-trimethyoxyacetophenone[6] (*cf.* Ia) have been condensed in the usual manner[7] with bis(2-chloroethyl)-amine hydrochloride and formaldehyde in ethanol. A number of attempts to use a similar procedure for preparing nitrogen mustard derivatives of acetophenone and *p*-methoxyacetophenone were unrewarding. A variety of acidic,[5-7] neutral,[8a-e] and basic conditions[8f] commonly employed in Mannich reactions also proved uniformly unsuccessful. Unreacted bis(2-chloroethyl)amine (as its perchlorate derivative) usually was recovered in high yield.

On the basis of previous Mannich reaction mechanism studies[9] the reaction between bis(2-chloroethyl)amine and a readily enolizable ketone, such as acetophenone, should be more favorable under strong acid conditions.[10] The recent study of Cummings and Shelton[9] suggests that an acid medium would enhance formation of intermediates related to A and B, but at low pH values the rate of reaction would be independent of pH.

In essential agreement with the above observation it was found eventually that a solvent composed of 1:2 concentrated hydrochloric

(4) Refer to R. Jones, Jr., W. B. Kessler, H. E. Lessner, and L. Rane, *Cancer Chemotherapy Rep.*, **10**, 99 (1960); L. F. Larionov, *Cancer Research*, **21**, 99 (1961); L. F. Larionov, *Cancer Chemotherapy Rep.*, **11**, 165 (1961); and D. A. Karnofsky, *CA-Bull. Cancer Progr.*, **11**, 58 (1961), for leading literature citations.

(5) R. F. Phillips, C. H. Shunk, and K. Folkers, *J. Am. Chem. Soc.*, **70**, 1661 (1948).

(6) G. R. Pettit and D. S. Alkalay, *J. Org. Chem.*, **25**, 1363 (1960).

(7) *Cf.* G. de Stevens and A. Halamandaris, *ibid.*, **26**, 1614 (1961); R. L. Hinman, R. D. Ellefson, and R. C. Campbell, *J. Am. Chem. Soc.*, **82**, 3988 (1960); T. H. Waid and A. Taurins, *Can. J. Chem.*, **38**, 1983 (1960); and F. F. Blicke and F. J. McCarty, *J. Org. Chem.*, **24**, 1069 (1959).

(8) For example, see (a) P. Da Re, L. Verlicchi, and I. Setnikar, *ibid.*, **25**, 1097 (1960); (b) J. H. Burckhalter and D. R. Dill, *ibid.*, **24**, 562 (1959); (c) H. W. Heine, M. B. Winstead, and R. P. Blair, *J. Am. Chem. Soc.*, **78**, 672 (1956); (d) C. C. Bombardieri and A. Taurins, *Can. J. Chem.*, **33**, 923 (1955); (e) H. Hellmann and I. Löschmann, *Chem. Ber.*, **87**, 1684 (1954); and (f) K. A. Jensen and B. Alkede, *Acta Chim. Scand.*, **6**, 201 (1952).

(9) Leading references and a discussion pertinent to this subject have been presented by T. F. Cummings and J. R. Shelton, *J. Org. Chem.*, **25**, 419 (1960).

(10) Since bis(2-chloroethyl)amine might be expected to behave as a weak base in the Mannich reaction, an acid medium could increase the possibility of condensation. *Cf.*, S. V. Lieberman and E. C. Wagner, *ibid.*, **14**, 1001 (1949), and a discussion of acid-catalyzed enolization by E. S. Gould, "Mechanism and Structure in Organic Chemistry," H. Holt and Co., New York, N. Y. 1959, p. 374.

acid–ethanol would supply the necessary environment. Nitrogen mustard Mannich base derivatives of acetophenone and several phenyl-substituted acetophones (*i.e.*, Ic-f) were prepared in reasonable yields (47–72%) employing this solvent. Potassium permanganate oxidation of β-bis(2-chloroethyl)amino-4-methoxypropiophenone (Ic) to *p*-anisic acid supported the structural assignments.

While acetone was converted to 4-[bis(2-chloroethyl)amino]butan-2-one (II) perchlorate (m.p. 115-116°) in good yield[11] (83%) using the 1:2 hydrochloric acid–ethanol procedure, several attempts to effect an analogous condensation in the case of cyclohexanone failed to yield Mannich base III. A sampling of mild acid[5-7] and neutral[8a-e] methods met with similar lack of success. However, when the reaction was carried out in a cold mixture of formalin (37%) and dilute sodium hydroxide (4%)[8f] the perchlorate salt of 2-[bis(2-chloroethyl)-amino]methylcyclohexanone(III) was obtained in 46% yield.

The comparatively short reaction period (1 hr. or less) required, using these conditions, allows this base-catalyzed[9,12] Mannich reaction to compete favorably with ethyleneimonium ion[13](C) formation for available bis(2-chloroethyl)amine.

Hydrochloride derivatives of the Mannich base nitrogen mustards described in the Experimental section are presently being evaluated by the Cancer Chemotherapy National Service Center. Preliminary biological studies[14] have been carried out with the Walker 256 tumor, in non-inbred rats; and the Dunning leukemia tumor, in inbred albino Fischer F-344 rats.

Acetophenone nitrogen mustards Ib, Ie, If, and cyclohexanone Mannich base III were employed against the Walker 256 tumor. Although each of these substances inhibited tumor growth none exhibited a therapeutic index (dose producing 10% deaths divided by the dose leading to 90% inhibition of tumor weight) greater than 2. The survival time of rats, implanted subcutaneously, with Dunning leukemia and treated with nitrogen mustard Mannich bases Ic and

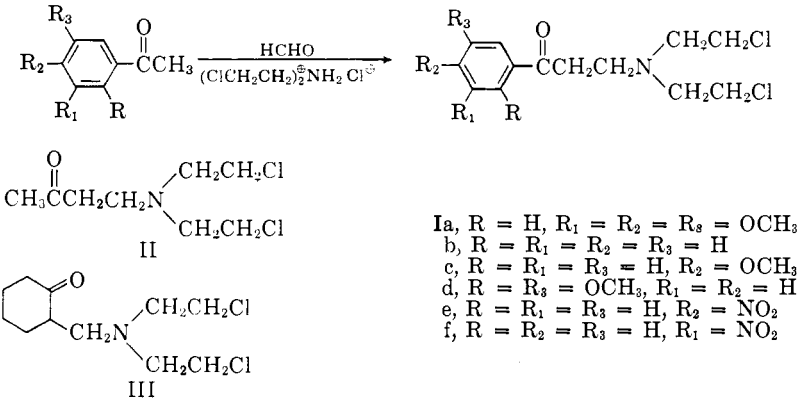
(11) Previously obtained in 70% yield: see ref. 5.

(12) An increase in cyclohexanone carbanion formation at the expense of carbonium ion (B) concentration may be more important with ketones of this type.

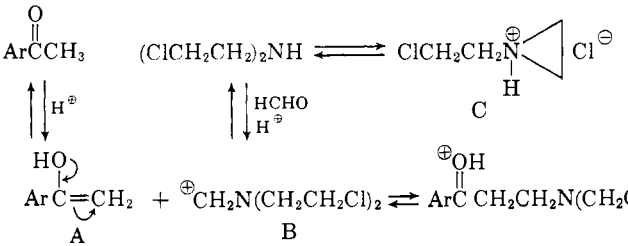
(13) Consult: J. F. Allen and N. B. Chapman, *J. Chem. Soc.*, 1076 (1961); J. F. Allen and N. B. Chapman, *ibid.*, 1482 (1960); T. L. Fletcher and W. H. Wetzel, *J. Org. Chem.*, **25**, 1348 (1960); and V. M. Rakova, A. D. Chinaeva, and A. Ya. Berlin, *Zhur. Obshchei Khim.*, **29**, 3962 (1959), for recent investigations related to this subject.

(14) Dr. Howard Bond, Cancer Chemotherapy National Service Center, has kindly informed us that a more nearly complete biological evaluation of these substances will be summarized in a future *Cancer Chemotherapy Screening Data* supplement to *Cancer Research*.

Id increased, respectively, thirteen and nine days.



Ia, R = H, $R_1 = R_2 = R_3 = OCH_3$
b, $R = R_1 = R_2 = R_3 = H$
c, $R = R_1 = R_3 = H$, $R_2 = OCH_3$
d, $R = R_3 = OCH_3$, $R_1 = R_2 = H$
e, $R = R_1 = R_3 = H$, $R_2 = NO_2$
f, $R = R_2 = R_3 = H$, $R_1 = NO_2$



Experimental[15]

Bis(2-chloroethyl)amine Perchlorate.—The perchlorate salt of bis(2-chloroethyl)amine crystallized from an aqueous solution of the hydrochloride following treatment with 70% perchloric acid. Two recrystallizations from ethanol–water afforded a pure specimen as colorless leaflets, m.p. 169.5–170°.

Anal. Calcd. for $C_4H_{10}Cl_3NO_4$: C, 19.81; H, 4.12; Cl, 43.86; N, 5.78. Found: C, 20.32; H, 4.25; Cl, 44.00; N, 5.40.

β-Bis(2-chloroethyl)aminopropiophenone (Ib) Perchlorate.—A mixture of acetophenone (2.7 g., 0.035 mole), paraformaldehyde (0.9 g.) and bis(2-chloroethyl)amine hydrochloride[16] (5.5 g., 0.03 mole) in ethanol (14 ml.)-concd. hydrochloric acid (7 ml.) was heated at reflux for 30 min. Two additional quantities (0.9 g. total) of paraformaldehyde were added at 10-min. intervals. The reac-

(15) Melting points are uncorrected and were observed using open Kimble glass capillary tubes in a silicone oil-bath. The infrared spectrum of each new compound was found to be consistent with the assigned structure. Dr. R. A. Hill of this laboratory recorded these spectra. With the exception of chlorine determinations which were carried out in the laboratory of Drs. Weiler and Strauss, Oxford, England, elemental analyses were provided by Dr. A. Bernhardt, Max Planck-Institut, Mülheim (Ruhr), Germany.

(16) F. G. Mann, *J. Chem. Soc.*, 461 (1934).

tion mixture then was concentrated, *in vacuo* at 35–40°, to a dark, viscous oil. Following addition of 50% aqueous perchloric acid (10 ml.) and refrigeration (overnight) the colorless crystalline perchlorate was collected, washed with ether, and dried; yield, 4.8 g. (47%), m.p. 65–68°. Three recrystallizations from ethanol (at temperatures below 60°) raised the melting point to 70-71°.

Anal. Calcd. for $C_{13}H_{18}Cl_3NO_5$: C, 41.59; H, 4.83; Cl, 28.64; N, 3.74. Found: C, 42.03; H, 4.84; Cl, 28.78; N, 3.93.

Treating an ethanol solution of the perchlorate with picric acid in the same solvent led to the **picrate derivative of Ib.** Two recrystallizations from acetone gave a pure specimen as yellow needles, m.p. 134°.

Anal. Calcd. for $C_{19}H_{20}Cl_2N_4O_8$: C, 45.35; H, 4.01; Cl, 14.10; N, 11.13. Found: C, 45.63; H, 4.05; Cl, 13.75; N, 10.58.

The propiophenones to be described were prepared employing the general procedure noted above (*cf.*, Ib).

β-Bis(2-chloroethyl)amino-4-methoxypropiophenone (Ic) Perchlorate.—Conversion of *p*-methoxyacetophenone (3.2 g.) to the crude **perchlorate derivative of Ic** provided a 53% yield (5.9 g.) of colorless crystals melting at 125–127°. Two recrystallizations from ethanol gave an analytical sample as colorless prisms, m.p. 128°.

Anal. Calcd. for $C_{14}H_{20}Cl_3NO_6$: C, 41.62; H, 4.99; Cl, 26.30; N, 3.47. Found: C, 41.94; H, 5.13; Cl, 26.20; N, 3.50.

In another experiment, the concentrated reaction mixture was dissolved in acetone and cooled. The colorless crystalline **hydrochloride** which separated weighed 4.7 g. (48%) and melted at 84–89°. Four recrystallizations from acetone led to a pure specimen, as colorless needles, m.p. 105–105.5°.

Anal. Calcd. for $C_{14}H_{20}Cl_3NO_2$: C, 49.36; H, 5.90; Cl, 31.24; N, 4.11. Found: C, 49.60; H, 6.11; Cl, 31.38; N, 3.79.

Oxidation of β-Bis(2-chloroethyl)amino-4-methoxypropiophenone (Ic).—The hydrochloride derivative of Ic (0.4 g.) was added to a solution of potassium permanganate (1.5 g.) and sodium hydroxide (0.3 g.) in water (40 ml.). Following a 1 hr. period of reflux, precipitated material was collected and the filtrate was concentrated *in vacuo* to *ca.* 10 ml. Acidification, using dilute sulfuric acid, precipitated a crude specimen of ***p*-anisic acid** (0.13 g.) melting at 168–172°. Two recrystallizations (Norit-A) from water gave pure material (0.025 g.), m.p. 184-184.5°. The product was identical (mixture melting point determination) with an authentic sample (m.p. 184.5-185°) prepared by potassium permanganate oxidation of *p*-methylanisole.

β-Bis(2-chloroethyl)amino-2,5-dimethoxypropiophenone (Id) Hydrochloride.—The crude oily product obtained from 2,5-dimethoxyacetophenone (8.8 g.) was dissolved in acetone and cooled. The **hydrochloride derivative of Id** which separated weighed 13.2 g. (72%) and melted at 133–135°. An analytical sample crystallized from acetone as colorless plates, m.p. 133.5–134°.

Anal. Calcd. for $C_{15}H_{22}Cl_3NO_3$: C, 48.58; H, 5.98; Cl, 28.70; N, 3.78. Found: C, 49.22; H, 6.09; Cl, 28.85; N, 3.70.

The **picrate derivative of Id** was prepared by adding an ethanol solution of the hydrochloride to picric acid in ethanol. A pure sample crystallized from ethanol as fine yellow threads, m.p. 102-102.5°.

Anal. Calcd. for $C_{21}H_{24}Cl_2N_4O_{10}$: C, 44.79; H, 4.26; N, 9.94. Found: C, 44.85; H, 4.36; N, 10.08.

β-Bis(2-chloroethyl)amino-4-nitropropiophenone (Ie) Perchlorate.—The first crop (2.4 g.) of **nitrogen mustard (Ie) perchlorate** prepared from *p*-nitroacetophenone (4.4 g.) melted at 124–125°. Recooling the mother liquor led to a second crop (2.8 g.) melting at 138–141°: total yield, 59%. The 124–125° polymorph was converted to the higher melting form by recrystallization from ethanol using a seed crystal of the 138–141° material. An analytical sample was obtained as colorless prisms, m.p. 143–143.5°, by recrystallizing the higher-melting substance from ethanol.

Anal. Calcd. for $C_{13}H_{17}Cl_3N_2O_7$: C, 37.20; H, 4.08; N, 6.69. Found: C, 37.30; H, 4.08; N, 6.39.

The free base (Ie), prepared from the perchlorate using cold dilute sodium hydroxide solution, was dissolved in ether, dried over magnesium sulfate, and treated with hydrogen chloride. A pure sample of the **hydrochloride** derivative, m.p. 114–115.5°, crystallized from acetone as colorless plates.

Anal. Calcd. for $C_{13}H_{17}Cl_3N_2O_3$: C, 43.89; H, 4.81; N, 7.87. Found: C, 43.68; H, 4.93; N, 7.90.

Microanalytical determinations for chlorine in both the perchlorate and hydrochloride salts consistently gave values 1 to 2% less than calculated.

β-Bis(2-chloroethyl)amino-3-nitropropiophenone (If) Perchlorate.—Cooling an aqueous perchloric acid solution of the crude product from *m*-nitroacetophenone (4.4 g.) afforded 4.6 g. of colorless crystals melting at 86–90°. A second crop (4.4 g.) melted at 93–95° and a third (0.2 g.) at 87–90°: total yield 68%. Repeated recrystallization of the second crop from ethanol yielded an analytical sample as colorless prisms, m.p. 104.5–105.5°.

Anal. Calcd. for $C_{13}H_{17}Cl_3N_2O_7$: C, 37.20; H, 4.08; Cl, 25.33; N, 6.69. Found: C, 37.41; H, 4.08; Cl, 25.15; N, 6.72.

A pure sample of the **hydrochloride** derivative recrystallized from acetone in colorless rosettes, m.p. 121.5–122°.

Anal. Calcd. for $C_{13}H_{17}Cl_3N_2O_3$: C, 43.89; H, 4.81; Cl, 29.91; N, 7.87. Found: C, 43.71; H, 4.74; Cl, 29.80; N, 7.72.

2-[Bis(2-chloroethyl)amino]methylcyclohexanone (III) Perchlorate.—A solution of bis(2-chloroethyl)amine hydrochloride[16] (1.1 g.) in 37% formalin (1.5 ml.) was added with stirring and cooling (ice-bath) to a mixture of cyclohexanone (2.3 g.) and sodium hydroxide (0.3 g.) in water (5 ml.). Stirring, with cooling, was continued for 1 hr. before extracting the reaction mixture with ether (precooled). The ethereal solution was washed with ice-water and then three 2-ml. portions of cold 50% aqueous perchloric acid. In several experiments a small quantity of bis(2-chloroethyl)amine perchlorate (m.p. 167–169.5°) separated at this stage and was removed by filtration. Before collecting the **perchlorate derivative of Mannich base III** (0.93 g., 46%), m.p. 125–127°, the combined perchloric acid extract was refrigerated for several days. Two recrystallizations from ethanol gave an analytical sample as colorless prisms, m.p. 128.5–129°.

Anal. Calcd. for $C_{11}H_{20}Cl_3NO_5$: C, 37.48; H, 5.71; N, 3.97. Found: C, 37.30; H, 5.78; N, 4.07.

The **hydrochloride** was prepared as was the corresponding derivative of propio-

phenone le. Several recrystallizations from acetone provided a pure specimen as colorless plates, m.p. 110–110.5°.

Anal. Calcd. for $C_{11}H_{20}Cl_3NO$: C, 45.77; H, 6.99; Cl, 36.85. Found: C, 45.32; H, 7.07; Cl, 36.79.

6-Acylamido- and 6-Acylamido-9(or 7)-acylpurines[1]

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Both 6-acylamido-9(or 7)-acylpurines and 6-acylamidopurines were synthesized. Various chemical, spectral and chromatographic characteristics of the above compounds are described. Neither rat liver cell fractions, homogenates of mammalian organs nor proteolytic enzymes hydrolyze 6-acylamidopurines. Anticancer screening yielded negative results.

Compounds such as 6-chloropurine[2] and 6-mercaptopurine[2,3] have pronounced effects on cell division. 6-Methylpurine alters cellular enzyme levels.[4] Other compounds in which the amino group of adenine is alkyl or aryl substituted, *e.g.*, puromycin[5,6] and kinetin, inhibit protein synthesis and cell growth.[2] The pharmacological action of puromycin has been associated with the alkyl substituted amino group; substitution of the 6-dimethylaminopurine moiety by adenine resulted in a loss of biological effect.[7] An analog of kinetin, 6-N-β-indolylethyladenine causes a disturbance of mitosis in normal and malignant cells and lyses tissue cultures of sarcomas and car-

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