Some 9-Bis(2-chloroethyl)aminoalkylpurines[1]

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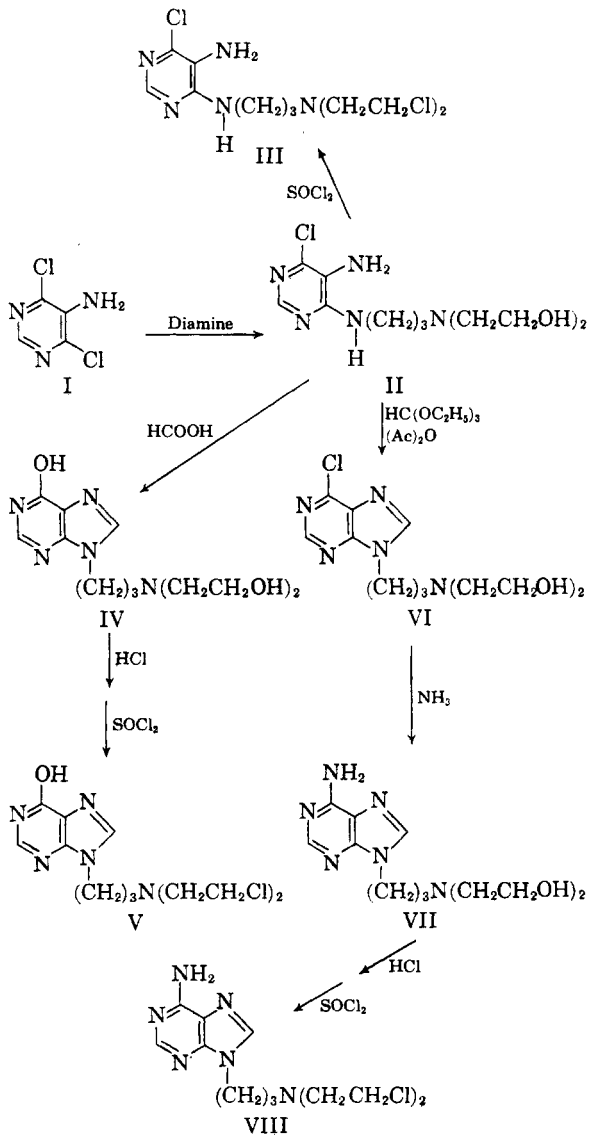
The purine mustard derivatives reported herein differ from those in the preceding paper in that the alkyl mustard side chain is attached in the 9-position, that normally involved in coupling with ribose or 2-deoxyribose in forming RNA or DNA.

The hypoxanthine derivative (V) has shown an interesting spectrum of activity against a number of experimental tumors in mice and will be given more extensive antitumor testing.

REACTION SCHEME



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EXPERIMENTAL

4-{3-[Bis(2-hydroxyethyl)amino]propylamino}-5-amino-6-chloropyrimidine (II). A mixture of 9.84 g. of 4,6-dichloro-5-aminopyrimidine and 19.44 g. of 3-[bis(2-hydroxyethyl)-amino]propylamine in 130 ml. of water was refluxed for 5 hr. The aqueous solution was extracted with chloroform continuously for 20 hr. The chloroform extract was distilled to dryness under reduced pressure. The viscous residue solidified upon scratching the surface of the flask with a spatula. The crude solid was recrystallized from ethyl acetate to give 16 g. (92%) of yellowish white needles, m.p. 116–117°. This was recrystallized again from ethyl acetate to give colorless needles, m.p. 116–117°.

Anal. Calcd. for $C_{11}H_{20}N_5ClO_2$: C, 45.59; H, 6.95; N, 24.17; Cl, 12.24. Found: C, 45.61; H, 7.10; N, 24.65; Cl, 12.57.

Ultraviolet absorption: 0.1*N* sodium hydroxide, λ_{max} 263 mμ, ε 10.1 × 10³; λ_{max} 289 mμ, ε 9.9 × 10³; ethanol, λ_{max} 267.5 mμ, ε 12.2 × 10³; λ_{max} 294 mμ, ε 13.0 × 10³.

4-{3-[Bis(2-chloroethyl)amino]propylamino}-5-amino-6-chloropyrimidine hydrochloride (III). To a solution of 0.3 g. of II in 8.0 ml. of chloroform 10 ml. of thionyl chloride was added slowly. A yellow solid precipitated instantly. The mixture was refluxed excluding moisture for 1 hr. and was kept overnight at room temperature. The chloroform and the excess thionyl chloride were removed by distilling under reduced pressure. To the yellowish brown residue 15 ml. of absolute ethanol was added. The mixture was refluxed until all the residue went into solution. The solution, after being cooled to room temperature, was filtered. To the filtrate 80 ml. of anhydrous ether was added. A large amount of greenish yellow granular solid separated; after the solid settled to the bottom of the flask, the liquid was decanted. The residue was washed three times with anhydrous ether by decantation. The precipitate was filtered and quickly transferred into a drying apparatus and dried at room temperature (3 mm.) for 10 hr.; yield, 0.26 g. (70%); m.p. 54–65°.

A portion of the product was dried for 4 hr. at 56° (1.5 mm.).

Anal. Calcd. for $C_{11}H_{18}N_5Cl_3 \cdot HCl$: C, 36.38; H, 5.27; N, 19.29; Cl, 39.07. Found: C, 36.59; H, 5.59; N, 19.36; Cl, 36.40. Ionic Cl Calcd., 9.76. Ionic Cl found, 11.12 (determined by conductometric titration).

Ultraviolet absorption: ethanol, λ_{max} 295 mμ, ε 6.5 × 10³; λ_{max} 266 mμ, ε 6.2 × 10³.

9-{3-[Bis(2-hydroxyethyl)amino]propyl}-hypoxanthine (IV). A solution of 3.5 g. of II dissolved in 200 ml. of formic acid (98–100%) was refluxed for 6 hr. The formic acid was removed by distillation under reduced pressure. The brown viscous residue was heated on a steam bath for another hour under reduced pressure. To the viscous residue, 10 ml. of concd. ammonium hydroxide was added. The mixture was heated gently until all the viscous residue went into solution. To the solution, 150 ml. of absolute ethanol was added. The precipitate of ammonium formate and ammonium chloride was filtered. Light tan prism-like crystals precipitated upon leaving the filtrate in the refrigerator overnight; yield, 2.8 g. (80%); m.p. 165–170°.

The crude product was recrystallized from 95% ethanol, m.p. 171–172°. *Anal.* Calcd. for $C_{12}H_{19}N_5O_3 \cdot 2H_2O$: C, 45.41; H, 7.30; N, 22.08. Found: C, 45.42; H, 6.65; N, 22.23.

Ultraviolet absorption: 0.1*N* hydrochloric acid, λ_{max} 249 mμ, ε 12.1 × 10³; 0.1*N* sodium hydroxide, λ_{max} 255 mμ, ε 20.6 × 10³; ethanol, λ_{max} 250 mμ, ε 9.8 × 10³.

9-{3-[Bis(2-chloroethyl)amino]propyl}-hypoxanthine dihydrochloride (V). Hydrogen chloride gas was passed through a suspension of 1.8 g. of IV in 40 ml. of absolute ethanol for 5 min. The hydrochloride of the 9-substituted hypoxanthine became a sticky mass on standing in the hot ethanolic solution. The ethanol was removed by distillation under reduced pressure. To the brown viscous residue, 40 ml. of thionyl chloride was added and the mixture was refluxed for 40 min.

The excess thionyl chloride was removed by distillation under reduced pressure. To the residue, 50 ml. of absolute ethanol was added and the mixture was refluxed until all the residue went into solution. The solution, after being cooled to room temperature, was filtered and anhydrous ether was added dropwise until it became slightly cloudy. The cloudy solution was kept in the refrigerator overnight. Light yellow needles separated and were filtered and dried at room temperature (28°, 3 mm.) for 10 hr.; yield, 1.8 g. (72.6%); m.p. 264–266° (darkening at 183°). For microanalysis, the compound was dried at 78° (3 mm.) for 3 hr.

Anal. Calcd. for $C_{12}H_{17}N_5Cl_2O \cdot 2HCl \cdot C_2H_5OH$: C, 38.46; H, 5.76; N, 16.02; Cl, 32.44; Ionic Cl, 16.22. Found: C, 38.64; H, 5.80; N, 16.51; Cl, 31.69, 32.32; Ionic Cl, 16.48 (determined by conductometric titration).

Ultraviolet absorption: ethanol, λ_{max} 251 mμ, ε 11.4 × 10³.

9-{3-[Bis(2-hydroxyethyl)amino]propyl}-6-chloropurine (VI). *Method A.* A solution of 10.2 g. of II in 50 ml. of diethoxymethyl acetate was boiled under reflux for 5 hr. The dark brown solution was distilled to dryness under reduced pressure. In order to remove the diethoxymethyl acetate completely, the viscous residue was vacuum dried at room temperature (0.5 mm.) for 2 hr. The brown viscous material, 9-{3-[bis(2-hydroxyethyl)amino]propyl}-6-chloropurine monoacetate, weighed 14 g. The residue was dissolved in 30 ml. of cold water. The solution was made alkaline (*p*H 11) with concd. ammonium hydroxide and extracted with three 200-ml. portions of chloroform. The chloroform extract, after being dried over anhydrous magnesium sulfate, was distilled to dryness under reduced pressure. The brown residue was further dried at room temperature (0.5 mm.) for 2 hr.; yield, 8.5 g. (84%).

Ultraviolet absorption: 0.1*N* hydrochloric acid, λ_{max} 263 mμ, ε 8.1 × 10³; ethanol, λ_{max} 264 mμ, ε 6.5 × 10³.

Method B. A solution of 1.1 g. of II in a mixture of 25 ml. of ethyl orthoformate and 25 ml. of acetic anhydride was refluxed for 2 hr. and then distilled to dryness under reduced pressure. The brown viscous residue was dried at room temperature (0.5 mm.) for 2 hr. in order to ensure the complete removal of acetic anhydride and ethyl orthoformate. The residue was dissolved in 15 ml. of water. The aqueous solution was made alkaline with concd. ammonium hydroxide. The basic solution was then extracted with three 50-ml. portions of chloroform. The chloroform solution, after being dried over anhydrous magnesium sulfate, was distilled to dryness under reduced pressure. The viscous residue was dried at room temperature (0.5 mm.) for 2 hr.; yield, 0.6 g. (53%).

Ultraviolet absorption: 0.1*N* hydrochloric acid; λ_{max} 263 mμ, ε 7.8 × 10³; ethanol, λ_{max} 265 mμ, ε 6.0 × 10³.

9-{3-[Bis(2-hydroxyethyl)amino]propyl}adenine (VII). A mixture of 3.0 g. of VI and 35 ml. of ammonium hydroxide saturated with ammonia gas at 3° was sealed in an autoclave and heated at 135° for 6 hr. The autoclave was cooled to room temperature overnight. The brown solution was evaporated to dryness on a steam bath. To the residue, 40 ml. of 2-propanol was added. The mixture stood in the open air for 2 days. The viscous residue solidified as a grayish granular solid, and some white crystals of 9-substituted adenine crystallized on the wall of the flask. Twenty milliliters more of 2-propanol was added to the mixture. The solid and crystals were collected by filtration, washed with solvent and dried; yield, 2.1 g. (67%); m.p. 166–171°. The crude solid was recrystallized from 2-propanol and water (95:5), to give white solid; m.p. 172–175°; yield, 1.9 g. (61%).

Anal. Calcd. for $C_{12}H_{20}N_6O_2 \cdot 2H_2O$: C, 45.56; H, 7.65; N, 26.57. Found: C, 45.35; H, 7.45; N, 26.60.

Ultraviolet absorption: 0.1*N* hydrochloric acid, λ_{max} 260 mμ, ε 14.2 × 10³; 0.1*N* sodium hydroxide, λ_{max} 263 mμ, ε 26.9 × 10³; ethanol, λ_{max} 261 mμ, ε 13.3 × 10³.

9-{3-[Bis(2-chloroethyl)amino]propyl}adenine dihydrochloride (VIII). Hydrogen chloride gas was passed for a few minutes into a suspension of 0.5 g. of well pulverized 9-{3-[bis(2-hydroxyethyl)amino]propyl}adenine (0.5 g.) of VII in 20 ml. of absolute ethanol. Most of the adenine hydrochloride became a viscous mass on standing in the hot ethanol solution. The mixture was distilled to dryness under reduced pressure, 25 ml. of thionyl chloride was added to the viscous residue, and the mixture was refluxed for 0.5 hr. The excess thionyl chloride was removed by distillation under reduced pressure. The viscous residue was protected from moisture and refluxed in 60 ml. of absolute ethanol for 10 min. The undissolved brown solid was removed and the filtrate was kept in the refrigerator overnight. Orange crystals separated, m.p. 243–246°; yield, 0.35 g. (50%).

Benzene (5 ml.) was added to the filtrate. The solution was concentrated by distillation to half its volume and kept in the refrigerator overnight. More crystals separated; yield, 0.08 g.; m.p. 244–247°. The combined yield was 0.43 g. (61%). A portion of the product was further dried at room temperature (3 mm.) for 2 hr.

Anal. Calcd. for $C_{12}H_{18}N_6Cl_2 \cdot 3HCl \cdot H_2O$: C, 32.41; H, 5.21; N, 18.90; Cl, 39.87; Ionic Cl, 23.92. Found: C, 32.64, 32.52; H, 4.78, 4.90; N, 19.14, 19.24; Cl, 39.22, 39.08; Ionic Cl, 24.83 (determined by conductometric titration). After further drying at 56° (3 mm.) for 2 hr., it lost the molecule of water.

Anal. Calcd. for $C_{12}H_{18}N_6Cl_2 \cdot 3HCl$: C, 33.78; H, 4.96; N, 19.70; Cl, 41.56. Found: C, 34.29, 34.03; H, 5.05, 4.93; N, 19.74, 19.57; Cl, 37.32, 37.06.

The product was then dried at 78° (3 mm.) for 2 hr., and it lost a molecule of hydrogen chloride.

Anal. Calcd. for $C_{12}H_{18}N_6Cl_2 \cdot 2HCl$: C, 36.94; H, 5.17; N, 21.54; Cl, 36.35. Found: C, 36.74, 36.50; H, 5.06, 5.26; N, 21.88, 21.72; Cl, 35.15, 35.33.

Ultraviolet absorption: ethanol, λ_{max} 260 mμ, ε 14.8 × 10³.

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Alkaloids of Tobacco Smoke. III. Methyl and Ethyl 3-Pyridyl Ketone as Constituents of Burley Tobacco Cigarette Smoke[1]

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As we have concluded our work on the identification of the alkaloids of Burley tobacco smoke, we wish to report some results obtained since the appearance of the preceding paper[2] on this subject.

It has been found that previously unidentified Fractions 4 and 6 correspond to methyl 3-pyridyl ketone and ethyl 3-pyridyl ketone, respectively. A tentative identification was accomplished by comparative gas chromatography on two different columns of the isolated fractions and the known ketones. Confirmatory identification was obtained by comparative paper chromatography in two solvent systems of their 2,4-dinitrophenylhydrazones. The data are recorded in Table I. This marks the first identification of the methyl ketone

(1) Presented at the Organic Section of the Southeastern Regional, A. C. S. Meeting, Richmond, Virginia, November 6, 1959.

(2) L. D. Quin, *J. Org. Chem.*, 24, 914 (1959).