[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA]

Synthesis of Some 9-[2-(Diethylamino)ethyl]-6-substituted Purines as Potential Antimetabolites[1]

HSI HU LIN AND CHARLES C. PRICE

Received June 2, 1960

Several 9-[2-(diethylamino)ethyl]-6-substituted purines were synthesized from 9-[2-(diethylamino)ethyl]-6-chloropurine (VII). This compound (VII) was prepared by direct chlorination of the corresponding 9-substituted hypoxanthine (IX) which was, in turn, obtained by cyclization of 4-[2-(diethylamino)ethylamino]-5-amino-6-chloropyrimidine (VI) in formic acid. The diaminochloropyrimidine (VI) was prepared by treatment of 4,6-dichloro-5-aminopyrimidine with *N,N*-diethylethylenediamine.

In the synthesis of various potential antagonists of the natural purines, Robins and Lin[2] reported that 9-methyl-6-chloropurine has shown the same order of activity against Adenocarcinoma 755 in C-57 black mice as 6-chloropurine,[3,4] while two other 9-methyl-6-substituted purines have shown less activity against this tumor. 9-Ethyl-6-chloropurines[5] and 9-propyl-6-chloropurine,[6] synthesized by Montgomery and Temple, have also shown the same kind of activity.

The results make the investigation of other 9,6-disubstituted purines of great interest. There were two reasons for synthesizing 9-[2-(diethylamino)-ethyl]-6-substituted purines. The first was to see how the antitumor activity of the 6-chloropurine and 6-mercaptopurine would be affected by attaching a basic 2-(diethylamino)ethyl radical on the 9-position of the purine antimetabolites. The second was for preliminary studies of possible procedures for synthesizing 9-substituted-purine nitrogen mustards as cytotoxic agents.[7]

In this investigation, treatment of 4,6-dichloro-5-nitropyrimidine (I) with an aqueous solution of excess *N,N*-diethylethylene diamine at *p*H 8 did not give the expected monosubstituted derivative but the disubstituted derivative. This was under conditions similar to those employed by Robins and Lin[2] for the synthesis of 4-methylamino-5-nitro-6-chloropyrimidine. Several other conditions were also explored by changing solvent, *p*H, reaction temperature and ratio of the two reactants. In spite of these trials, only the disubstituted product and the starting material were isolated. Failure to obtain the monosubstituted compound could arise from too small a difference between the rates for the first and second nucleophilic substitution.

In view of the above reasoning, the nitro group of the dichloronitropyrimidine was reduced to the amino group in order to deactivate the pyrimidine ring against nucleophilic attack and hopefully to enlarge the difference between the first and second nucleophilic substitution rates. When a mixture of one equivalent of the dichloroaminopyrimidine (II) and two equivalents of *N,N*-diethylethylene diamine in water was refluxed, a quantitative yield of 4-[2-(diethylamino)ethylamino]-5-amino-6-chloropyrimidine was obtained.[8] In 1954 Brown prepared 4-methylamino-5-amino-6-chloropyrimidine by heating aqueous methylamine and the pyrimidine II at 130° in a sealed tube. Montgomery and Temple,[5] using conditions similar to Brown's, succeeded in synthesizing 4-ethylamino-5-amino-6-chloropyrimidine in good yield.

When 4-[2-(diethylamino)ethylamino]-5-amino-6-chloropyrimidine (VI) was refluxed with formic acid, cyclization took place to give 9-[2-(diethylamino)ethyl]-hypoxanthine (IX) in good yield.

As the loss of a chlorine atom of various chloro-substituted 4,5-diaminopyrimidines upon formylation and cyclization with formic acid,[9,2,5] or with formamide,[10] has been reported previously, this behavior is not unexpected.

Montgomery[11] reported the synthesis of 2-chloropurine, 6-chloropurine and 2,6-dichloropurine by cyclization of the appropriate chloro-4,5-diaminopyrimidine in ethyl orthoformate and acetic anhydride combination. 9-Methyl-6-chloropurine[2] was prepared in the same manner. Temple and Montgomery[5] claimed that diethoxymethyl acetate was a better cyclizing agent than the ethyl orthoformate–acetic anhydride combination in the synthesis of 9-ethyl-6-chloropurine. The parent com-

(1) This investigation was supported in part by research grant CY-2714 from the National Institute of Health, Public Health Service.

(2) R. K. Robins and H. H. Lin, *J. Am. Chem. Soc.*, **79**, 490 (1957).

(3) H. E. Skipper, J. R. Thompson, and R. K. Robins, The Southern Research Institute, Birmingham, Alabama, unpublished data.

(4) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

(5) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **79**, 5238 (1957).

(6) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **80**, 409 (1958).

(7) H. H. Lin and C. C. Price, *J. Org. Chem.*, **25**, 226 (1960).

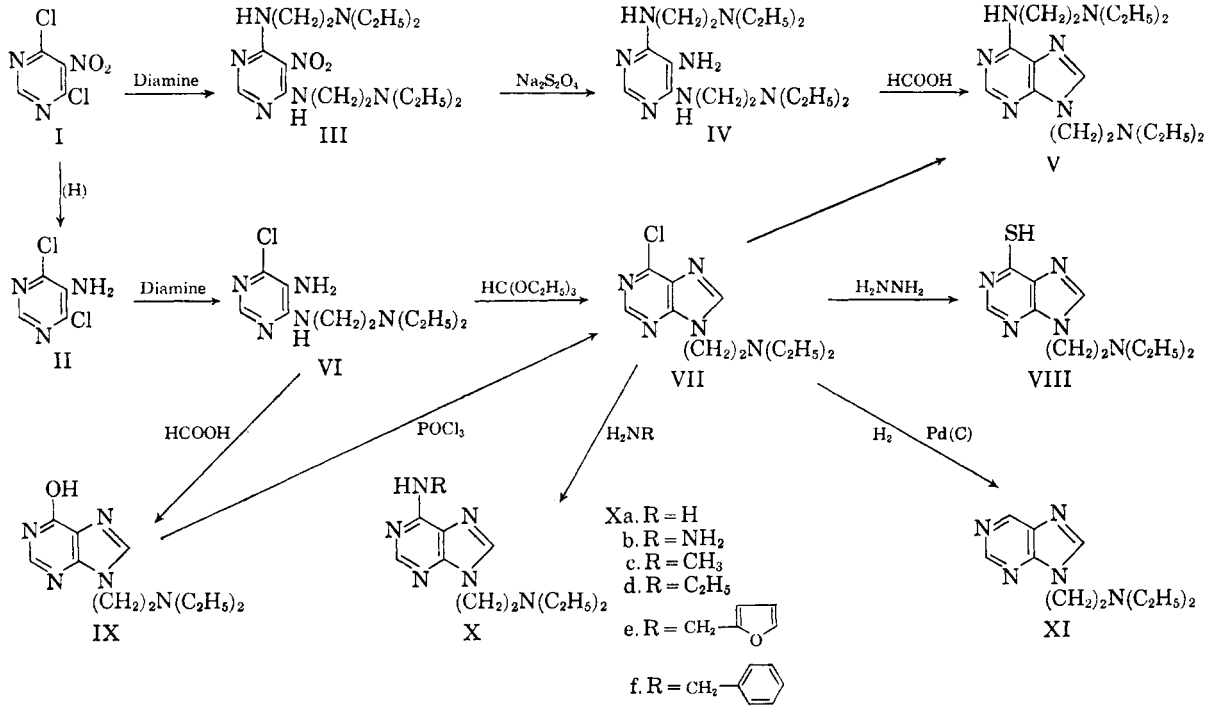
(8) D. J. Brown, *J. Appl. Chem.*, **4**, 72 (1954).

(9) R. K. Robins, K. L. Dille, and B. E. Christensen, *J. Org. Chem.*, **19**, 930 (1954).

(10) R. K. Robins, K. L. Dille, C. H. Willits, and B. E. Christensen, *J. Am. Chem. Soc.*, **75**, 263 (1953).

(11) J. A. Montgomery, *J. Am. Chem. Soc.*, **78**, 1928 (1956).

REACTION SCHEME



pound 9-[2-(diethylamino)ethyl]-6-chloropurine (VII) was prepared in low yield by cyclization in ethyl orthoformate–acetic anhydride combination. This compound (VII) was also prepared in excellent yield by direct chlorination of the corresponding hypoxanthine (IX) with phosphorus oxychloride.

The parent compound (VII) and thiourea in boiling 2-ethoxyethanol (b.p. 138°) gave 9-[2-(diethylamino)ethyl]-6-mercaptopurine (VIII). Most of the starting material was recovered when compound VII and thiourea were refluxed in absolute ethanol whereas 6-mercaptopurine,[4] 9-methyl-6-mercaptopurine,[2] and 9-ethyl-6-mercaptopurine[5] were prepared by refluxing the corresponding chloropurine with thiourea in ethanol. Montgomery and Temple[6] also reported that the condensation between thiourea and 9-propyl or 9-butyl-6-chloropurine required a solvent which has a higher boiling point than that of absolute ethanol.

9-[2-(Diethylamino)ethyl]purine (XI) was prepared[5] by hydrogenolysis of compound VII, using 10% palladium-on-charcoal as catalyst. Treatment of compound VII with concentrated ammonium hydroxide in an autoclave at 125° gave 9-[2-(diethylamino)ethyl]adenine (X*a*), which showed that replacement of the 6-chlorine atom with ammonia can be carried out using water as solvent and at a temperature lower than that used by Bendich, Russell, and Fox[4] or by Robins and Lin.[2]

9-[2-(Diethylamino)ethyl]-6-methylaminopurine (Xc) and 9-[2-(diethylamino)ethyl]-6-ethylaminopurine (Xd) were obtained by heating compound VII with the corresponding aqueous amine in a pressure bottle at 100°. 9-[2-(Diethylamino)ethyl]-6-hydrazinopurine (Xb) was prepared by merely refluxing compound VII in aqueous hydrazine. When compound VII was refluxed with furfurylamine in 1-butanol, benzylamine in 2-ethoxyethanol and with *N*,*N*-diethylethylene diamine in 2-ethoxyethanol, the corresponding 6-substituted aminopurines (Xe; Xf; V) were obtained. 9-[2-(Diethylamino)ethyl] - 6 - [2 - (diethylamino)ethylamino]-purine (V) was also prepared by another route. Treatment of 4,6-dichloro-5-nitropyrimidine (I) with *N*,*N*-diethylethylene diamine gave 4,6-bis[2-(diethylamino)ethylamino]-5-nitropyrimidine (III). Catalytic reduction of III with sodium hydrosulfite gave 4,6-bis[2-(diethylamino)ethylamino]-5-aminopyrimidine (IV) which was cyclized successfully with formic acid to give the corresponding purine (V). The product was judged to be identical with the product from the amination of compound VII on the basis of mixture melting point and identical ultraviolet absorption spectra.

In general, the condensation of compound VII with nucleophilic reagents required more vigorous reaction conditions, namely higher temperature and more polar medium than the condensation of 9-unsubstituted, 9-methyl- and 9-ethyl-6-chloropurine with the same nucleophilic reagents. This could be attributed to greater basicity of the 9-nitrogen, permitting greater electron release to the pyrimidine ring thus decreasing the ease of nucleophilic displacement of chlorine.

The ultraviolet absorption maxima in the spectra of 9-[2-(diethylamino)ethyl]-6-substituted purines are listed in Table I and II. As was expected, the ultraviolet absorption spectra of 9-[2-(diethyl-

TABLE I

THE ULTRAVIOLET ABSORPTION MAXIMA OF SEVERAL 9-[2-(DIETHYLAMINO)ETHYL]-6-SUBSTITUTED PURINES

6-R	0.1*N* HCl, λ_{max}, mμ	ϵ	0.1*N* NaOH, λ_{max}, mμ	ϵ
OH	249	10.4×10^3	255	12.7×10^3
SH	322	22.7×10^3	309	26.5×10^3
Cl	264.6	6.7×10^3		
H	263	5.8×10^3	260	7.6×10^3

TABLE II

THE ULTRAVIOLET ABSORPTION MAXIMA OF SOME 9-[2-(DIETHYLAMINO)ETHYL]-6-SUBSTITUTED AMINOPURINES

6-NHR,R =	0.1*N* HCl, λ_{max}, mμ	ϵ	0.1*N* NaOH, λ_{max}, mμ	ϵ
—H	260	15.2×10^3		
—NH_2	262	17.7×10^3		
—CH_3	263	18.6×10^3	266	18.1×10^3
—CH_2CH_3	264	11.7×10^3	268	13.7×10^3
—$CH_2C_4H_3O$	266	17.6×10^3	269	12.8×10^3
—$CH_2C_6H_5$	265	16.7×10^3	269.5	17.5×10^3
—$CH_2CH_2NEt_2$	266	22.6×10^3	269	30.1×10^3

amino)ethyl]-6-substituted purines are very similar to those of the 9-unsubstituted, 9-methyl-[2] and 9-ethyl-6-substituted purines.

EXPERIMENTAL[12]

4,6-Bis[2-(diethylamino)ethylamino]-5-nitropyrimidine (III). 4,6-Dichloro-5-nitropyrimidine (5.8 g.) was dissolved in 70 ml. of absolute ethanol. To the solution, 7 g. of *N,N*-diethylethylenediamine in 50 ml. of absolute ethanol was added slowly. The solution was refluxed for 2 hr. and distilled to half its original volume. Yellowish crystals of 4,6-bis-2-(diethylamino)ethylamino-5-nitropyrimidine dihydrochloride separated on cooling. After being filtered, washed with ethanol, and dried at 110°, they yielded 11.7 g. The dihydrochloride was dissolved in 110 ml. of cold water and made alkaline with 5% sodium hydroxide. A yellow oil separated instantly. On cooling in the refrigerator overnight, the oil crystallized as needles, which were filtered, washed with cold water, and dried at room temperature in a vacuum desiccator; yield, 9.3 g. (87%), m.p. 54–55°. The crude product was recrystallized from a mixture of acetone and water and vacuum dried at room temperature, m.p. 55°.

Anal. Calcd. for $C_{16}H_{31}N_7O_2$: C, 54.36; H, 8.84; N, 27.74. Found: C, 54.73; H, 8.77; N, 27.14.

4,6-Bis[2-(diethylamino)ethylamino]-5-aminopyrimidine (IV). 4,6-Bis[2-(diethylamino)ethylamino]-5-nitropyrimidine (5.8 g.) was suspended in 200 ml. of water (90°) and 32 g. of sodium hydrosulfite (90%) was added slowly. The yellow oily diaminonitropyrimidine dissolved at the end of the addition. The solution was heated to boiling for 5 min., filtered and cooled to room temperature. Sodium hydroxide (10%) was added slowly to the solution until it reached *p*H 10. The white oil which separated was extracted with about 200 ml. of ether. The ether layer was dried over anhydrous magnesium sulfate for 1 hr. and filtered. The filtrate was distilled under reduced pressure to a volume of 30 ml. Colorless prisms which crystallized out of the ether solution were filtered and dried in a vacuum desiccator; yield, 4.1 g. (80%), m.p. 107–108°. A portion was recrystallized from anhydrous ether and dried at 56° to give colorless prisms, m.p. 108°.

Anal. Calcd. for $C_{16}H_{33}N_7$: C, 59.40; H, 10.28; N, 30.31. Found: C, 59.13; H, 10.34; N, 30.57.

9-[2-(Diethylamino)ethyl]-6-[2-(diethylamino)ethylamino]-purine trihydrochloride (V). *Method A.* 4,6-Bis[2-(diethylamino)ethylamino]-5-aminopyrimidine (3.3 g.) dissolved in 150 ml. of formic acid (98–100%), was refluxed for 3 hr. and distilled under reduced pressure to dryness. The yellow oily residue was heated under reduced pressure on a steam bath for 0.5 hr. and 50 ml. of alcoholic ammonia was added. The separated crystals of ammonium formate were removed by filtration. The filtrate was evaporated to dryness on a steam bath and then extracted with chloroform with vigorous stirring. The chloroform solution was filtered and hydrogen chloride gas was passed through. White crystals of 9,6-disubstituted aminopurine trihydrochloride which separated were filtered at once and dried in a vacuum desiccator; yield, 3.1 g. (50%), m.p. 247–251°. The crude product was recrystallized from absolute ethanol to give white crystals, m.p. 251–252° (vacuum dried at 78°).

Anal. Calcd. for $C_{17}H_{34}N_7Cl_3$: C, 46.10; H, 7.74; N, 22.14; Cl, 24.02. Found: C, 46.40; H, 7.64; N, 22.10; Cl, 23.83.

Method B. *N,N*-Diethylethylenediamine (470 mg.) and 9-[2-(diethylamino)ethyl]-6-chloropurine (510 mg.) dissolved in 20 ml. of 2-ethoxyethanol were refluxed for 1 hr. and distilled under reduced pressure to dryness. The residue was extracted with 60 ml. of chloroform and the chloroform solution was filtered. Hydrogen chloride gas was passed through the filtrate. White crystalline solid which separated was filtered, washed with chloroform and then with absolute ethanol, and dried; yield, 350 mg. (52%); m.p. 244–250°. The crude product was recrystallized from absolute ethanol, m.p. 250–252°. No depression was found on the mixture melting point of the product from method (A) and (B). The ultraviolet absorption spectra were identical in 0.1 *N* hydrochloric acid.

4-[2-(Diethylamino)ethylamino]-5-amino-6-chloropyrimidine (VI). 4,6-Dichloro-5-aminopyrimidine (4.92 g.) was suspended in a solution which was made by dissolving 7.10 g. of *N,N*-diethylethylenediamine in 110 ml. of water. The suspension was refluxed for 5 hr. Yellowish brown oil of 4-[2-(diethylamino)ethylamino]-5-amino-6-chloropyrimidine separated out of the aqueous solution. The mixture was kept in the refrigerator overnight. Yellowish needles crystallized out from the solution and the oil. The crystals were filtered and dried; yield, 8.1 g. (90%), m.p. 76–81°. A small amount of the crude product was recrystallized from water to give light yellowish needles, m.p. 74–80°.

Anal. Calcd. for $C_{10}H_{18}N_5Cl.2H_2O$: C, 42.93; H, 7.92; N, 25.04; Cl, 12.68. Found: C, 43.27; H, 7.86; N, 25.30; Cl, 13.08.

9-[2-(Diethylamino)ethyl]hypoxanthine (IX). Dehydrated 4-[2-(diethylamino)ethylamino]-5-amino-6-chloropyrimidine (9.4 g.), dissolved in 160 ml. of formic acid (98–100%), was refluxed for 6 hr. and distilled under reduced pressure to dryness. The brown viscous residue was heated on a steam bath under reduced pressure for another hour. To the viscous residue, 25 ml. of concd. ammonium hydroxide and 10 ml. of water were added successively. White crystalline solid separated. The crude product was recrystallized from water to give tan prisms, m.p. 181°, yield, 7.63 g. (84%).

Anal. Calcd. for $C_{11}H_{17}N_5O$: C, 56.14; H, 7.28; N, 29.77. Found: C, 56.97; H, 7.28; N, 29.49.

9-[2-(Diethylamino)ethyl]-6-chloropurine (VIII). *Method A.* 9-[2-(Diethylamino)ethyl]hypoxanthine (6.5 g.), suspended in 260 ml. of phosphorus oxychloride, was refluxed for 6 hr. The dark brown solution was distilled under reduced pressure nearly to dryness. The viscous residue was poured into about 500 g. of crushed ice with frequent stirring. Ammonia gas was passed through the ice cold acidic solution until it reached *p*H 11. The cloudy basic solution was extracted with four 200-ml. portions of chloroform. The combined ex-

(12) All melting points are uncorrected and were taken on a Fisher-Johns melting point block and copper block.

tract was distilled under reduced pressure to dryness after being dried over anhydrous magnesium sulfate overnight. The viscous brown residue, after being vacuum dried at room temperature for 2 hr., was recrystallized from petroleum ether (b.p. 30–60°) in a Dry Ice–acetone bath to give white prisms which, on standing at room temperature, changed to light yellow liquid, yield 6.3 g. (90%). A small amount of this was recrystallized again under the same conditions and vacuum dried at 78°.

Anal. Calcd. for $C_{11}H_{16}N_5Cl$: C, 52.06; H, 6.36; N, 27.60; Cl, 13.97. Found: C, 52.38; H, 6.39; N, 27.75; Cl, 12.84.

Method B. 4-[2-(Diethylamino)ethylamino]-5-amino-6 chloropyrimidine (0.88 g.) was suspended in a mixture of 25 ml. of ethyl orthoformate and 25 ml. of acetic anhydride. The solution was refluxed for 5 hr. and distilled to dryness under reduced pressure. The brown viscous residue was further dried over a steam bath at 2 mm. pressure for 1 hr. and about 10–15 g. of crushed ice was then added along with cold concd. ammonium hydroxide to *p*H 11. The cold basic solution was extracted with four 50-ml. portions of chloroform. The combined extract, after being dried over anhydrous magnesium sulfate overnight, was distilled under reduced pressure to dryness. The brown viscous residue after being dried at room temperature (2 mm.) for 1 hr. was recrystallized from petroleum ether (b.p. 30–60°) in a Dry Ice–acetone bath to give white prism-like crystals which, on standing at room temperature, liquified. The light-yellowish liquid was dried at 78° (3 mm.) for 5 hr.; yield, 0.46 g. (52%). The ultraviolet absorption spectra is identical with that of the product from method (A).

9-[2-(Diethylamino)ethyl]adenine (Xa). Concentrated ammonium hydroxide (25 ml., 80%) was added to 0.8 g. of 9-[2-(diethylamino)ethyl]-6-chloropurine. The mixture was sealed in an autoclave and heated at 125° for 5 hr. The autocalve was cooled to room temperature overnight. Colorless prisms separated and were filtered and dried; yield, 0.51 g. (69%); m.p. 180–181.5°. A portion of the product was recrystallized from water to give colorless prisms, m.p. 181–182°.

Anal. Calcd. for $C_{11}H_{18}N_6$: C, 56.38; H, 7.74; N, 35.87. Found: C, 56.35; H, 7.69; N, 35.89.

9-[2-(Diethylamino)ethyl]-6-mercaptopurine (VIII). 9-[2-(Diethylamino)ethyl]-6-chloropurine (0.6 g.) and 0.2 g. of thiourea dissolved in 2-ethoxyethanol (20 ml.) were refluxed for 2 hr. and distilled under reduced pressure nearly to dryness. To the dark brown residue, 0.25 g. of sodium hydroxide in 15 ml. of water was added. The basic solution was then heated on a steam bath for 0.5 hr., boiled gently for a few minutes with charcoal and filtered. The alkaline solution was adjusted to *p*H 6–7 with concd. hydrochloric acid and evaporated to dryness. The dry residue was extracted with hot absolute ethanol. The undissolved sodium chloride was removed by filtration. As the filtrate became turbid upon evaporation, colorless needles separated. The crystals were filtered and dried; yield, 0.3 g. (44%), m.p. 247–251°. The crude product was recrystallized from absolute ethanol twice to give colorless needles, m.p. 249–251°.

Anal. Calcd. for $C_{11}H_{17}N_5S.HCl$: C, 45.90; H, 6.30: N, 24.34; S, 11.14; Cl, 12.32. Found: C, 45.77; H, 6.34; N, 24.24; S, 10.81; Cl, 12.09.

9-[2-(Diethylamino)ethyl]-6-hydrazinopurine (Xb). A solution of aqueous hydrazine (0.26 g., 60%) and 9-[2-(diethylamino)ethyl]-6-chloropurine (0.5 g.) dissolved in 20 ml. of water was refluxed for 2 hr. The solution was evaporated to dryness on a steam bath. The crystalline residue was extracted with two 40-ml. portions of chloroform. The extract after being dried over anhydrous magnesium sulfate for 1 hr. was evaporated to dryness. Light brown needles crystallized from the viscous residue on cooling, yield, 0.4 g. (81%), m.p. 126–131°. The crude product was recrystallized from benzene with activated charcoal to give colorless needles, yield, 0.31 g. (63%), m.p. 133–134°.

Anal. Calcd. for $C_{11}H_{19}N_7$: C, 53.00; H, 7.68; N, 39.32. Found: C, 52.61; H, 7.60; N, 39.96.

9-[2-(Diethylamino)ethyl]-6-methylaminopurine (Xc). A glass pressure bottle which contained 30 ml. of aqueous methylamine (40%) and 0.5 g. of 9-[2-(diethylamino)ethyl]-6-chloropurine was heated in a steam bath for 3 hr. and the solution was evaporated to dryness. On cooling the viscous residue, light yellow needles separated. The solid mixture was extracted with chloroform (60 ml.) and the extract was evaporated to dryness. The residue was recrystallized with activated charcoal from absolute ethanol to give colorless needles, yield, 0.23 g. (47%), m.p. 134–135°.

Anal. Calcd. for $C_{12}H_{20}N_6$: C, 58.04; H, 8.12; N, 33.84. Found: C, 58.50; H, 8.08; N, 33.96.

9-[2-(Diethylamino)ethyl]-6-ethylaminopurine dihydrochloride (Xd). A mixture of aqueous ethylamine (70%) and 1.6 g. of 9-[2-(diethylamino)ethyl]-6-chloropurine in a glass pressure bottle was heated on a steam bath overnight (about 20 hr.). The reaction mixture was evaporated to dryness on a steam bath. The light yellow residue was extracted with 100 ml. of chloroform. The chloroform extract after being dried over anhydrous magnesium sulfate for 1 hr. was distilled to dryness. To the viscous residue, 70 ml. of absolute ethanol was added. Hydrogen chloride gas was passed through the solution and a large amount of white crystalline solid separated. The crude product was filtered and dried, yield, 1.32 g. (66%), m.p. 260–268°. A portion of the crude product was recrystallized from 85% aqueous ethanol to give white needles, m.p. 267–270°.

Anal. Calcd. for $C_{13}H_{22}N_6.2HCl$: C, 46.56; H, 7.22; N, 25.07; Cl, 21.15. Found: C, 46.61; H, 7.07; N, 25.22; Cl, 20.81.

9-[2-(Diethylamino)ethyl]-6-furfurylaminopurine dihydrochloride (Xe). Furfurylamine (0.4 g.) and 9-[2-(diethylamino)ethyl]-6-chloropurine (0.52 g.) in 30 ml. of 1-butanol were refluxed for 6 hr. and distilled to dryness under reduced pressure. The residue was extracted with 60 ml. of anhydrous ether with constant scratching and stirring. The granular solid furfurylamine hydrochloride which was suspended in the ether extract was removed by filtration. The filtrate was distilled to dryness. The viscous residue was dissolved in 30 ml. of absolute ethanol and hydrogen chloride gas was passed through for a few minutes. Tan needles separated as the ethanolic solution was kept overnight in a refrigerator. The crude product was filtered and dried in a desiccator; yield, 0.28 g. (35%), m.p. 208–220°. The crude product was recrystallized three more times from absolute ethanol to give white needles, m.p. 241°, yield, 0.2 g. (25%).

Anal. Calcd. for $C_{16}H_{22}N_6O.2HCl$: C, 49.61; H, 6.25; N, 21.70; Cl, 18.31. Found: C, 48.71; H, 6.64; N, 21.80; Cl, 17.97.

9-[2-(Diethylamino)ethyl]-6-benzylaminopurine dihydrochloride (Xf). Benzylamine (0.36 g.) and 9-[2-(diethylamino)-ethyl]-6-chloropurine (0.42 g.) dissolved in 20 ml. of 2-ethoxyethanol, were refluxed for 1 hr. and distilled under reduced pressure to dryness. To the residue, 20 ml. of water was added. The resultant aqueous solution was extracted with 60 ml. of benzene. The benzene solution, after being dried over anhydrous magnesium sulfate for an hour, was distilled to dryness under reduced pressure. To the residue, 20 ml. of absolute ethanol was added and the alcoholic solution was made acidic by passing in hydrogen chloride gas for a few minutes. Upon standing overnight in the refrigerator, the white crystalline needles which separated out of the dark brown solution were filtered and dried; yield, 0.19 g. (30%), m.p. 220–223°. The crude product was recrystallized from absolute ethanol to give white needles, m.p. 223–224°.

Anal. Calcd. for $C_{18}H_{24}N_6.2HCl$: C, 54.41; H, 6.59; N, 21.15; Cl, 17.85. Found: C, 54.40; H, 6.58; N, 21.10; Cl, 17.85.

9-[2-(Diethylamino)ethyl]purine dihydrochloride (XI). A solution of 0.3 g. of 9-[2-(diethylamino)ethyl]-6-chloropurine in a 1:1 mixture of ethanol-water (40 ml.) containing 10% palladium on charcoal catalyst (0.2 g.) and 0.2 g. of magnesium oxide was hydrogenated at 40 lb./in.^{2} for 2 hr.

The hydrogenation was complete within 2 hr. and the catalyst was removed by filtration. The filtrate was evaporated to dryness and the residue was redissolved in 40 ml. of water and made alkaline with concd. ammonium hydroxide. The aqueous solution was then extracted with chloroform. The chloroform solution, after being dried over anhydrous magnesium sulfate, was filtered and distilled to dryness. The residue was dissolved in 25 ml. of absolute ethanol. Hydrogen chloride gas was passed through the alcoholic solution for a few minutes. Light brown needles separated upon cooling the solution in the refrigerator; yield, 0.16 g. (46.5%), m.p. 178–182°. The crude product was recrystallized from absolute ethanol to give tan needles, m.p. 179–182°.

Anal. Calcd. for $C_{11}H_{17}N_5.2HCl$: C, 45.21; H, 6.55; N, 23.97; Cl, 24.27. Found: C, 44.59; H, 6.38; N, 24.42; Cl, 24.00.

PHILADELPHIA, PA.

[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE]

Phenazine Syntheses. X.[1a] 2,8-Disubstituted Phenazines Made as Intermediates for New Vital Stains, Together with Two New Vital Stains Related to Neutral Red

DONALD L. VIVIAN,[1b] RICHARD M. HOGART, AND MORRIS BELKIN

Received October 5, 1959

The synthesis and some of the properties of a homologous series of 2-alkylamino-8-chlorophenazines are described, together with the preparation of 2,8-dibromophenazine and two new vital stains related to neutral red: 2-methylamino-8-*n*-propylaminophenazine, and 2,8-bis(methylamino)-3-methylphenazine.

Earlier work[2,3] had shown that 2,8-diaminophenazines, when converted to their hydrochlorides or other salts, possessed the property of "localized" or "particulate" staining of living cells,[4] such as is exhibited by Neutral Red, which is the hydrochloride of 2-amino-8-dimethylamino-3-methylphenazine. It was likewise shown that this ability to act as a vital stain was peculiar to these 2,8-disubstituted phenazines, and was not possessed by their 2,7-disubstituted analogs. (In the report which follows, the free bases alone will be described, with the understanding that it is the hydrochloride or similar salt that actually produces the vital staining.)

It was originally proposed, therefore, to prepare a series of 2,8-bis(alkylamino)phenazines, in order to study their vital staining ability, as well as their other properties. The planned method of preparation involved replacement of both halogens of 2,8-dihalophenazines by amino or alkylamino groups, using the same method of relatively high-temperature sealed-tube reactions as was earlier found feasible with monohalogenated phenazines.[3] When primary alkylamines were used in this procedure, however, it was found that the results were not completely satisfactory with 2,8-dichlorophenazine, for a mixture of difficultly separable compounds always resulted. The chief reasons for the multiplicity of products are probably the following: (1) The greater activity of the secondary amines which result from replacement of the first chlorine. (2) Instability of some of the reaction products at the relatively high temperatures necessary for replacement of the second chlorine. (3) The tenacity with which a small portion of the chlorine is retained, possibly due to complex formation.

Because of these complex mixtures resulting from the higher temperature reactions necessary with the dichloro compounds, complete purification has been achieved only with two 2,8-bis(alkylamino) phenazines. This paper, therefore, deals chiefly with products in which only one of the two chlorines has been replaced. These are readily obtained from the 2,8-dichlorophenazine by lower-temperature reactions than those which yield the mixtures already referred to, for it has been found that the first chlorine atom can be replaced at a much lower temperature than is required to replace the second one. Thus, replacement of the first chlorine by the more reactive amines, such as methylamine, can be effected by long reaction at as low a temperature as 100°.

In later work it is planned to proceed with the original idea of making a series of both symmetrically and unsymmetrically substituted 2,8-bis-(alkylamino) phenazines, attempting to overcome some of the difficulties encountered with 2,8-dichlorophenazine by taking advantage of the greater reactivity of the bromine atoms in the 2,8-dibromophenazine described below, as well as by determining whether long-continued reaction will ultimately result in complete replacement of all of the chlorine in 2,8-dichlorophenazine. It is also planned to study the action of secondary amines on 2,8-dihalophenazines.

(1)(a) Paper IX. *J. Org. Chem.*, 21, 1188 (1956).
(1)(b) Present address: College of Pharmacy, University of Arizona, Tucson, Ariz.
(2) D. L. Vivian and M. Belkin, *Nature*, 178, 154 (1956).
(3) D. L. Vivian, *J. Org. Chem.*, 21, 565 (1956).
(4) M. Belkin and M. J. Shear, *Am. J. Cancer*, 24, 483 (1937).