Potential Folic Acid Antagonists. I. Deaza Analogs of Methotrexate. I[1]

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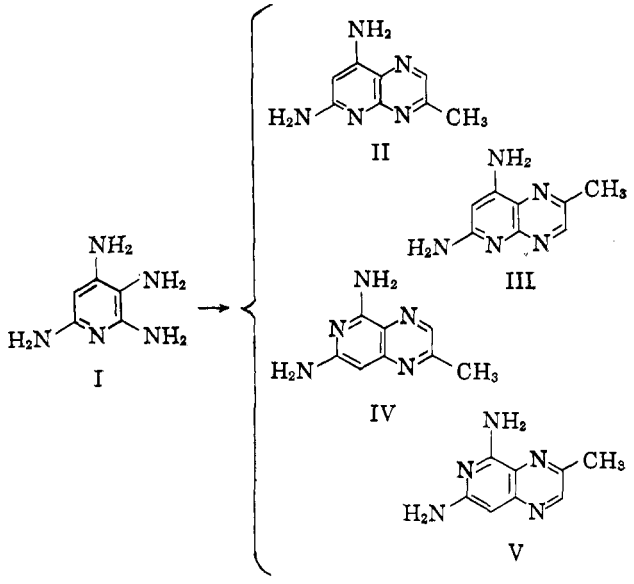
Nitration of diethyl 4-chloro-2,6-pyridinecarbamate (VI) gave diethyl 4-chloro-3-nitro-2,6-pyridinedicarbamate (VII), which reacted with aminoacetone semicarbazone to give diethyl 4-acetonylamino-3-nitro-2,6-pyridinecarbamate semicarbazone (VIII). Hydrolysis of the semicarbazone group of VIII followed by reduction of the nitro group of IX gave diethyl 1,2-dihydro-3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XII) which was oxidized to diethyl 3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XIII), the desired "1-deazapteridine" ring system. Basic hydrolysis of XIII gave 5,7-diamino-3-methylpyrido[3,4-*b*]pyrazine (XVIII). Bromination of either XIII or XVIII took place on the ring, presumably in the 8-position, rather than on the methyl group.

Deaza analogs of Aminopterin or Methotrexate are of interest for the determination of the structural features necessary for binding and therefore inhibiting the enzymes involved in folic acid metabolism.[3] The synthesis of such compounds is, however, potentially more difficult than the synthesis of Aminopterin or Methotrexate themselves. The numerous variations of the original synthesis of folic acid,[4] some of which have been applied to the synthesis of Aminopterin and Methotrexate,[5] involve the reaction of a 3-carbon compound with a 4,5-diaminopyrimidine, a reaction which could give rise to two isomeric products. The reaction of tetraaminopyrimidine and pyruvaldehyde does, in fact, give rise to 2,4-diamino-6-methylpteridine and 2,4-diamino-7-methylpteridine.[5] The reaction of 2,3,4,6-tetraaminopyridine (I)[6] with pyruvaldehyde may be more complex because 2,3,4,6-tetraaminopyridine lacks an element of symmetry possessed by the pyrimidine. Thus four isomers (II–V) could be formed in this case.[7] Because of this added complication we investigated the application of Boon and Leigh's method for the unambiguous synthesis of 2-amino-6-methylpteridin-4(3*H*)-one[13] to the synthesis of 2,4-diamino-6-methyl-1-deazapteridine [5,7-diamino-3-methylpyrido[3,4-*b*]pyrazine (XVIII)], a compound that should be convertable *via* bromination or oxidation of the methyl group to "1-deazaaminopterin" or "1-deazamethotrexate." (See Scheme I.)



The starting compound for the proposed synthetic route, diethyl 4-chloro-3-nitro-2,6-pyridinedicarbamate (VII), was prepared by the nitration of diethyl 4-chloro-2,6-pyridinedicarbamate (VI)[14] with fuming nitric acid in concentrated sulfuric acid. Aminoacetone[15] was converted to its semicarbazone[13] which reacted readily with VII in ethanol at room temperature to give diethyl 4-acetonylamino-5-nitro-2,6-pyridinedicarbamate semicarbazone (VIII). The semicarbazone (VIII) was hydrolyzed in warm 1 *N* hydrochloric acid to the parent ketone (IX) which in turn reacted rapidly with hydrazine in alcohol to give the corresponding hydrazone (X).

Catalytic reduction of the nitro group of IX with Raney nickel resulted in spontaneous cyclization of the intermediate diethyl 4-acetonylamino-3-amino-2,6-pyridinedicarbamate to diethyl 1,2-dihydro-3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XII), isolated as an ethanolate. Oxidation of the dihydro compound (XII) with potassium permanganate in acetone gave diethyl 3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XIII). The nitro group of the hydrazone (X) was also reduced with hydrogen and Raney nickel catalyst. The intermediate diethyl 4-acetonylamino-3-aminopyridine-2,6-dicarbamate hydrazone was not characterized but was immediately hydrolyzed in dilute acetic acid to the corresponding ketone. Under the conditions of this hydrolysis, cyclization and some oxidation occurred giving a mixture of XII and XIII.

Treatment of XIII with alcoholic potassium hydroxide resulted in stepwise removal of its carbethoxy groups. Thus after two hours reflux, the principal

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(3) For an example of this type of study, see S. F. Zahrzewski, *J. Biol. Chem.*, **238**, 1485 (1963).

(4) Many of these variations are described by E. L. R. Stokstad in "The Vitamins," W. H. Sebrell, Jr., and R. S. Harris, Ed., Academic Press, Inc., New York, N. Y., 1954, p. 91.

(5) D. R. Seeger, D. B. Cosulich, J. M. Smith, Jr., and M. E. Hultquist, *J. Am. Chem. Soc.*, **71**, 1753 (1949).

(6) J. A. Montgomery and N. F. Wood, unpublished data.

(7) Surprisingly few syntheses of the 1-deazapteridine (pyrido[3,4-*b*]-pyrazine) ring system have been reported,[8-12] and none of these are applicable to the problem at hand. Albert and Hampton found that the reaction of 2,3,4-triaminopyridine with glyoxal gave a mixture of isomers, 5-aminopyrido[3,4-*b*]pyrazine and 8-aminopyrido[2,3-*b*]pyrazine.[11]

(8) E. Koenigs, G. Kinner, and W. Weiss, *Ber.*, **57**, 1172 (1924).

(9) E. Koenigs, H. Buren, and G. Jung, *ibid.*, **69**, 2690 (1936).

(10) O. Bremer, *Ann.*, **529**, 290 (1937).

(11) A. Albert and A. Hampton, *J. Chem. Soc.*, 4985 (1952).

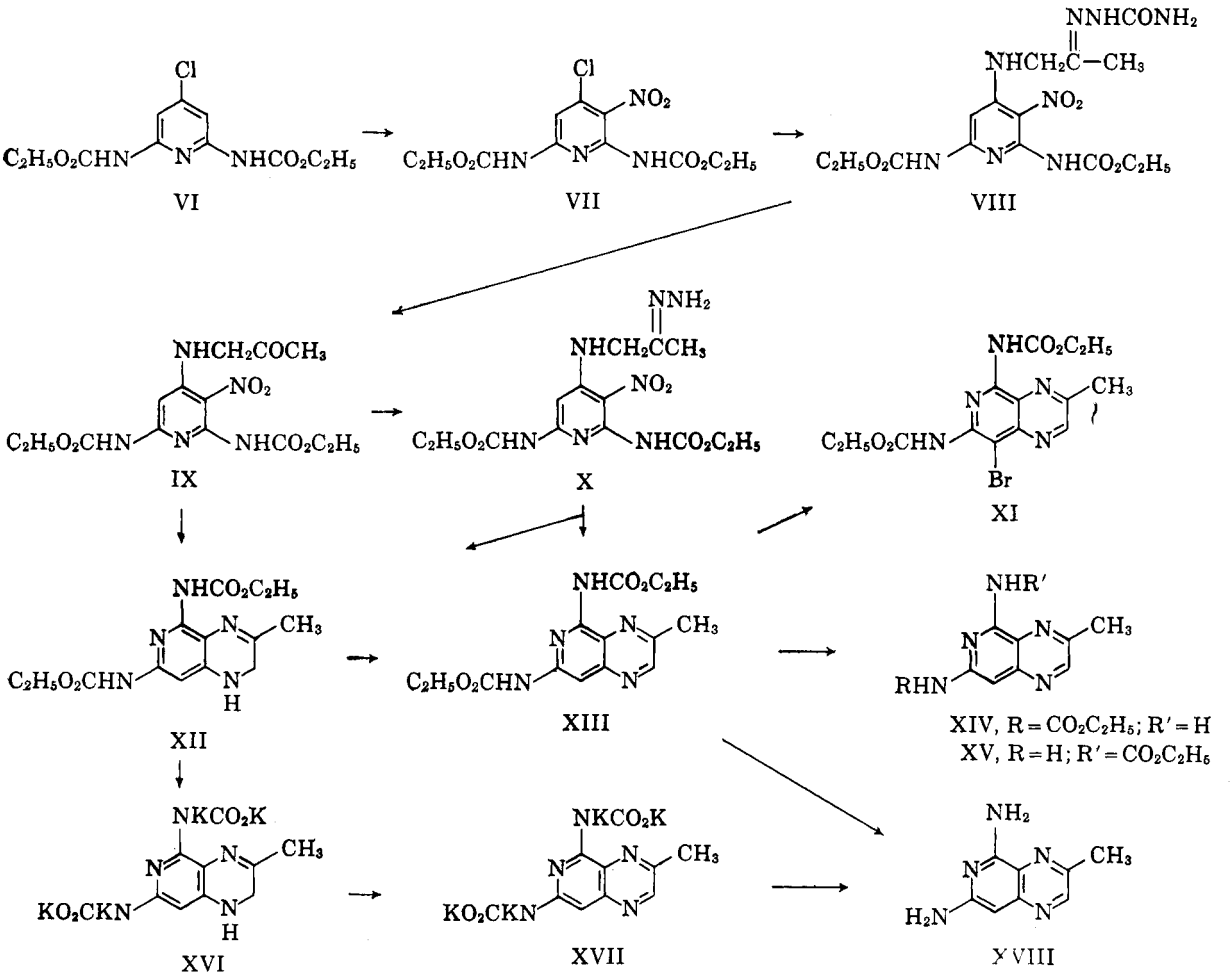
(12) M. Israel and A. R. Day, *J. Org. Chem.*, **24**, 1455 (1959).

(13) W. R. Boon and T. Leigh, *J. Chem. Soc.*, 1497 (1951).

(14) D. G. Markees and G. W. Kidder, *J. Org. Chem.*, **78**, 4130 (1956).

(15) A. Albert and S. Matsuura, *J. Chem. Soc.*, 5131 (1961).

SCHEME I



product of the isolation procedure employed was ethyl 5(or 7)-amino-3-methylpyrido[3,4-*b*]pyrazine-7(or 5)-carbamate (XIV or XV), whereas at the end of seven hours reflux a good yield of 5,7-diamino-3-methylpyrido[3,4-*b*]pyrazine (XVIII) was isolated, and none of the monocarbamate (XIV or XV) was detected in the reaction mixture. Treatment of XIII with methanolic ammonia gave, after 36 hours at room temperature, a mixture of the monocarbamate (XIV or XV) and an unidentified second component.

An alternative procedure for the preparation of 5,7-diamino-3-methylpyrido[3,4-*b*]pyrazine (XVIII) consisted of saponification of the carbethoxy groups of XII with ethanolic potassium hydroxide, followed by permanganate oxidation of the intermediate tetrapotassium 1,2-dihydro-3-methylpyrido[3,4-*b*]pyrazine-2,6-dicarbamate (XVI) to XVII. The solubility of XVI in aqueous alkali facilitated the permanganate oxidation. Acidification of the solution of XVII gave the unstable dicarbamic acid which spontaneously decomposed to XVIII. Attempts to convert XVI to 5,7-diamino-1,2-dihydro-3-methylpyrido[3,4-*b*]pyrazine prior to oxidation were unsuccessful due to its instability.

Although 5,7-diamino-3-methylpyrido[3,4-*b*]pyrazine (XVIII) failed to undergo bromination with *N*-bromosuccinimide in carbon tetrachloride in the presence of benzyl peroxide,[16] diethyl 3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XIII) gave a monobromo derivative (XI) under the same conditions. That XI was diethyl 8(2)-bromo-3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate instead of the desired diethyl 3-bromomethylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate was shown by a comparison of the proton magnetic resonance spectrum of XI and XIII (see Fig. 1). The integral of the band at 2.78 p.p.m. in the spectrum of XIII showed that this band represents the three equivalent protons of the methyl group of XIII. This same band with an integral equivalent to three protons is also present in the spectrum of XI. Conversely the band at 8.06 p.p.m. in the spectrum of XIII representing one of the ring protons (at either position 2 or 8) is missing from the spectrum of XI. Although it has not been definitely established that the bromine is in fact at position 8 rather than 2, this seems likely since, in general, bands due to aromatic protons adjacent to a hetero atom (such as f) occur at a lower field than those in other positions.[17] Also *N*-bromosuccinimide, even in the presence of benzoyl peroxide, will readily brominate the pyridine nucleus at position 3 (or 5) if there is an amino or acetamido group at C-2.[18] Further treatment of XI with *N*-bromosuccinimide resulted in a high recovery of unchanged starting material.

(16) Z. U. Pushkareva and L. U. Alekseeva, [*Zh. Obshch. Khim.*, **32**, 1058 (1962)] describe the bromination of 2-amino-6-methylpteridin-4(3*H*)-one by a similar procedure.

(17) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 64.

(18) R. P. Mariella and E. P. Belcher, *J. Am. Chem. Soc.*, **74**, 1916 (1952).

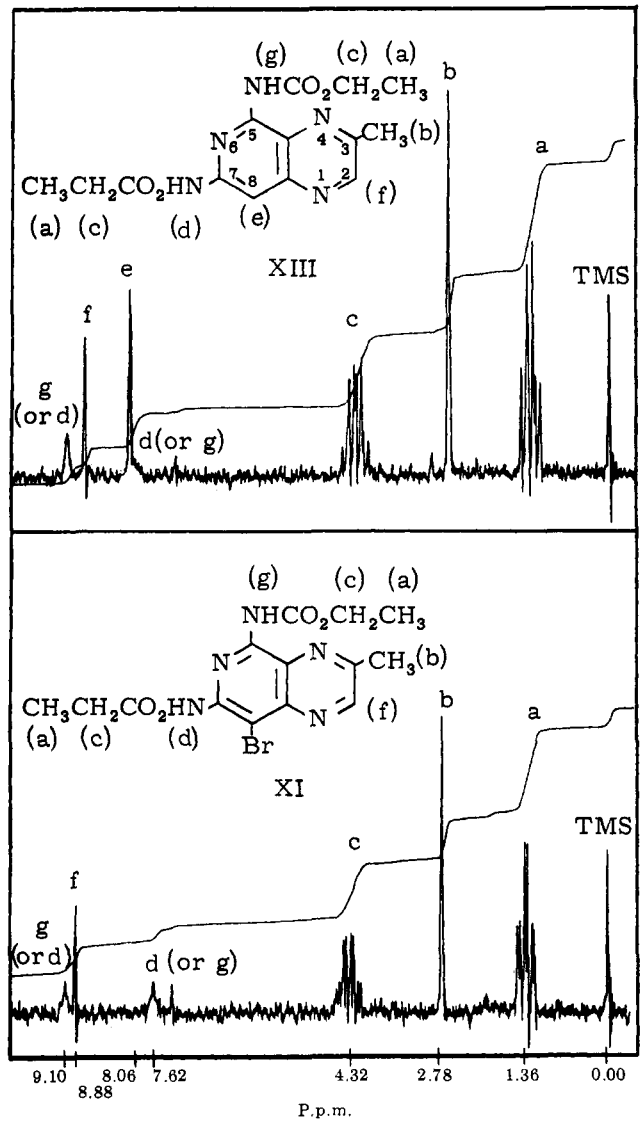


Fig. 1.—Proton magnetic resonance spectrum (60 Mc./sec.) of XI and XIII in chloroform; field increases from left to right, chemical shifts in p.p.m., relative to internal tetramethylsilane.

Bromination of XVIII with bromine in acetic acid, a procedure used for the preparation of 2-amino-6-bromomethylpteridin-4(3*H*)-one,[19,20] gave a material, whose analyses indicated was an impure bromo derivative of 5,7-diamino-3-methylpyrido[3,4-*b*]pyrazine hydrobromide. Treatment of this material with aqueous bicarbonate gave the free base which was not obtained analytically pure, but a comparison of its p.m.r. spectrum with that of XVIII indicated that ring bromination took place in this case also giving 5,7-diamino-8-bromo-3-methylpyrido[3,4-*b*]pyrazine.

Other approaches to the desired deaza analogs currently under investigation are (1) oxidation of the methyl group of XIII and (2) the use of a suitably substituted aminoacetone for aminoacetone itself in the cyclization reaction.

Experimental

Melting points below 260° were determined on a Kofler Heizbank and are corrected. The ultraviolet absorption spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer ("sh" designates shoulder and "b," broad), whereas the infrared absorption spectra were determined in pressed potassium bromide disks with a Perkin-Elmer Model 221 spectrophotometer. The p.m.r. spectra were determined in 10% (w./v.) deuteriochloroform solution with a Varian Associates Model A-60 spectrometer (probe temperature 42 ± 1°). R_f values were determined as previously described[22] in the following solvents: A, butyl alcohol–acetic acid–water (5:2:3); B, isopropyl alcohol–ammonium hydroxide–water (70:5:25); C, 0.1 *N* phosphate buffer, pH 6.7; D, water-saturated butyl alcohol.

Diethyl 4-Chloro-3-nitro-2,6-pyridinedicarbamate (VII).—A cold mixture of diethyl 4-chloro-2,6-pyridinedicarbamate[14] (VI, 10.0 g.) and sulfuric acid (Sp. Gr. 1.84, 30 ml.) was added with shaking to fuming nitric acid (Sp. Gr. 1.5, 100 ml.) kept at about 0° in an ice bath, additional sulfuric acid (*ca.* 2 ml.) being used to complete the transfer. The reaction vessel was removed from the ice bath and allowed to stand at room temperature for 2 hr. with occasional shaking. The solution was then poured slowly with stirring onto crushed ice (600 g.), causing precipitation of a white solid and formation of a blue coloration in the solution. Ice-cold 10% sodium hydroxide was added with stirring until the mixture acquired a permanent light yellow color. Throughout the neutralization, ice was added to maintain the temperature of the mixture at about 0°. The product was collected by filtration, washed with cold water, and dried over phosphorus pentoxide *in vacuo;* the yield was 10.8 g. (93%), m.p. 120°. The analytical sample was obtained by recrystallization from ethanol–water (3:2), m.p. 120°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1 and 7—230 (sh, 15.0), 296 (5.80), and 343 (sh, 2.89); pH 13—257 (13.9) and 302 (9.43); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3320, 3290 (NH); 2990, 2930 (CH); 1765 sh, 1750 sh, 1735, 1720 sh (C=O), 1600, 1580, 1530 (C=C, C=N); 1550, 1360 (NO_2); 1440 (C–CH_3); 1275 (C–O–C); 1230, 1075, 760, 701 (ring CH); R_f values: A, 0.96; B, 0.92; C, 0.47.

Anal. Calcd. for $C_{11}H_{13}ClN_4O_6$: C, 39.71; H, 3.94; Cl, 10.66; N, 16.84. Found: C, 39.41; H, 3.87; Cl, 10.6; N, 16.65.

Diethyl 4-Acetonylamino-3-nitro-2,6-pyridinedicarbamate Semicarbazone (VIII).—Aminoacetone semicarbazone hydrochloride[13] (63.4 g., 0.38 mole) was added to a cold solution of sodium ethoxide prepared from sodium (8.74 g., 0.38 mole) and ethanol (400 ml.). After 1.75 hr. the mixture was added to a solution of diethyl 4-chloro-3-nitro-2,6-pyridinedicarbamate (55.0 g., 0.165 mole) in ethanol (400 ml.) with stirring. The solution quickly turned bright yellow, and a yellow precipitate soon appeared. After stirring for 20 hr., the yellow solid was collected by filtration, stirred with water (300 ml.), and again collected by filtration; weight, 52.2 g., after drying *in vacuo* at 40°. An additional precipitate, obtained by allowing the mother liquid to stand at room temperature for an additional 2.5 days, was collected by filtration, stirred with water (150 ml.), and again collected by filtration; weight after drying *in vacuo* at 40° was 6.0 g., combined material, 58.2 g. (83%). The analytical specimen was obtained by recrystallization of a sample of this material (100 mg.) from ethanol (25 ml.), m.p. *ca.* 240° dec.; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—224 (28.3), 253 (26.4), 275 (17.1), and 331 (10.6, b); pH 7—360 (too insoluble for ε determination); pH 13—378 (7.38, b); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3485, 3340 (NH); 2980, 2920 (CH); 1735 (urethan C=O); 1695 (NHCONH); 1610 (NH); 1570 and 1485 (C=C, C=N); 1540, 1340 (NO_2); 1440 (C–CH_3); 1280 (C–O–C); 1195, 1020, 765 (ring CH).

Anal. Calcd. for $C_{15}H_{22}N_8O_7$: C, 42.25; H. 5.21; N, 26.28. Found: C, 41.96; H, 5.59; N, 25.68.

Diethyl 4-Acetonylamino-3-nitro-2,6-pyridinedicarbamate (IX).—Crude diethyl 4-acetonylamino-3-nitro-2,6-pyridinedicarbamate semicarbazone (VIII, 54.0 g.) was stirred with 1 *N* hydrochloric acid (1000 ml.) in a water bath kept at 50°. After 3.25 hr. the mixture was cooled, and he yellow precipitate was collected by filtration, washed with cold water (100 ml.), and dried over phosphorus pentoxide *in vacuo* for several hours at 40° and for 2 hr. at 100°; the yield was 46.3 g. (99%), m.p. 170°. For analysis a small amount of the above material was twice recrystallized from ethanol to give long yellow needles; m.p. 174–176° dec. (when placed on the block at 150° and taken rapidly to the m.p.); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—224 (23.2), 252 (23.8), 275 (16.0), and 331 (10.0, b); pH 7—225 (28.9), 246 (21.2), and

(19) D. J. Brown, *J. Chem. Soc.*, 1644 (1953).

(20) A similar procedure using a higher reaction temperature was successful for the preparation of 2,4-diamino-6-bromomethyl-8-deazapteridine.[21]

(21) V. Oakes and H. N. Rydon, U. S. Patent 2,924,599 (1960).

(22) J. A. Montgomery and K. Hewson, *J. Am. Chem. Soc.*, **82**, 463 (1960).

351 (11.7, b); pH 13—234 (21.8), 293 (8.24, b), and 350 (9.11, b); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3440, 3330 (NH); 2980, 2910 (CH): 1725, (C=O); 1610, 1570, 1480 (C=C, C=N); 1540 (NO_2); 1400 (C-CH_3); 1275 (C-O-C); 1190, 1025, 765 (ring CH); R_f values: A, 0.91; B, 0.89; C, 0.04; D, 0.86.

Anal. Calcd. for $C_{14}H_{19}N_5O_7$: C, 45.53; H, 5.19; N, 18.96. Found: C, 45.24; H, 5.54; N, 18.50.

Diethyl 4-Acetonylamino-3-nitro-2,6-pyridinedicarbamate Hydrazone (X).—A warm solution of diethyl 4-acetonylamino-3-nitro-2,6-pyridinedicarbamate (IX, 739 mg., 2.0 mmoles) in ethanol (100 ml.) was allowed to cool almost to room temperature, but before precipitation started a solution of hydrazine (100 mg., 3.13 mmoles) in ethanol (10 ml.) was added with stirring. Precipitation occurred and the mixture was stirred for 2 days; the precipitate was collected by filtration, washed with water and air-dried, yield 470 mg. (61%). Paper chromatography showed that this material contained some IX. After two 20-ml. extractions with boiling ethanol, the residue was recrystallized from dimethylformamide (10 ml.) and dried *in vacuo* over phosphorus pentoxide at 80°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—225 (27.2), 251 (26.8), 274 (17.2), and 332 (10.1, b); pH 7—225 (34.4), 245 (23.9), and 352 (12.9, b); pH 13—234 (30.6) and 374 (8.08, b); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3360, 3300 (NH); 2990, 2910 (CH); 1750 sh, 1735 (C=O); 1625 (NH); 1580, 1530, 1495 (C=C, C=N); 1560, 1340 (NO_2); 1460 (C-CH_3); 1275 (C-O-C); 1205, 1025, 770 (ring CH).

Anal. Calcd. for $C_{14}H_{21}N_7O_6$: C, 43.86; H, 5.53; N, 25.58. Found: C, 43.80; H, 5.56; N, 25.72.

Diethyl 8-Bromo-3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XI).—A mixture of diethyl 3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XIII, 319.3 mg., 1.0 mmole), *N*-bromosuccinimide (190 mg., 1.07 mmoles), benzoyl peroxide (7 mg.), and carbon tetrachloride (5 ml.) was refluxed for 45 min. After 15 min. the solution cleared but soon needles began to crystallize. The solid was collected by filtration, extracted with boiling water, and the residue of crude diethyl 8-bromo-3-methylpyrido[3,4-*b*]-pyrazine-5,7-dicarbamate (XI) was recrystallized from ethanol to give pale yellow needles, m.p. 222° dec.; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—264 (25.5), 310 (8.4, b), and 385 (5.3, b); pH 7—263 (31.9), 297 (3.8, b), and 368 (5.0, b); pH 13—269 (28.6) and 354 (3.00, b); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3380, 3260 (NH); 2985, 2915 (CH); 1765, 1710 (C=O); 1600, 1585, 1565, 1510 (C=C, C=N); 1480, 1410 (C-CH_3); 1230 (C-O-C); 1180, 1050, 765, 725 (ring CH); R_f values: A, 0.93; B, 0.92; C, 0.61; D, 0.90.

Anal. Calcd. for $C_{14}H_{16}BrN_5O_4$: C, 42.22; H, 4.05; N, 17.59; Br, 20.07. Found: C, 42.08; H, 4.10; N, 17.49; Br, 20.2.

Diethyl 1,2-Dihydro-3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XII).—A suspension of crude diethyl 4-acetonylamino-3-nitro-2,6-pyridinedicarbamate (IX, 25 g., 67 mmoles) in ethanol (750 ml.) was stirred with Raney nickel (25 g. weighed wet with ethanol) in the presence of hydrogen at atmospheric pressure and room temperature for 36 hr. The yellow color of the starting material had by then disappeared, and the rate of hydrogen uptake became very slow. Most of the colorless solid that had formed and the solution were decanted from the catalyst which was washed several times with warm ethanol (a total of 250 ml.). The combined reaction mixture and washings was heated to dissolve the solid, and the hot solution was filtered through a fluted filter paper. On refrigeration, colorless plates crystallized and were collected by filtration and dried *in vacuo* over phosphorus pentoxide at 56° for 2 hr.; the yield was 16.0 g. (65%); m.p. 188–190° dec. (when heated rapidly); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—255 (57.6) and 312 (8.82, b); pH 7—250 (38.0) and 299 (7.04); pH 13—248 (34.1) and 289 (7.75); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3580 (C_2H_5OH); 3430, 3340, 3280 (NH); 3180, 2985, 2920 (CH); 1760 sh, 1740 sh, 1720 (C=O); 1650 (NH); 1630, 1600, 1550, 1500 (C=C, C=N); 1440 (C-CH_3); 1220, 1035, 770 (ring CH); R_f values: A, 0.93, B, 0.91; C, 0.46; D, 0.92.

Anal. Calcd. for $C_{14}H_{19}N_5O_4 \cdot C_2H_5OH$: C, 52.30; H, 6.86; N, 19.06. Found: C, 52.08; H, 7.13; N, 19.27.

The presence of alcohol was confirmed by the infrared spectrum of this compound and by its behavior on melting.

When the above preparation was carried out using pure starting material (1.0 g.), a yield of 0.77 g. (77%) was obtained.

Diethyl 3-Methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XIII).—Diethyl 1,2-dihydro-3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XII, 4.1 g., 11.2 mmoles) was dissolved in warm acetone (400 ml.), and the solution was allowed to cool to room temperature. A 0.27% solution of potassium permanganate in acetone was slowly added with stirring over a period of about 2 hr. until the color of the permanganate persisted (about 550 ml. of permanganate solution was required). The manganese dioxide was removed by filtration, and the solution was evaporated to dryness *in vacuo*. The residue was washed with water (12 ml.), treated with boiling ethanol (30 ml.), and the solution was filtered. Hot water (30 ml.) was added to the hot filtrate, and the mixture was allowed to cool. The pale yellow needles that deposited were collected by filtration and dried *in vacuo* over phosphorus pentoxide at 78° for 2.5 hr.; the yield was 3.60 g. (100%); m.p. 206°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—242 (31.2), 301 (15.3), and 385 (5.65, b); pH 7—257 (34.9), 290 (6.59), and 376 (4.93, b); pH 13—259 (2.97), 345 (3.14, b), and 392 (4.66, b); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3380, 3260 (NH); 3080, 2990, 2930 (CH); 1755, 1725 (C=O); 1620, 1600, 1520 (C=C, C=N); 1460, 1440 (C-CH_3); 1260 (C-O-C); 1220, 1195, 1050, 770 (ring CH); R_f values: A, 0.93; B, 0.91; C, 0.37; D, 0.92.

Anal. Calcd. for $C_{14}H_{17}N_5O_4$: C, 52.66; H, 5.37; N, 21.93. Found: C, 52.68; H, 5.38; N, 21.94.

Reductive Cyclization of Diethyl 4-Acetonylamino-3-nitro-2,6-pyridinedicarbamate Hydrazone (X).—A suspension of diethyl 4-acetonylamino-3-nitro-2,6-pyridinedicarbamate hydrazone (126 mg., 0.33 mmole) in dimethylformamide (5 ml.) was stirred with Raney nickel (200 mg. weighed wet with water) in the presence of hydrogen at atmospheric pressure and room temperature. After 24 hr. the uptake of hydrogen had ceased so the catalyst was removed by filtration and the solution was evaporated to dryness *in vacuo*. The residue was dissolved in 2 *N* acetic acid (25 ml.) and the solution was refluxed for 1 hr. The solution was evaporated to dryness *in vacuo* and the residue was dissolved in hot ethanol. The brown crystals that deposited on cooling were collected by filtration; the weight after air-drying was 15 mg.; m.p. 160–165°; λ_{max} in mμ: pH 1—255 and 303; pH 7—250 and 296; pH 13—249. This product was identified as diethyl 1,2-dihydro-3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XII) by its melting point, ultraviolet absorption spectrum, and its chromatographic behavior.

When water was added to the filtrate remaining after collection of the first crop of crystals, a second crop was obtained and collected by filtration; the weight after air-drying was 40 mg.; m.p. 202–204°; λ_{max} in mμ; pH 1—242, 301, and 386; pH 7—256, 290, and 375; pH 13—259, and 395. This second product was identified as diethyl 3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XIII) by its melting point, ultraviolet absorption spectrum, and chromatographic behavior.

Ethyl 5(or 7)-Amino-3-methylpyrido[3,4-*b*]pyrazine-7(or 5)-carbamate (XIV or XV).—Diethyl 3-methylpyrido[3,4-*b*]pyrazine 5,7-dicarbamate (XIII, 1400 mg., 4.4 mmoles) was dissolved in a solution of potassium hydroxide (2000 mg., 35.7 mmoles) in ethanol (30 ml.), and the solution was refluxed for 2 hr. The bright red precipitate that formed on cooling was collected by filtration and suspended in water (5 ml.). 3 *N* Hydrochloric acid was added until the solution was slightly acid, and the mixture was refrigerated overnight. The resulting yellow precipitate was collected by filtration and washed with water, m.p. 179°. Recrystallization from ethanol (25 ml.) with charcoal gave an initial crop of XIV (XV) as brownish orange crystals (410 mg., 38%), m.p. 182°. Refrigeration of the solution for several days gave a second crop of orange crystals identified as 5,7-diamino-3-methylpyrido[3,4-*b*]pyrazine (XVII, 40 mg.), m.p. 225°. For the analysis of the monocarbamate XIV (XV), a small sample (50 mg.) was recrystallized from ethanol (5 ml.) to give golden rosettes (33 mg.), m.p. 184°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—238 (16.6), 307 (18.2), and 384 (4.1, b); pH 7—266 (33.5), 311 (7.4), and 396 (3.6 b); pH 13—266 (32.4), 311 (7.3), and 396 (3.4, b). $\bar{\nu}_{max}$ in cm.$^{-1}$: 3490, 3460, 3360, 3260 (NH); 3050, 2985, 2920 (CH); 1725 (C=O); 1620, 1580, 1535 (C=C, C=N); 1450 (C-CH_3); 1280 (C-O-C); 1230, 1190, 1040, 770 (ring CH); R_f values: A, 0.91; B, 0.82; C, 0.25; D, 0.83.

Anal. Calcd. for $C_{11}H_{13}N_6O_2$: C, 53.43; H, 5.30; N, 28.33. Found: C, 53.68; H, 5.32; N, 28.40.

In a second run 1400 mg. of XIII gave 447 mg. (41%) of XIV (XV) and 220 mg. (29%) of XVII.

5,7-Diamino-3-methylpyrido[3,4-*b*]pyrazine (XVIII). A.—Diethyl 1,2-dihydro-3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XII, 760 mg., 2.1 mmoles) was refluxed with a solution of potassium hydroxide (1250 mg., 22.3 mmoles) in ethanol for 3 hr. The orange precipitate that formed on cooling was collected by filtation and weighed 850 mg.

Anal. Calcd. for $C_{10}H_7K_4N_5O_2$: K, 37.46. Found: K, 39.2.

To a solution of this material in 0.12 *N* sodium hydroxide (20 ml.), 0.1 *N* potassium permanganate (12.5 ml.) was added slowly with stirring during a period of 30 min. After removal of the manganese dioxide by filtration, the solution was neutralized with dilute hydrochloric acid. Evaporation to a small volume *in vacuo* gave an orange precipitate of crude 5,7-diamino-3-methylpyrido[3,4-*b*]pyrazine (XVII, 90 mg.). Recrystallization of the crude product from water (5 ml.) with charcoal gave orange plates which were collected and dried *in vacuo* over phosphorus pentoxide at 76° for 4 hr.; the yield was 50 mg. (14%); m.p. 222–223° dec. (tends to sublime around 210° to give needles); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—244 (17.9) and 314 (16.8); pH 7—265 (22.6) and 312 (7.3); pH 13—266 (23.3) and 312 (7. 3); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3435, 3350, 3300 (NH); 3140, 2960(CH); 1650 (NH); 1600, 1580, 1540 (C=C, C=N); 1440 (C-CH_3); 1200, 1170, 1050, 780 (ring CH); R_f values: A, 0.71; B, 0.66; C, 0.29; D, 0.59.

Anal. Calcd. for $C_8H_9N_5$: C, 54.84; H, 5.18; N, 39.98. Found: C, 54.94; H, 5.29; N, 40.04.

B.—Diethyl 3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XIII, 140 mg., 0.44 mmole) was dissolved in a solution of potassium hydroxide (310 mg., 5.5 mmoles) in ethanol (5 ml.) to give an orange-red solution. On refluxing, the orange-red precipitate which first appeared gradually changed to orange-yellow. After 7 hr. the solution was allowed to cool to room temperature and the precipitate was collected by filtration. A solution of this precipitate in water (1 ml.) was neutralized with 3 *N* hydrochloric acid to give an orange precipitate of the crude product which was collected by filtration and washed with water; the yield was 59 mg. (77%), m.p. 226°. A second run using 5 g. of XIII gave 2.48 g. (91%) of pure XVIII, m.p. 226°.

Action of Methanolic Ammonia on Diethyl 3-Methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XIII).—The dicarbamate (XIII, 50 mg.) was dissolved in methanol (5 ml.), saturated with ammonia at 0°, and the solution was sealed in a Parr bomb. At intervals, samples of the solution were examined by paper chromatography. After 15 hr., the reaction mixture was found to contain a very complex mixture of derivatives, none of which appeared to be 5,7-diamino-3-methylpyrido[3,4-*b*]pyrazine (XVII). Only one component appeared yellow in daylight and gave a yellow fluorescence under ultraviolet light, and this material corresponded in R_f values (A, 0.89; B, 0.85; C, 0.30; D, 0.84) and appearance to XIV (or XV). After 36 hr., only two components were present in the reaction mixture; on standing for a further 3 days no more significant changes were noted. One component (R_f: C, 0.15) exhibited a green-blue fluorescence under ultraviolet light, but was invisible in daylight, while the other (R_f: C, 0.25) gave a yellow fluorescence under ultraviolet light and appeared yellow in daylight. This material was identified as XIV (or XV).

Action of *N*-Bromosuccinimide on Diethyl 8-Bromo-3-methylpyrido[3,4-*b*]pyrazine-5,7-dicarbamate (XI).—*N*-Bromosuccinimide (75 mg.), dibenzoyl peroxide (4 mg.), and XI (160 mg.) were refluxed in chloroform (3 ml.) for 11 hr. After standing at room temperature for several days, the solution was evaporated to dryness *in vacuo*. The residue was washed with boiling water and recrystallized from ethanol (30 ml.) to give pale yellow needles which weighed 119 mg. The melting point and infrared spectrum of the product were identical with those given by the starting material.

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Cyclic Imidocarbonate Hydrochlorides from the Reaction of Cyanogen Chloride with Dithiols and Diols

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The reaction of ethanedithiol with cyanogen chloride in nonpolar solvents to give 2-imino-1,3-dithiolane hydrochloride is acid-catalyzed only in the presence of catalytic amounts of an alcohol. Hydrogen chloride accelerates the reaction of cyanogen chloride with mercaptoethanol to give 2-imino-1,3-oxathiolane hydrochloride and with ethylene glycol to give 2-imino-1,3-dioxolane hydrochloride. These reactions are quite general. Some physical properties of a number of cyclic imidocarbonate hydrochlorides and of 2-imino-1,3-dithiolane itself are described.

2-Imino-1,3-dithiolane hydrochloride (I) and its tin double salt were first characterized by Miolati.[1] The free imine was reported by Miolati to be an unstable oil.[1] His preferred synthesis of I involved a tin and hydrochloric acid partial reduction of ethylene thiocyanate followed by treatment of the tin–hydrochloride double salt with hydrogen sulfide.

Reaction of ethanedithiol with a cyanogen halide appeared to us to be a more attractive route to I. Formation of 2-imino-1,3-dithiolanes has been postulated as an intermediate step in the formation of thiocyanate ion in the quantitative estimation of *vic*-dithiols using cyanogen chloride in an aqueous system.[2,3] However, no iminodithiolane salts were actually isolated or identified from such a procedure.

Reaction of Dithiols, Mercapto Alcohols, and Diols with Cyanogen Chloride.—Reaction of ethanedithiol and cyanogen chloride (eq. 1) proceeded slowly in refluxing benzene. I precipitated in high purity but low yield. In subsequent reactions, using ethylene

(1)

I

glycol dimethyl ether or acetonitrile as solvents, the yield was raised to as high as 66%. The reaction was still erratic, however, with long reaction times generally being required. When commercial chloroform saturated with hydrogen chloride was used, a rapid, exothermic reaction resulted. However, the reaction when run in toluene or in washed and dried chloroform

(1) A. Miolati, *Ann.*, **262**, 61 (1891). The systematic name used by *Chemical Abstracts* for I is "cyclic ethylene dithioimidocarbonate hydrochloride."

(2) W. N. Aldridge, *Biochem. J.*, **42**, 52 (1948).

(3) J. R. Siegel and D. H. Rosenblatt, *J. Am. Chem. Soc.*, **80**, 1753 (1958).