1-Deaza-6-methylthiopurine Ribonucleoside[1]

JOHN A. MONTGOMERY AND KATHLEEN HEWSON

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

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7-Methylthio-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XI), the 1-deaza analog of the highly cytotoxic 6-methylthiopurine ribonucleoside, was prepared in two ways. In the first method, 2-amino-4-chloro-3-nitropyridine (III), the minor product of the amination of 2,4-dichloro-3-nitropyridine, was converted in three steps to 7-chloro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XII), which was allowed to react with sodium methyl mercaptide to give XI. In the second method, 2,4-dichloro-3-nitropyridine was allowed to react with sodium methyl mercaptide to give 2-chloro-4-methylthio-3-nitropyridine, which was then converted in three steps to 7-methylthio-3*H*-imidazo[4,5-*b*]pyridine (XIV). Fusion of XIV with tetra-*O*-acetylribofuranose gave predominantly the tri-*O*-acetyl derivative of XI from which the acetyl groups were removed by treatment with methanolic ammonia. Although much less cytotoxic than 6-methylthiopurine ribonucleoside, XI was cytotoxic to HEp-2 cells resistant to 6-mercaptopurine.

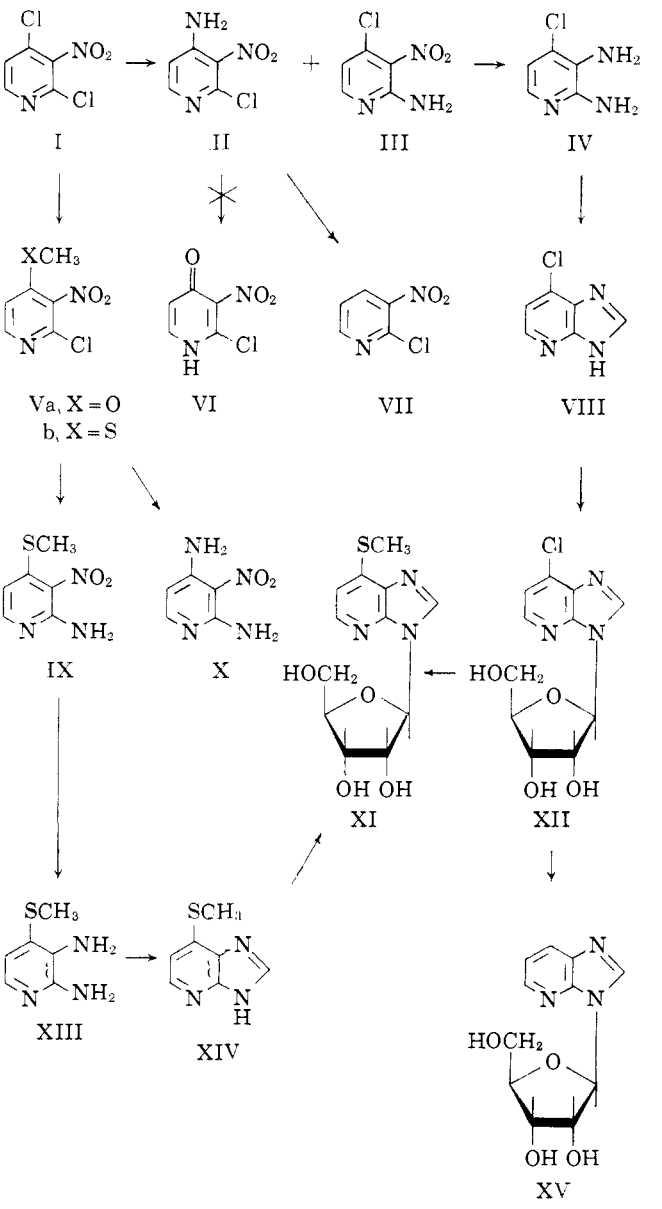
In a previous paper the rationale behind and the results from the synthesis and biologic evaluation of 3-deaza-6-methylthiopurine ribonucleoside were discussed.[2] Briefly, the purpose of this work is to determine the importance of the ring nitrogens to the biologic functioning of purines and their ribonucleosides. This information may prove helpful in the design of new agents more selective in their toxicity. The deaza analogs of 6-methylthiopurine ribonucleoside were chosen for study because of the great cytotoxicity shown by this purine ribonucleoside.[3] We now wish to report the synthesis of 1-deaza-6-methylthiopurine ribonucleoside (7-methylthio-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]-pyridine, XI).

Although the major product of the monoamination under mild conditions of 2,4-dichloro-3-nitropyridine (I) is 4-amino-2-chloro-3-nitropyridine (II),[2] a small amount of the isomeric 2-amino-4-chloro-3-nitropyridine (III) is also formed and can be separated from II by means of column chromatography on silica gel (see Scheme I). Catalytic reduction of III gave 2,3-diamino-4-chloropyridine (IV), which was cyclized to 7-chloro-3*H*-imidazo[4,5-*b*]pyridine (VII)[4] by means of diethoxymethyl acetate. Fusion of VIII with tetra-*O*-acetylribofuranose followed by treatment with methanolic ammonia gave 7-chloro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XII). The proof of structure of XII was accomplished by catalytic hydrogenolysis of the chloro group which gave 3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XV), a known compound.[5]

Displacement of the chloro group of XII with sodium methyl mercaptide proved difficult but was accomplished by heating the reaction mixture in a bomb at 100° for 18 hr. 1-Deaza-6-methylthiopurine ribonucleoside (XI) was obtained in 30% yield.

In a search for a more practical method to prepare larger amounts of XI, the conversion of II *via* diazotization to 2-chloro-3-nitro-4-pyridone (VI) was attempted. In mineral acid with sodium nitrite the diazotization failed. With isoamyl nitrite in glacial acetic acid a new pyridine was formed, but instead of VI, the only product isolated was found to be 2-chloro-

SCHEME I



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3-nitropyridine (VII),[6] identified by its melting point and proton magnetic resonance spectrum. Failure of this route caused us to turn to a study of the reaction of I with other nucleophiles. Reaction of I with 1 equiv of sodium methoxide gave 2(4)-chloro-4(2)-methoxy-3-nitropyridine (Va),[7] but replacement of the chloro group of Va without replacement of the methoxy group was not possible, and a mixture of 2,4-diamino-3-nitropyridine (X)[8] and another compound, probably 4(2)-amino-2(4)-methoxy-3-nitropyridine, that was difficult to resolve, was obtained. Since the methylthio group is known to be a poorer leaving group than the methoxy group in nucleophilic displacement reactions,[9] 2-chloro-4-methylthio-3-nitropyridine (Vb) was prepared by reaction of I with methyl mercaptan dissolved in ethanol containing 1 equiv of sodium methoxide. Reaction of Vb with alcoholic ammonia did, in fact, result in the displacement of the chloro group only and 2-amino-4-methylthio-3-nitropyridine (IX) was obtained in good yield. Reduction of IX by means of stannous chloride in concentrated hydrochloric acid gave 2,3-diamino-6-methylthiopyridine (XIII), which was cyclized with diethoxymethyl acetate to 7-methylthio-3*H*-imidazo[4,5-*b*]pyridine (XIV). Fusion of XIV with tetra-*O*-acetylribofuranose followed by treatment of the intermediate acetylated nucleoside with alcoholic ammonia gave XI in reasonable yield and a small amount of its α anomer.

Biologic Activity.—Table I shows a comparison of the cytotoxicity of 1-deaza-6-methylthiopurine ribonucleoside (XI) with that of 6-methylthiopurine ribonucleoside and the cytotoxicty of 1-deazapurine ribonucleoside with that of purine ribonucleoside. Although XI and XV are only about 0.25% as cytotoxic as the corresponding purine ribonucleosides, they are in fact cytotoxic, and the cytotoxicity of XI to the HEp-2/MP cell line may constitute useful biologic activity. In contrast, the ED_{50} of the isomeric 3-deaza-6-methylthiopurine ribonucleoside for HEp-2/MP cells is greater than 340 μmoles/l. (highest level tested).[2] These preliminary results indicate that, although N-3 of the purine ring appears essential to the biologic activity of the purine nucleosides, N-1 may not be *essential* even though it is obviously contributory. Further investigations of the 1-deazapurine ribonucleosides is in progress in these laboratories.

Table I

Compd	ED_{50}, μmoles/l.[a] HEp-S/S	HEp-2/MP
6-Methylthiopurine ribonucleoside	0.34	0.24
XI	>340	95
Purine ribonucleoside	1.0	...
XV	400	...
6-Mercaptopurine	1.4	1760

[a] ED_{50} is that concentration of compound inhibiting cell growth to 50% of controls. Cells were grown on glass and growth was measured by the determination of protein content [V. I. Oyama and H. Eagle, *Proc. Soc. Exptl. Biol. Med.*, **91**, 305 (1956)] after 4 days growth in the presence of the compound.

Experimental Section

The melting points reported were determined on a Kofler Heizbank and are corrected. The ultraviolet spectra were determined in solution with a Cary Model 14 spectrophotometer, and the infrared spectra were determined in pressed KBr disks with a Perkin-Elmer Model 521 spectrophotometer. The optical rotations were determined in the solvents specified with a Rudolph Model 80 polarimeter. SilicAR TLC-7 (Mallinckrodt) silica gel was used for most of the chromatographic separations. Spots were detected with an ultraviolet light after spraying the plates with Ultraphor WT highly concentrated (BASF Colors & Chemicals, Inc., Charlotte, N. C.).

2-Amino-4-chloro-3-nitropyridine (III).—A solution of 2,4-dichloro-3-nitropyridine[8] (I, 10 g, 52 mmoles) in ethanolic NH_3 (250 ml of absolute ethanol saturated at 5° with dry NH_3) was allowed to stand at room temperature for 6 hr before it was refrigerated overnight. The reaction solution was evaporated to dryness *in vacuo* and the residue was triturated with three 15-ml portions of boiling ligroin to extract unchanged starting compound (36%). The insoluble solid was triturated with four 40-ml portions of boiling chloroform and the filtrate was cooled to room temperature. The resulting $CHCl_3$ slurry was poured onto a silica gel column 3.75 × 25 cm containing 70 g of silica gel wet packed with $CHCl_3$). The column was eluted with chloroform until the fastest traveling yellow band had been eluted. Evaporation of this eluent to dryness *in vacuo* gave essentially pure III (490 mg, 5.4%) suitable for use as an intermediate. Crystallization of this material from benzene gave the analytical sample in several crops: yield 360 mg (4%), mp 176°. Thin layer chromatography on silica gel H (E. Merck, Darmstadt) using chloroform–ethyl acetate (1:1) as the eluent showed a single spot; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—220 (sh), 265 (sh), 327 (broad) (3.2), 352 (broad) (3.3); pH 7—226 (13.6), 250 (sh), 385 (3.3); pH 13—226 (13.8), 250 (sh), 385 (3.3); $\bar{\nu}_{max}$ in cm^{-1}: 3460, 3270, 3105 (NH, CH); 1630, 1580, 1545, 1520 (NH, C=C, C=N).

Anal. Calcd for $C_5H_4ClN_3O_2$: C, 34.59; H, 2.33; N, 24.21. Found: C, 34.67; H, 2.55; N, 24.31.

2-Chloro-4-methoxy-3-nitropyridine (Va).—An anhydrous solution of 2,4-dichloro-3-nitropyridine[8] (I, 676 mg, 3.5 mmoles) and sodium methoxide (3.5 ml of 1 *N* methanolic $NaOCH_3$) in methanol (10 ml) was allowed to stand at room temperature for 18 hr before it was evaporated to dryness *in vacuo*. The residue was triturated with water and the insoluble solid was collected by filtration and dried *in vacuo* to give 600 mg (91%) of crude product. Recrystallization of the crude product from methanol gave the pure material in two crops: yield 515 mg (78%), mp 77°. Thin layer chromatography using benzene–chloroform (2:1) as the eluent showed a single spot; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1,7,13—215 (sh) (11.5), 250 (1.4); $\bar{\nu}_{max}$ in cm^{-1}: 3080, 2995, 2960–2900, 2850 (CH); 1595, 1565, 1540 (C=C, C=N, C—NO_2); 1375 (C–NO_2); 1040 (C–OC).

Anal. Calcd for $C_6H_5ClN_2O_3$: C, 38.21; H, 2.68; N, 14.85. Found: C, 38.18; H, 2.93; N, 14.86.

2-Chloro-4-methylthio-3-nitropyridine (Vb).—Sodium methoxide (837 mg) in ethanol (15.5 ml) was added dropwise to a cold solution of 2,4-dichloro-3-nitropyridine (I, 3.00 g, 15.5 mmoles) in ethanol (100 ml) saturated with methyl mercaptan. After the addition was complete the reaction mixture was stirred for 1 hr at 5° before it was filtered, and the filtrate evaporated to dryness *in vacuo*. The resulting residue was triturated with boiling cyclohexane (150 ml) and filtered through dry Celite to remove additional inorganics. The crude product that precipitated from the filtrate was collected in three crops. Essentially pure material, suitable for use as an intermediate, was obtained by recrystallization of the crude product from ethanol with Norit treatment; yield 1.96 g (62%), mp 98°. Thin layer chromatography using benzene–chloroform (2:1) as the eluent showed one spot. An analytical sample Vb was obtained by cyclohexane recrystallization of the purified material: mp 100°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—220 (sh), 263 (11.5); pH 7—220 (sh), 263 (11.5); pH 13—263 (12.0); $\bar{\nu}_{max}$ in cm^{-1}: 3005, 2915 (CH); 1565, 1525 (C=C, C=N, C–NO_2); 1430 (CH_3); 1360 (C–NO_2).

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(7) By analogy one would assume preferential attack at C-4 of I, but confirmation of the structure of Va was not attempted; however, only one product was detected.

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Anal. Calcd for $C_6H_5ClN_2O_2S$: C, 35.22; H, 2.47; N, 13.69. Found: C, 35.60; H, 2.78; N, 13.63.

7-Chloro-3H-imidazo[4,5-*b*]pyridine (VIII).—A solution of 2-amino-4-chloro-3-nitropyridine (III, 300 mg, 1.7 mmoles) in ethanol (50 ml) containing glacial acetic acid (0.1 ml, 1.7 mmoles) was hydrogenated at atmospheric pressure in the presence of platinum oxide catalyst (30 mg). After the theoretical amount of hydrogen had been consumed, the catalyst was removed by filtration in a nitrogen atmosphere, and the filtrate was evaporated to dryness *in vacuo*. The resulting diaminopyridine was dissolved in diethoxymethyl acetate (10 ml), and the solution was allowed to stand at room temperature overnight before it was evaporated to dryness. Ethanol solutions of the residue were evaporated to dryness several times before it was dissolved in boiling water (40 ml) and the solution was filtered through Celite. The filtrate was evaporated to dryness to give 150 mg (56%) of crude product. Recrystallization of the crude product from boiling water with Norit treatment gave essentially pure material; yield 128 mg (48%), mp 168°. Thin layer chromatography using chloroform–ethyl acetate (1:1) as the eluent showed a trace of fluorescent contaminant; λ_{max} in mμ (ε × 10^{-3}): pH 1—243 (infl), 250 (infl), 259 (infl), 273 (sh) (8.4), 279.5 (9.3); pH 7—251 (5.9), 277 (8.7), 282 (infl); pH 13—287 (10.7); ν_{max} in cm^{-1}: 3070, 3010, 2980, 2900, 2800-2550 (CH, acidic H); 1620, 1605, 1575 (C=C, C=N).

2-Amino-4-methylthio-3-nitropyridine (IX).—A solution of 2-chloro-4-methylthio-3-nitropyridine (Vb, 1.60 g, 7.8 mmoles) in ethanolic ammonia (450 ml of absolute ethanol saturated at 5° with dry NH_3) was heated at 60° for 18 hr in a glass-lined bomb. The reaction solution was evaporated to dryness *in vacuo*, and the residue was triturated with three 15-ml portions of water. The insoluble solid was collected by filtration and triturated with three 5-ml portions of ethanol before it was dried *in vacuo*; yield 1.27 g (88%), mp 218° (sublim). Thin layer chromatography using chloroform–ethyl acetate (1:1) as the eluent showed a single spot. The analytical sample was obtained by recrystallization from ethanol; mp 220°; λ_{max} in mμ (ε × 10^{-3}): pH 1—209 (14.0), 262.5 (19.0), 352 (10.0); pH 7—221 (19.4), 242.5 (14.8), 265 (sh), 354 broad (5.8); pH 13—224 (16.7), 242 (15.2), 265 (sh), 354 broad (5.9); $\bar{\nu}_{max}$ in cm^{-1}: 3460, 3250 (NH); 3100, 2915 (CH); 1620, 1570, 1530, 1510 (NH_2, C=C, C=N); 1430 (CH_3).

Anal. Calcd for $C_6H_7N_3O_2S$: N, 22.69. Found: N, 22.56.

7-Methylthio-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XI). **A.**—A solution of 7-chloro-3-β-D-ribofuranosyl-3*H*-imidazopyridine (XII, 90 mg, 0.3 mmole) and sodium methoxide (0.6 ml of 1 *N* $NaOCH_3$ in methanol) in absolute methanol (15 ml) was saturated with methyl mercaptan at 5°. The resulting solution was heated in a glass-lined Parr bomb for 18 hr at 100°. The reaction solution was evaporated to dryness and the residue was extracted three times with 10 ml of a boiling mixture of acetone and ethyl acetate (1:4). After the insoluble solid was removed by filtration, the combined extracts were evaporated to dryness *in vacuo*. Trituration of the residue with ethyl acetate initiated crystallization. The crystals that precipitated were collected in several crops to give a total crude yield of 44 mg (47%). Recrystallization of the crude product from ethanol (3 ml) gave the pure material, yield 28 mg (30%), mp 206°, $[\alpha]^{28}D$ −73.3 ± 0.3 (0.98 g/100 ml of methanol). Thin layer chromatography using chloroform–methanol (9:1) as the eluent showed one spot which gave a positive Schiff–metaperiodate test; λ_{max} in mμ (ε × 10^{-3}): pH 1—225 (10.8), 280 (sh) (12.1), 288 (13.0); pH 7—218 (14.7), 282 (18.7), 287 (sh) (18.0); pH 13—282 (19.2), 287 (sh) (18.4); $\bar{\nu}_{max}$ in cm^{-1}: 3340, 3300-3120 (OH); 2990, 2950, 2920, 2905 (CH); 1590, 1570 (C=C, C=N); 1160, 1115, 1080 (COC); τ in ppm: 7.43 (H_2), 1.81 d and 2.88 d (H_5 and H_6), 3.98 d (H_1'), 4.78 m (OH), 5.47 m and 5.95 m (H_2', H_3', and H_4'), 6.36 m (H_5'), 7.40 (SCH_3).

Anal. Calcd for $C_{12}H_{15}N_3O_6S$: C, 48.48; H, 5.09; N, 14.13. Found: C, 48.61; H, 5.21; N, 14.06.

B.—A mixture of 7-methylthio-3*H*-imidazo[4,5-*d*]pyridine (XIV, 85 mg, 0.47 mmole), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (636 mg, 2 mmoles), and *p*-toluenesulfonic acid catalyst (8 mg) was fused at 180° (15 mm) for 15 min. The reaction melt was dissolved in $CHCl_3$ (10 ml), and the solution was washed ($NaHCO_3$, H_2O) before it was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The resulting residue was purified by means of preparative thin layer chromatography using chloroform–ethyl acetate (1:1) as the eluent. The acetylated ribonucleoside was extracted from the silica gel with ethyl acetate and the ethyl acetate was evaporated to dryness *in vacuo* to give 118 mg (59%) of purified product as an oil.

The chromatographically purified acetylated ribonucleoside (118 mg, 0.28 mmole) was dissolved in anhydrous ethanolic NH_3 (50 ml) and the solution was refrigerated for 72 hr. The solution was evaporated to dryness, the residue was dissolved in water, and the solution was extracted ($CHCl_3$). Evaporation of the water solution to dryness gave an oil, which solidified with trituration and seeding of an ethanol–ether suspension of the oil. The solid was collected by filtration, washed with ether, and dried *in vacuo*; yield 56 mg (67%), mp 200°. Thin layer chromatography using chloroform–methanol (9:1) as the eluent indicated the presence of a small amount of contaminant. Ethanol recrystallization of this material gave the pure product.

The contaminant was isolated by means of preparative thin layer chromatography. It gave a positive Schiff–metaperiodate test; $[\alpha]^{28}D$ +30.7 ± 2.4° (0.88 g/100 ml of methanol); λ_{max} in mμ (ε × 10^{-3}): pH 1—222, 279 (sh), 289 (9.4); pH 7—217, 281 (13.3), 287 (sh); pH 13—282 (13.1), 287 (sh). Since the ultraviolet spectrum indicates that the sugar is attached at N-3, the optical rotation establishes that the compound is the α anomer of XI. The pmr spectrum supports this assignment.

7-Chloro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XII). A mixture of 7-chloro-3*H*-imidazo[4,5-*b*]pyridine[4] (VIII, 115 mg, 0.8 mmole), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (480 mg, 1.5 mmoles), and *p*-toluenesulfonic acid catalyst (12 mg) was fused at 160° (20 mm) for 20 min. The reaction melt was dissolved in chloroform (5 ml), and the solution was washed ($NaHCO_3$, H_2O) before it was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The resulting residue was dissolved in ether and filtered through dry Celite, and the filtrate was streaked on a thin layer plate (20 × 20 cm plate coated with 1 mm of silica gel) for purification. The preparative thin layer plate was developed with chloroform–ethyl acetate (1:1) three times before the broad center band was scraped from the plate and eluted with methanol. Evaporation of the methanol solution gave the purified acetylated ribonucleoside as a glass (135 mg, 44%).

The acetylated ribonucleoside (150 mg, 0.4 mmole) was dissolved in anhydrous ethanolic NH_3 (30 ml) and the solution was refrigerated for 72 hr. The solution was evaporated to dryness, the residue was dissolved in water (5 ml), and the solution was extracted with $CHCl_3$ (5 ml). The water solution was evaporated to dryness and the resulting residue was triturated with ether. The insoluble solid that formed was collected by filtration, washed with fresh ether, and dried *in vacuo*; yield 76 mg (73%), mp indefinite above 100°. Thin layer chromatography using chloroform–methanol (9:1) as the eluent showed one spot which gave a positive Schiff–metaperiodate test; λ_{max} in mμ (ε × 10^{-3}): pH 1—253 (5.7), 274 (5.9), 280 (sh); pH 7—255 (5.9), 277 (5.8), 285 (sh); pH 13—257 (5.8), 278 (6.0), 286 (sh); $\bar{\nu}_{max}$ in cm^{-1}: 3400-3300 (OH); 3110, 2920, (CH); 1660, 1620, 1590 (C=C, C=N); 1130, 1105, 1075, 1050 (COC).

7-Methylthio-3*H*-imidazo[4,5-*b*]pyridine (XIV).—A solution of stannous chloride (2 g) in concentrated HCl (2.25 ml) was added dropwise with stirring to a mixture of 2-amino-4-methylthio-3-nitropyridine (IX, 500 mg, 2.7 mmoles) in ethanol (30 ml). After the addition was complete, the reaction mixture was warmed to 60° and stirred for an additional 2 hr. The resulting colorless reaction solution was cooled in an ice bath and neutralized with 6 *N* NaOH. The insoluble solid that precipitated was removed by filtration, and the filtrate was evaporated to dryness. The residue was triturated with three 10-ml portions of ethanol and filtered through dry Celite. The filtrate was evaporated to dryness *in vacuo*. The resulting purified 2,3-diamino-4-methylthiopyridine was dissolved in diethoxymethyl acetate (10 ml), the solution was allowed to stand at room temperature overnight before it was filtered through dry Celite, and the filtrate was evaporated to dryness *in vacuo*. Ethanol and water solutions of the residue were evaporated to dryness several times before the solid was triturated with ether–ethanol and the insoluble solid was collected by filtration to give the crude product (387 mg, 79%). Recrystallization of the crude product from water (20 ml) with Norit treatment gave the pure material, yield 337 mg (69%), mp 100°; resolidifies and remelts at 182°. Thin layer chromatography using chloroform–methanol (9:1) as the eluent showed a single spot; λ_{max} in mμ (ε × 10^{-3}): pH 1—226 (8.4), 289 (14.8), 308 (19.2); pH 7—215.5 (15.1), 281 (19.0), 286 (sh); pH 13—289 (17.2), 296 (sh); $\bar{\nu}_{max}$ in cm^{-1}: 3400-3200 broad (H_2O); 3095, 3050, 2985, 2950, 2915, 2800, 2720, 2630, 2570 (CH and acidic NH); 1598, 1575, 1550 (C=N,

C=C); 1475, 1430, 1420, 1365, 835, 810, 640, 600 (unassigned); τ in ppm: 7.43 (SCH_3), 2.94 d and 1.8 d (H_5 and H_6), 1.7 (H_2).

Anal. Calcd for $C_7H_7N_3S \cdot {}^7/_8H_2O$: C, 46.48; H, 4.88; N, 23.23. Found: C, 46.43; H, 4.76; N, 23.26.

3-β-D-Ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XV).[5]—A solution of 7-chloro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XII, 70 mg, 0.25 mmoles) in ethanol–water (5:10 ml) was hydrogenated at atmospheric pressure in the presence of MgO (9.8 mg, 0.2 mmole) and 5% Pd–C catalyst (10 mg). After the theoretical amount of hydrogen had been consumed, the catalyst and MgO were removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in water (1 ml) and filtered through dry Celite and the filtrate was refrigerated overnight. The crystals that formed were collected by filtration, washed, and dried *in vacuo;* yield 28 mg (45%), mp 226° (lit.[5] 220–222°), $[\alpha]^{28}D$ −78.0 ± 0.5° (0.99 g/100 ml of methanol). Thin layer chromatography using chloroform–methanol (9:1) as the eluent showed one spot which gave a positive Schiff–metaperiodate test: λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—236 (5.0), 274 (9.9), 281 (8.7); pH 7—244 (5.1), 277 (sh), 281 (8.5), 287 (6.6); pH 13—246 (4.8), 277 (sh), 281 (8.2), 286 (sh); [lit.[5] λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 0.5—236 (5.6), 275 (10.1), 281 (8.6); pH 5.66—243 (4.9), 281 (8.5), 287 (6.6)]; $\bar{\nu}_{max}$ in cm^{-1}: 3340, 3240, 3125, 2980, 2940, 2920, 2860 (OH, CH); 1595, 1580 (C=C, C=N); 1130, 1115, 1105, 1075, 1050 (CO–).

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Potential Carcinolytic Agents.[1] III. Fluoronitrogen Mustard Analogs of Cyclophosphamide[2]

ZINON B. PAPANASTASSIOU, ROBERT J. BRUNI, FRANCES POTTS FERNANDES, AND PHILIP L. LEVINS

Arthur D. Little, Inc., Acorn Park, Cambridge, Massachusetts 02140

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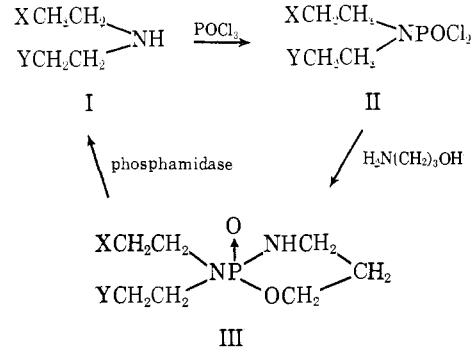
Two fluoro analogs of cyclophosphamide, 2-[bis(2-fluoroethyl)amino]-2H-1,3,2-oxazaphosphorinane 2-oxide and 2-[(2-chloro-2′-fluorodiethyl)amino]-2H-1,3,2-oxazaphosphorinane 2-oxide, were synthesized. These compounds and their precursors displayed little, if any, antitumor activity against rodent tumors.

Cyclophosphamide (IIIa) is one of the most effective biological alkylating agents for treating certain experimental rodent malignancies.[3] However, rather disappointing results were reported in treating neoplastic diseases in man with this agent.[4] Many structural modifications[5] of this compound have not produced any superior antitumor agents against animal tumors.

Recently a number of Russian investigators[6] have reported that some fluoroanalogs of nitrogen mustard derivatives exhibit antitumor properties (*e.g.*, 5-(2-chloro-2′-fluorodiethylamino)-6-methyluracil, see also ref 7). Also, Pettit and Smith[8] found that bis(2-fluoroethyl)amine hydrobromide inhibited the growth of Walker 256 carcinosarcoma (40% by 0.6 mg/kg, but the therapeutic index was less than 1). Our investigation on the mechanism of action of fluoroethylamines demonstrated that these compounds act, *in vitro*, like the corresponding chloroethylamines through the formation of an aziridinium intermediate but at a slower rate.[9] It was therefore of interest to examine if the oxazaphosphorinane moiety of cyclophosphamide (IIIa) could act as a carrier of the cytotoxic fluoro nor-nitrogen mustards Ib and c in transporting IIIb, IIIc, or an activated product derived from them[10] to the tumor site. Hydrolysis, catalyzed by phosphamidase, could then produce the active biological alkylating agents Ib and c.



III

a, X = Y = Cl
b, X = Y = F
c, X = Cl; Y = F

Straight-chain ω-fluoroalkylamines with an even number of carbon atoms are very toxic because they are oxidized by monoamine oxidases to precursors of

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(2) Cyclophosphamide: 2-[bis(2-chloroethyl)amino]-2H-1,3,2-oxazaphosphorinane 2-oxide.

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(9) P. L. Levins and Z. B. Papanastassiou, *J. Am. Chem. Soc.*, **87**, 826 (1965).

(10) N. Brock, *Acta Unio Intern. Contra Cancrum*, **20**, 45 (1964).