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Synthesis of Aristeromycin Analogs

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Reaction of aristeromycin (I) with acetyl bromide in acetonitrile, followed by treatment with hydrobromic acid afforded 3′β-bromo-3′-deoxyaristeromycin hydrobromide (V) and 2′β-bromo-2′-deoxyaristeromycin hydrobromide (VI). Catalytic reduction of V and VI with palladium on charcoal gave 3′-deoxyaristeromycin (X) and 2′-deoxyaristeromycin (XI), respectively. Treatment of V with sodium alkoxide yielded 2′,3′-anhydroaristeromycin (IV). Two carbocyclic analogs (XII and XV) of inosine and 6-mercaptopurine ribonucleoside, 8-bromoaristeromycin (XVI) and 8-hydroxyaristeromycin (XVII) were also prepared.

The authors have previously reported a novel reaction of N^4-acetylcytidine with acetyl bromide to form 1-(2′,5′-di-O-acetyl-3′-bromo-3′-deoxy-β-D-xylofuranosyl)-N^4-acetylcytosine.[2] The formation of this product could be explained *via* conversion of the 2′,3′-*cis* diol function to a reactive 2′,3′-acetoxonium ion and subsequent ring-opening of the acetoxonium ion by nucleophilic "downward" attack of the bromide anion on $C_{3'}$.[3] This paper deals mainly with application of this anomalous reaction to aristeromycin (I), which is an antibiotic isolated from the culture broth of *Streptomyces citricolor* and has been assigned the (1′*R*, 2′*S*, 3′*R*, 4′*R*)-9-β-[2′α,3′α-dihydroxy-4′-β-(hydroxymethyl)cyclopentyl]adenine structure.[5]

The reaction mixture of I with acetyl bromide in acetonitrile at 120° for 3 hr was subjected to thin-layer chromatography (TLC, silica gel, $CHCl_3$–MeOH, 4: 1), which showed a single ultraviolet (UV) absorbing spot. Treatment of the product with methanolic ammonia, however, gave two UV absorbing substances on a paper electrophoreogram (0.05M borate, pH 9.2), one of which migrated the same distance as I while the other remained near the origin. These observations suggest that reaction of I with acetyl bromide may lead to formation of the 2′,3′-*trans* bromohydrin acetate (II) in addition to the tri-O-acetyl derivative (III) of I, and that treatment of the mixtures with methanolic ammonia may yield 2′,3′-anhydroaristeromycin (IV) from II and I from III, respectively. With the aim of hydrolyzing the presumed bromohydrin acetate (II) to the bromohydrin (V), the reaction mixture of I with acetyl bromide was treated with hydrobromic acid. The product was subjected to TLC, which revealed the presence of two UV absorbing compounds (*Rf* 0.50, 0.38) in addition to I (*Rf* 0.20). The mixture was chromatographed on a silica gel column. The two compounds thus obtained, one as crystals (mp 188°, *Rf* 0.50) and the other as resin (*Rf* 0.38), had the same chemical compositions ($C_{11}H_{15}O_2N_5Br_2$) as that of the hydrobromide of the bromohydrin. The nuclear magnetic resonance (NMR) spectrum (d_6-DMSO) of the crystals showed the location of the bromo function at C_3' by the 0.6 ppm downfield shift of H_3' relative to that in I. Treatment of the crystals with sodium alkoxide gave a compound as needles (mp 175°,

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[2] R. Marumoto and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), 22, 128 (1974).

[3] There have been also reported similar reactions *via* 2′,3′-oxonium ion.[4]

[4] A.F. Russell, S. Greenberg, and J.G. Moffatt, *J. Am. Chem. Soc.*, 95, 4025 (1973); M.J. Robins, R. Mengel, and R.A. Jones, *ibid.*, 95, 4074 (1973).

[5] T. Kishi, M. Muroi, T. Kusaka, M. Nishikawa, K. Kamiya, and K. Mizuno, *Chem. Commun.*, 1967, 852; *idem, Chem. Pharm. Bull.* (Tokyo), 20, 940 (1972).

$C_{11}H_{13}O_2N_5$), which showed by paper electrophoresis (PE) a single UV absorbing spot near the origin. The crystalline and resinous compounds were thus assigned the structures of the hydrobromide of 3′β-bromo-3′-deoxyaristeromycin (V, D-xylo type 3′-bromo-3′-deoxyaristeromycin) and the hydrobromide of 2′β-bromo-2′-deoxyaristeromycin (VI, D-arabino type 2′-bromo-2′-deoxyaristeromycin), respectively. The compound obtained by the reaction of V with sodium alkoxide was thus identified as IV. Compounds, V and VI were formed in a ratio of about 5:1. This fact shows that the bromide ion makes its nucleophilic "downward" attack not only on C_3', but also on C_2' with subsequent ring-opening of the reactive 2′,3′-acetoxonium ion to give II and 2′β-bromo-3′α-acetate (VII). In the reaction of acetyl bromide with N^4-acetylcytidine, however, the bromide ion mainly attacked C_3'.2)

Treatment of V with alkali hydroxide afforded a mixture of two UV absorbing compounds (VIII and IX). Compound (VIII) migrated to the cathode on paper electrophoresis and possessed a UV absorbance maximum at 297 nm in neutral solution. Purification of this compound was unsuccessful because of its instability. VIII was assigned as carbocyclic analog of 5-amino-1-(3-deoxy-β-D-xylofuranosyl)imidazole-4-carboxamidine-$N^5 \rightarrow 3'$-cyclonucleoside based on its cationic character and bathochromic shift of its UV spectrum.6) On the other hand, compound (IX) migrated slightly to the cathode and was assigned as carbocyclic analog of 5-amino-1-(3-deoxy-β-D-xylofuranosyl)imidazole-4-carboxamide-$N^5 \rightarrow 3'$-cyclonucleoside based on its UV spectrum.6)

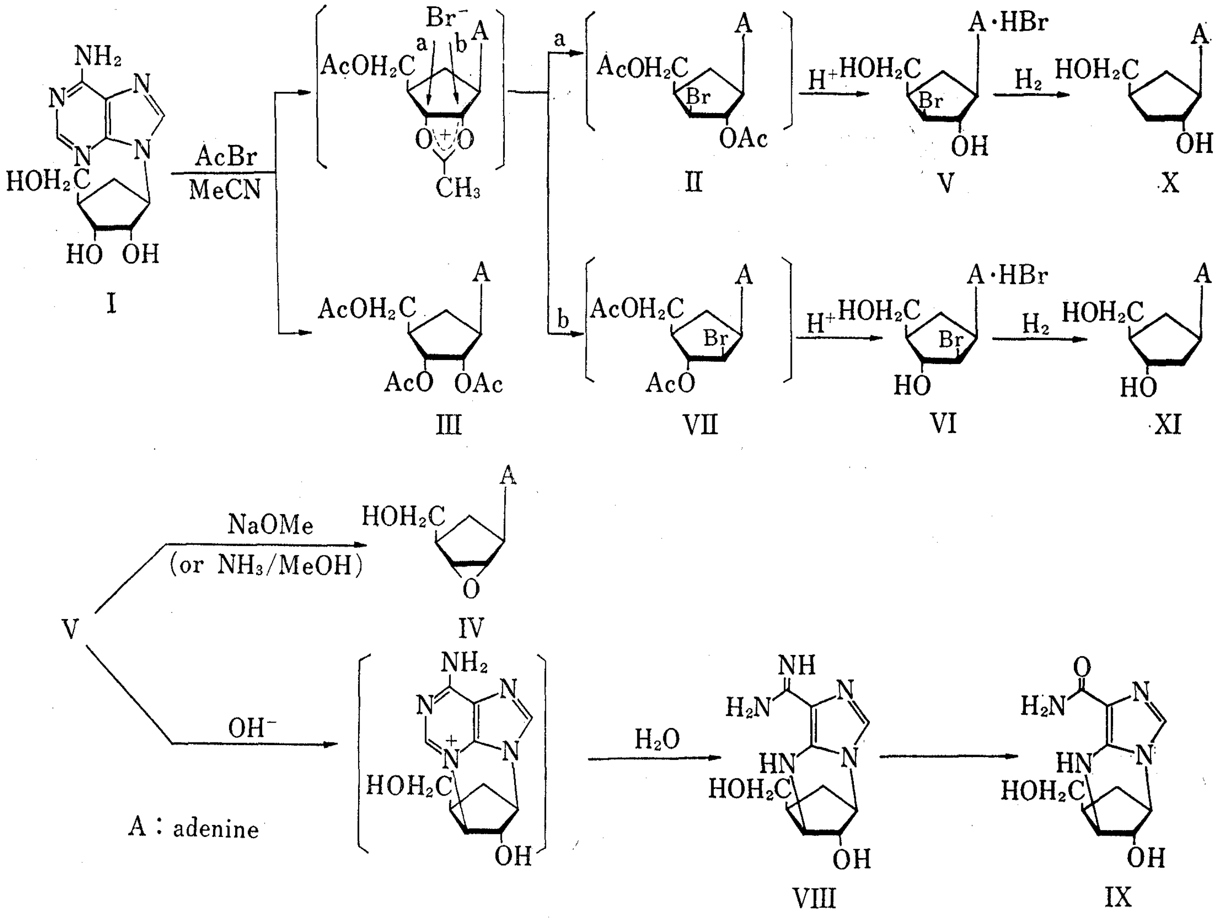

Chart 1

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6) Robins and coworkers have reported intramolecular degradation of adenosine 2′,3′-ribo-epoxide to 5-amino-1-(3-deoxy-β-D-xylofuranosyl)imidazole-4-carboxamidine-$N^5 \rightarrow 3'$-cyclonucleoside ($\lambda_{\max}^{\mathrm{pH}\,7}$ nm: 293, $\lambda_{\min}^{\mathrm{pH}\,7}$ nm: 252) and 5-amino-1-(3-deoxy-β-D-xylofuranosyl)imidazole-4-carboxamide-$N^5 \rightarrow 3'$-cyclonucleoside ($\lambda_{\max}^{\mathrm{pH}\,7}$ nm: 275, $\lambda_{\min}^{\mathrm{pH}\,7}$ nm: 221).7)

7) M.J. Robins, Y. Fouron, and R. Mengel, *J. Org. Chem.*, **39**, 1564 (1974).

In analogy with adenosine 2′,3′-ribo-epoxide,[6] the reaction of V with alkali hydroxide might give a ribo-type epoxide, on which the nucleophilic "downward" attack of the N^3 atom may occur to afford $N^3 \rightarrow 3'$-cyclo-intermediate. Attack of water on the C_2 of the intermediate, followed by hydrolysis of the resulting ring-opened N^5-formyl derivative, may lead to VIII, which further undergoes hydrolysis, ultimately yielding IX.

Catalytic reduction of V and VI with palladium on charcoal afforded crystalline 3′-deoxyaristeromycin (X, mp 217°, $C_{11}H_{15}O_2N_5 \cdot H_2O$) and 2′-deoxyaristeromycin (XI, mp 183°, $C_{11}H_{15}O_2N_5 \cdot 1/2H_2O$), respectively.[8a] The 100 MHz NMR spectrum (d_6-DMSO) of X showed a multiplet (5H, $2H_{2'}$, $2H_{5'}$, $1H_{4'}$) at δ 1.6—2.6 ppm, another multiplet (1H, $H_{3'}$) at δ 4.12 ppm and a quintet (1H, $H_{1'}$) at δ 5.06 ppm. When the methine proton of $C_{1'}$ was irradiated, a change of the multiplet at δ 1.6—2.6 ppm was observed but no change of the multiplet at 4.12 ppm. This observation is further evidence for the correctness of the structure XI (Chart 1).

Deamination of I with nitrous acid afforded a carbocyclic analog (XII) of inosine.[8b] Benzoylation of XII followed by chlorination with the Vilsmeier reagent gave an analog (XIII) of 6-chloro-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine. Treatment of XIII with thiourea gave an analog (XIV) of 6-mercapto-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine. Compound XIV was hydrolyzed with sodium methoxide to an analog (XV) of 6-mercaptopurine ribonucleoside.[8b] Bromination of I with bromine in a buffer solution gave 8-bromoaristeromycin (XVI), which was treated with hydrochloric acid to afford 8-hydroxyaristeromycin

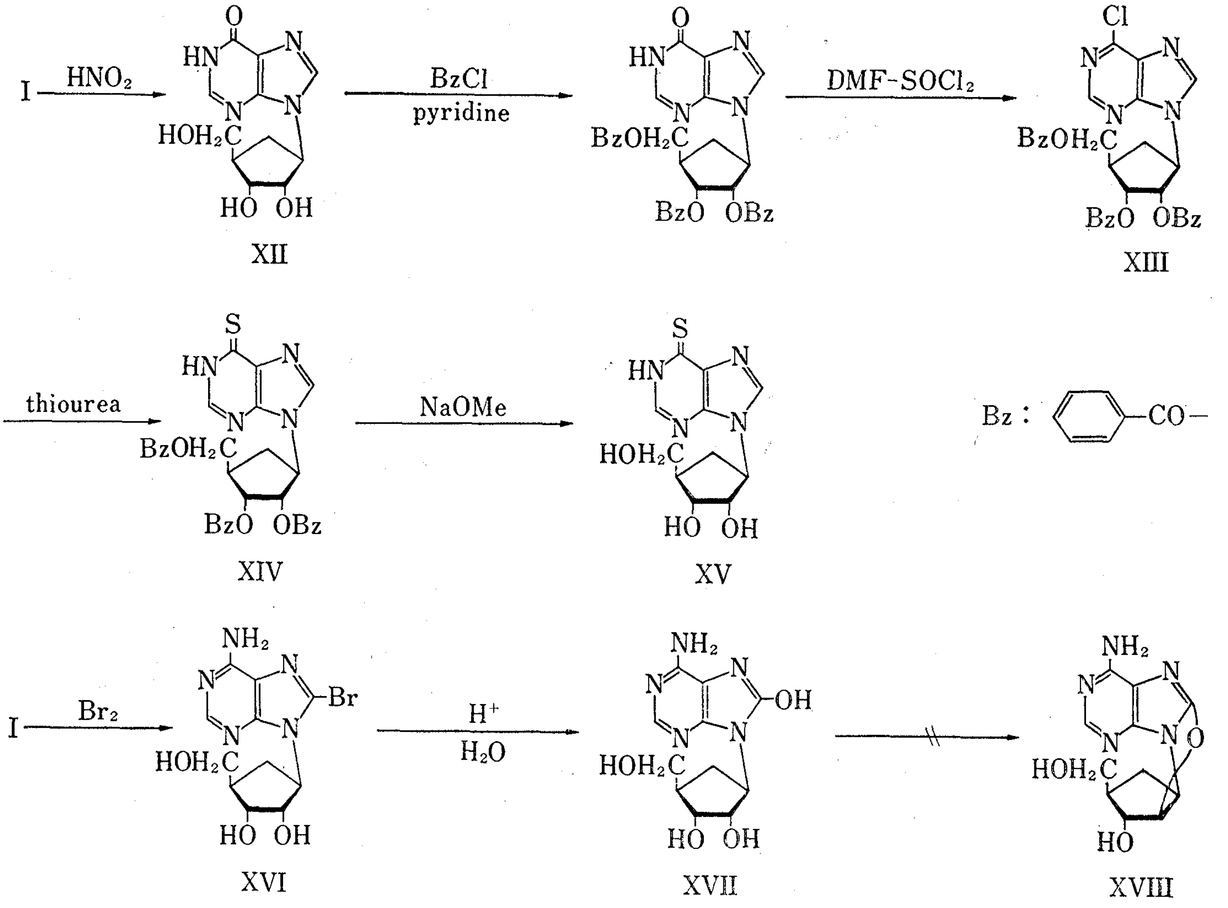

Chart 2

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[8] The corresponding racemic compound has been synthesized by the different route: *a*) T.F. Shealy and C.A. O'Dell, *Tetrahedron Letters*, **1969**, 2231; *b*) Y.F. Shealy and J.D. Clayton, *J. Pharm. Sci.*, **62**, 1252 (1973).

(XVII). Treatment of XVII with diphenyl carbonate in dimethylformamide⁹⁾ or with partially hydrolyzed phosphorous oxychloride in ethyl acetate¹⁰⁾ followed by hydrolysis with methanolic ammonia did not give the expected 8,2′-O-cycloaristeromycin (XVIII), but led to the recovery of XVII (Chart 2).

Experimental¹¹⁾

3′β-Bromo-3′-deoxyaristeromycin Hydrobromide (V) and 2′β-Bromo-2′-deoxyaristeromycin Hydrobromide (VI)——To a suspension of I (1.2 g, 4.5 mmoles) in CH_3CN (40 ml) was added AcBr (2.4 ml, 32 mmoles). The mixture was refluxed for 3 hr (bath temperature 120°) and evaporated to dryness *in vacuo*. A portion (10 mg) of the residue was dissolved in 20% MeOH–NH_3 (0.5 ml) and the solution was kept at room temperature for 20 hr. PE of the sample revealed the presence of two UV absorbing substances, one of which migrated the same distance as I and the other remained near the origin (formation ratio *ca.* 2: 1). All the above-described residue was dissolved in MeOH (20 ml) and to the solution was added 40% aq. HBr (2 ml). The mixture was stirred at room temperature for 1 hr and then evaporated to dryness *in vacuo*. The residue was purified by silica gel column chromatography (24 g, $CHCl_3$–MeOH, 17: 3). Fractions (Nos. 25—30, one fraction 10 g) were combined to be evaporated to dryness. Trituration of the residue with EtOH (4 ml) gave colorless crystals (V). Yield 300 mg. mp 188. *Rf* (TLC): 0.50. NMR (d_6-DMSO) δ: 1.7—2.8 (3H, m, $H_{4'}$, $2H_{5'}$), 3.64 (2H, broad d, $2H_{6'}$), 4.2—5.0 (3H, m, $H_{1'}$, $H_{2'}$, $H_{3'}$), 8.54 and 8.59 (2H, 2s, H_2, H_8), 8.7—9.5 (3H, broad s, $-NH_3^+Br^-$). *Anal.* Calcd. for $C_{11}H_{15}O_2N_5Br_2$: C, 32.29; H, 3.69; N, 17.12; Br, 39.07. Found: C, 32.86; H, 3.60; N, 16.65; Br, 35.61. Fractions (Nos. 31—40) and the mother liquor of V were combined to be purified by silica gel column chromatography (20 g, $CHCl_3$–MeOH, 17: 3). Fractions showing a single UV absorbing spot on TLC (*Rf* 0.38) were combined. Evaporation of the solution to dryness *in vacuo* afforded a resinous compound (VI). Yield 100 mg.

2′,3′-Anhydroaristeromycin (IV)——A solution of V (100 mg) in 1 N NaOMe (10 ml) was kept at room temperature for 20 hr and poured into ice-water (20 ml). The mixture was neutralized with conc. HCl. TLC of the sample showed a single UV absorbing spot of *Rf* 0.55. The solution was purified by silica gel column chromatography (7 g, $CHCl_3$–MeOH, 9: 1) and the pure fraction was evaporated to dryness. The residue was recrystallized from MeOH to give colorless crystals (60 mg). mp 175° (decomp.). NMR (d_6-DMSO) δ: 1.8—2.8 (2H, m, $2H_{5'}$), 3.6 (1H, m, $H_{4'}$), 3.7 (2H, m, $H_{6'}$), 3.93 (1H, d, $H_{3'}$), 4.18 (1H, d, $H_{2'}$), 7.38 (2H, broad s, $-NH_2$), 8.34 and 8.40 (2H, s, H_2, H_8). *Anal.* Calcd. for $C_{11}H_{13}O_2N_5$: C, 53.43; H, 5.30; N, 28.33. Found: C, 52.79; H, 5.15; N, 28.15.

Treatment of V with aq. NaOH (Formation of VIII and IX)——To a solution of V (100 mg) in water (5 ml) was added 1 N NaOH (1 ml). The mixture was heated at 80° for 30 min and neutralized with 1 N HCl. PE of the sample revealed the presence of two UV absorbing compounds: Compound A (formation ratio, 40%, UV $\lambda_{max}^{pH\,2}$ nm: 210, 297; $\lambda_{min}^{pH\,2}$ nm: 250; $\lambda_{max}^{pH\,7}$ nm: 210, 297; $\lambda_{min}^{pH\,7}$ nm: 255; $\lambda_{max}^{pH\,12}$ nm: 288; $\lambda_{min}^{pH\,12}$ nm: 250), which was assumed to be VIII, migrated to the cathode the same distance as I and became yellow in the atmosphere. Compound B (formation ratio, 60%, $\lambda_{max}^{pH\,2}$ nm: 255, 282; $\lambda_{min}^{pH\,2}$ nm: 229, 264; $\lambda_{max}^{pH\,7}$ nm: 280; $\lambda_{min}^{pH\,7}$ nm: 228; $\lambda_{max}^{pH\,12}$ nm: 281; $\lambda_{min}^{pH\,12}$ nm: 234), which was assumed to be IX, migrated slightly to the cathode.

3′-Deoxyaristeromycin (X)——To a solution of V (300 mg) in 80% aq. MeOH (10 ml) was added AcONa (600 mg) and 10% Pd-charcoal (500 mg). The mixture was shaken in a H_2 atmosphere at 760 mmHg and room temperature for 2 days. The catalyst was filtered off, the filtrate evaporated to dryness *in vacuo* and the residue was dissolved in water (30 ml). The solution was adsorbed on a column of charcoal and the column was eluted with H_2O–EtOH–BuOH–28% NH_4OH (24: 20: 5: 1, 200 ml). The eluate was evaporated to dryness *in vacuo* and the residue was recrystallized from MeOH to afford colorless crystals (150 mg). mp 217°. $[\alpha]_D^{25} = -223°$ ($c=0.5$, H_2O). *Rf* (TLC): 0.36. NMR (d_6-DMSO) δ: 1.5—2.5 (4H, $2H_{3'}$, $2H_{6'}$), 4.5 (2H, m, $H_{1'}$, $H_{2'}$), 7.0 (2H, broad s, $-NH_2$), 8.02 (2H, H_2, H_8). *Anal.* Calcd. for $C_{11}H_{15}O_2N_5 \cdot H_2O$: C, 49.43; H, 6.41; N, 26.20. Found: C, 49.40; H, 5.79; N, 26.15.

2′-Deoxyaristeromycin (XI)——To a solution of VI (100 mg) in 50% aq. MeOH (4 ml) was added AcONa (200 mg) and 10% Pd-charcoal (200 mg). The mixture was similarly treated as in the case of IX to give colorless crystals (50 mg). mp 183°. $[\alpha]_D^{25} = +49°$ ($c=0.5$, H_2O). *Rf* (TLC): 0.31. NMR (d_6-DMSO) δ: 1.6—2.6 (5H, m, $2H_{2'}$, $2H_{5'}$, $H_{4'}$), 3.5 (2H, d, $2H_{6'}$), 4.4 (1H, m, $H_{3'}$), 7.4 (2H, s, NH_2), 8.3 (2H, 2a, H_2, H_8). *Anal.* Calcd. for $C_{11}H_{15}O_2N_5 \cdot H_2O$: C, 49.43; H, 6.41; N, 26.20. Found: C, 49.45; H, 5.90; N, 25.56.

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9) A. Hampton and A.W. Nichol, *Biochem.*, **5**, 2076 (1966); M. Ikehara and T. Tezuka, *Tetrahedron Letters*, **1972**, 1169.

10) Y. Furukawa and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **16**, 2286 (1968).

11) All melting points were uncorrected. Paper electrophoresis (PE) was performed on Whatman No. 1 filter paper at 22 V/cm for 60 min using 0.05 M borate buffer (pH 9.2). Thin-layer chromatography (TLC) was carried out on 0.25 mm layer of E. Merck DC-Alufolien Kieselgel F 254 ($CHCl_3$–MeOH, 4: 1).

Carbocyclic Analog (XII) of Inosine——To a solution of I (4 g) in water (400 ml) was added $NaNO_2$ (21 g). AcOH (20 ml) was added dropwise to the ice-cooled stirred mixture. The solution was kept at room temperature for 1 day, diluted with water (1.3 liters) and stirred thoroughly with charcoal (27 g). The mixture was poured onto a previously prepared column of charcoal (13 g). After the fluid was passed through, the column was washed with water (1 liter) and eluted with H_2O–EtOH–BuOH–28% NH_4OH (24: 20: 5: 1, 2 liters). The eluate was evaporated to dryness. A pale yellow powder (3.5 g) was recrystallized from H_2O–EtOH (3: 7) to give colorless crystals. mp 235°. UV $\lambda_{\max}^{H_2O}$ nm (ε): 251 (1.19×10^4). *Anal.* Calcd. for $C_{11}H_{14}O_4N_4$: C, 49.60; H, 5.30; N, 21.04. Found: C, 49.69; H, 5.22; N, 21.05.

Carbocyclic Analog (XIII) of 6-Chloro-9-(2,3,5-tri-O-benzoyl-*β*-D-ribofuranosyl)purine——Benzoyl chloride (6 ml, 52 mmoles) was added dropwise to a warmed suspension at 55° of XII (3.5 g, 13 mmoles) in pyridine (100 ml). The mixture was warmed at 60—65° for 2 hr and kept at room temperature for 20 hr. Pyridine hydrochloride was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in $CHCl_3$ and the solution was successively washed with saturated aq. $NaHCO_3$ and water. The $CHCl_3$ layer was evaporated to dryness to give a yellow brown foam (tri-O-benzoate of XII). Yield 5.7 g. TLC of the sample showed a single UV absorbing spot. A portion (1.06 g) of the compound was dissolved in $CHCl_3$ (10 ml). To the solution were added DMF (0.33 ml) and $SOCl_2$ (1.0 g). The mixture was refluxed for 2 hr and evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ (30 ml). The solution was shaken with water (30 ml). The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to dryness. The oil was recrystallized from EtOH (10 ml) to afford colorless crystals (800 mg). mp 174.5°. $\lambda_{\max}^{EtOH}$ nm: 231, 267; $\lambda_{\max}^{EtOH}$ nm: 217.5, 255. Beilstein reaction: (+).

Carbocyclic Analog (XIV) of 6-Mercapto-9-(2,3,5-tri-O-benzoyl-*β*-D-ribofuranosyl)purine——A solution of XIII (745 mg) and thiourea (244 mg) in EtOH (10 ml) was refluxed for 1 hr. Upon cooling colorless needles crystallized out of the solution, which were collected by filtration and washed successively with EtOH and water. Yield 700 mg. mp 268°. $\lambda_{\max}^{EtOH}$ nm: 232, 285, 328; $\lambda_{\max}^{EtOH}$ nm: 262.

Carbocyclic Analog (XV) of 6-Mercaptopurine Ribonucleoside——To a suspension of XIV (700 mg) in MeOH (10 ml) was added 1 N NaOMe (2 ml). The mixture was stirred at room temperature for 2 hr and evaporated to dryness. A suspension of the residue in water (40 ml) was adjusted to pH 7 with 1 N HCl and washed with $CHCl_3$ (20+10+10 ml). The aq. layer was adjusted to pH 4 with AcOH to deposit colorless crystals. Yield 160 mg. mp 280° (decomp.). $[\alpha]_D^{25} = -289°$ ($c=0.5$, DMF). UV $\lambda_{\max}^{pH\ 1}$ nm: 228, 324; $\lambda_{\max}^{H_2O}$ nm: 230, 324; $\lambda_{\max}^{pH\ 13}$ nm: 235, 311.5. *Anal.* Calcd. for $C_{11}H_{14}O_3N_4S \cdot 1/2H_2O$: C, 45.35; H, 5.19; N, 19.23; S, 11.01. Found: C, 45.80; H, 4.70; N, 18.88; S, 10.54.

8-Bromoaristeromycin (XVI)——To a suspension of I (1 g) in 1 M acetate buffer (pH 4, 60 ml) was added saturated bromine water (60 ml). The mixture was stirred at room temperature for 20 hr, to which was added Na_2SO_3 (0.5 g). The solution was desalted with a column of charcoal (10 g) and the eluate (300 ml) was concentrated to give colorless prisms. Yield 750 mg. mp 250°. $\lambda_{\max}^{H_2O}$ nm: 266. *Anal.* Calcd. for $C_{11}H_{14}O_3N_5Br$: C, 38.38; H, 4.10; N, 20.35. Found: C, 37.36; H, 3.75; N, 19.90.

8-Hydroxyaristeromycin (XVII)——A solution of XVI (300 mg) in 1 N HCl (10 ml) was refluxed for 30 min (bath temperature 150°), adjusted to pH 3 with 1 N NaOH and desalted with a column of charcoal (5 g). The eluate (100 ml) was concentrated, to which was added EtOH. Recrystallization of the needles from water afforded colorless feathery crystals. Yield 230 mg. mp 149—151°. $[\alpha]_D^{25} = -120°$ ($c=0.5$, DMF). UV $\lambda_{\max}^{pH\ 1}$ nm: 264, 283; $\lambda_{\max}^{pH\ 13}$ nm: 282. *Anal.* Calcd. for $C_{11}H_{15}O_4N_5 \cdot 1/2H_2O$: C, 45.51; H, 5.55; N, 24.13. Found: C, 45.47; H, 5.41; N, 23.56.

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