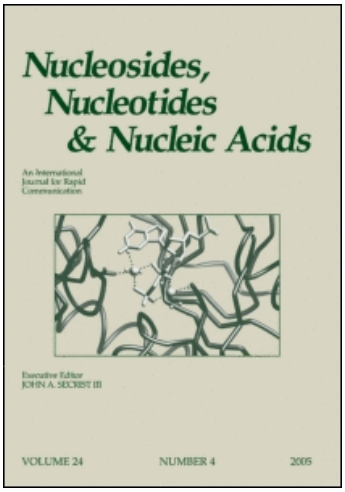

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Thiocyanation of Tubercidin and Its Derivatization to 6-Propyl-and 6-Cyano Derivatives (Nucleosides and Nucleotides. 41[1].)

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THIOCYANATION OF TUBERCIDIN AND ITS DERIVATIZATION TO 6-PROPYL- AND 6-CYANO DERIVATIVES (NUCLEOSIDES AND NUCLEOTIDES. 41.)[1]

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ABSTRACT

Thiocyanation of tubercidin with thiocyanogen chloride gave the 5-thiocyanate, which was converted to the 5-methylthio, 5-methylsulfonyl as well as 6-propyl and 6-cyano derivatives. The 6,5'-O-cyclotubercidins were also prepared.

Tubercidin (7-β-D-ribofuranosyl-4-aminopyrrolo[2,3-d]-pyrimidine[2]) is a nucleoside antibiotic especially active to Mycob. tuberculosis BCG and also cytotoxic to various mammalian cells.[3] These properties are explained on the basis that it is a deaza analog of adenosine. The 5-position of tubercidin (1) is unique since usual purine nucleosides do not possess a carbon atom in this position. The 5-cyanotubercidin (toyocamycin[4]) is also an active antibiotic. Therefore it is expected that other 5-substituted derivatives may well be effective compounds. The present paper deals with the introduction of a sulfur substituent into the 5-position of tubercidin and its further transformations.

Witkop and coworkers have reported the synthesis of 5-thiocyanatouridines by direct thiocyanation.[5] We have also extended this procedure for the preparation of 5-alkylthio and alkylsulfonyl derivatives of uridines.[6] It can be expec-

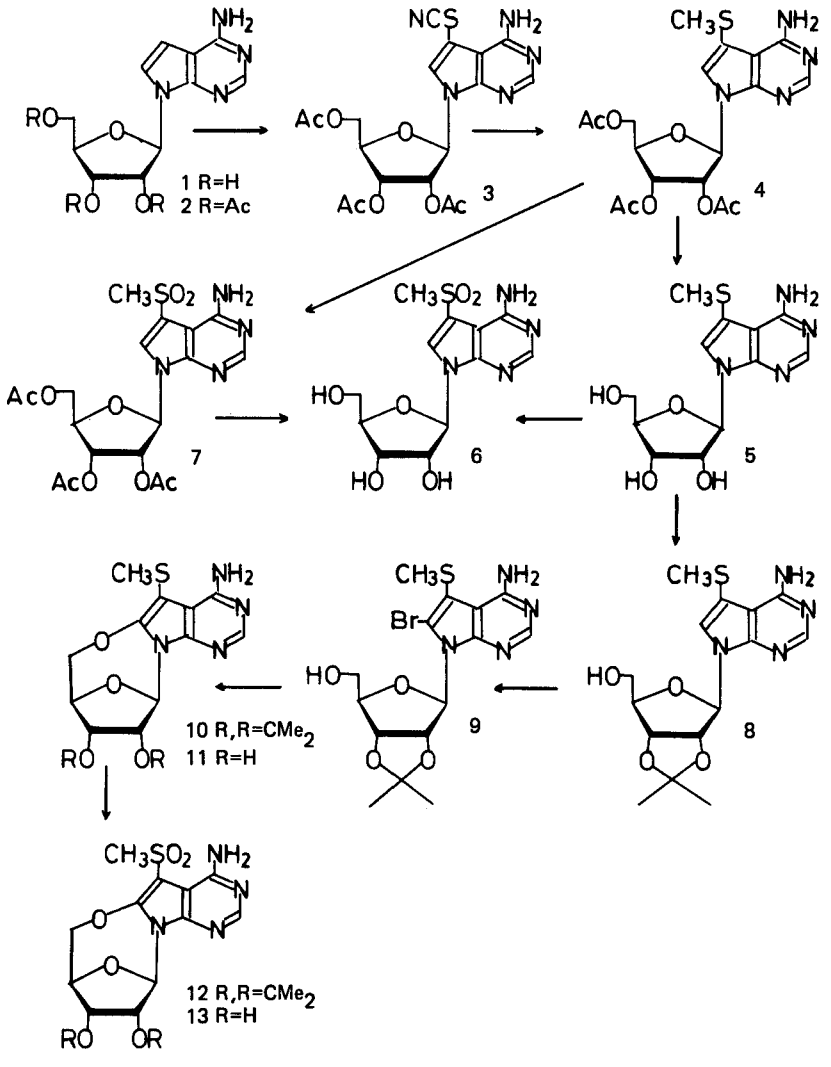
ted that 5 and 6 positions of the pyrrolopyrimidine system are susceptible to electrophilic attack, as evidenced by the halogenation of tubercidin.[7,8]

Treatment of 2',3',5'-tri-O-acetyltubercidin (2) with thiocyanogen chloride[5] in acetic acid afforded a mono thiocyanate (3). Treatment of 3 with 2-mercaptoethanol in ethanol followed by methyl iodide in the presence of sodium bicarbonate gave the methylthio derivative (4). Deacetylation of 4 gave crystalline 5-methylthiotubercidin (5). Treatment of 5 with potassium permanganate in 50% acetic acid under cooling gave the 5-methylsulfone (6). Compound 6 was also obtained by the initial oxidation of 4 to 7 followed by deacetylation.

From the NMR spectra of these derivatives as well as those of other reported derivatives of tubercidin, it is certain that the postition of thiocyanation was at 5 and not at 6. For a chemical proof of the position of substitution, the following transformation of 5 was undertaken. Acetonation of 5 gave 2',3'-O-isopropylidene-5-methylthiotubercidin (8). Treatment of 8 with N-bromosuccinimide in methylene chloride gave a monobromo derivative (9). Compound 9 was also prepared from 5'-O-acetyl-2',3'-O-isopropylidenetubercidin by thiolation and bromination. Treatment of 9 with sodium hydride in dimethylformamide[9] gave a cyclonucleoside, which was determined as 6,5'-O-cyclo-5-methylthio-2',3'-O-isopropylidenetubercidin (10). Deacetonation of 10 gave the free cyclotubercidin (11). Oxidation of 10 with permanganate gave the 5-methylsulfone (12) and deprotection of 12 also gave the free 6,5'-O-cyclo-5-methylsulfonyltubercidin (13).

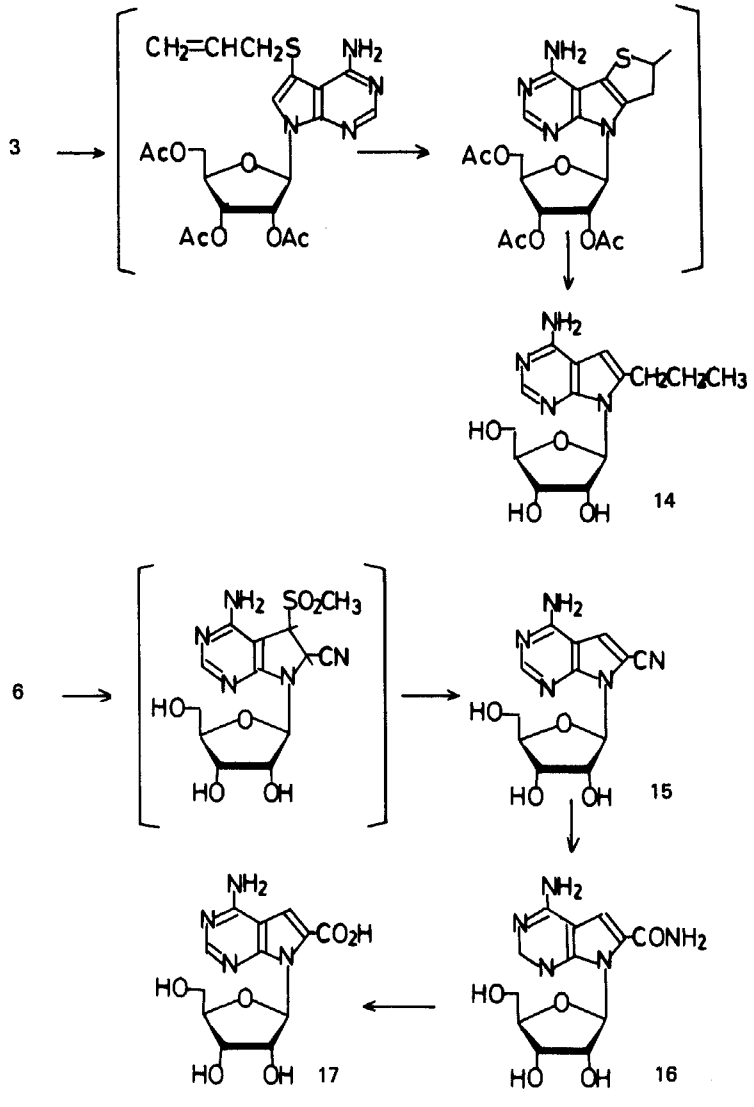
The spectrometric properties of 10-13 are consistent with the 5'-O-cyclo structures. If the initial substitution of tubercidin with thiocyanogen chloride was at the 6-position, then the resultant bromination should be at 5, and the cyclonucleoside would have been 5-bromo-6,5'-O-cyclotubercidin, an already documented derivative.[7]

The sulfur function of the 5-position of tubercidin was utilized for the introduction of a carbon unit to the base



moiety in a manner corresponding to the thio-Claisen rearrangement of 6-allylthiouridine to 5-propyluridine.[10]

Treatment of 3 with 2-mercaptoethanol and allyl bromide gave a complex mixture, consisting of 5-allylthio derivative and already rearranged intermediates. The mixture was refluxed in benzene and the product was desulfurized with Raney Ni and then deacetylated to afford 6-propyltubercidin (14, Scheme 2).



The nucleophilic substitution of the methylsulfonyl group of 6 with cyanide ion was also attempted. Treatment of 6 with sodium cyanide in dimethylformamide at 0° gave a product in high yield containing a cyano group. The physical properties, however, were different from those of toyocamycin[3,4] and the substitution went through the addition of a cyanide ion to the 6-position and elimination of methanesulfinic acid to furnish 6-cyanotubercidin (15). Similar substitution had been found in the reaction of a 5-bromouridine with cyanide ion,

giving 6-cyanouridine.[11] In the latter case the further addition-elimination of cyanide ion affording 5-cyanouridine was not observed with 6-cyanotubercidin. The 6-cyano group of 15 was hydrolyzed to give 6-carbamoyltubercidin and 6-carboxytubercidin, respectively (16 and 17).

The introduction of carbon units into the 5-position of tubercidin was thus not realized by the reactions described in the present paper, but the direct introduction has been achieved by the morpholinomethylation of tubercidin and this will be reported separately.[12]

EXPERIMENTAL

UV spectra were measured on a Shimadzu UV-300 spectrophotometer. IR spectra were taken on a Hitachi 215 spectrophotometer. Mass spectra were taken on a JEOL JMS-D 300 mass spectrometer or JMS-Q 10A spectrometer at 70 eV. NMR spectra were taken on a JEOL JNM-FX 100 FT spectrometer with tetramethylsilane as an internal standard. Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and were uncorrected.

2',3',5'-Tri-O-acetyltubercidin (2)---- Tubercidin (10 g, supplied from Kaken Kagaku Co. Ltd.) was suspended in 100 mL of pyridine. Acetic anhydride (20 mL) was added to the ice cooled suspension and it was stirred overnight at room temperature. To the solution was added 20 mL of MeOH and the solvent was evaporated *in vacuo*. The residue was taken up in 300 mL of MeOH and the solution was refluxed for 24 hr. After evaporation of the solvent the residue was partitioned with $CHCl_3$ and H_2O and the organic layer was dried over Na_2SO_4 and evaporated to leave 14.33 g (97%) of 2 as a non-crystalline powder. UV: λmax (H_2O) 269 nm, λmin (H_2O) 245 nm. This was used for the next step without purification.

2',3',5'-Tri-O-acetyl-5-thiocyanatotubercidin (3)---- Chlorine gas was absorbed into 100 mL of AcOH at 10° until 7.65 g of the gas was dissolved. To this solution was added KSCN (11.52 g) with stirring for 30 min at room temperature. Compound 2 (5 g, 12.8 mmol) was added to the solution and

after 3.5 hr 11 mL of cyclohexene was added to exhaust excess ClSCN. After removal of the insoluble material by filtration, the filtrate was concentrated and the residue was taken up in $CHCl_3$ and H_2O, neutralized with $NaHCO_3$ and the organic layer was concentrated to a small volume and applied to a column of silica gel (150 g, 4.8x18 cm). The column was washed with $CHCl_3$ and eluted with $CHCl_3$-MeOH (100:1) to give 4.73 g (83%) of crude 3. Crystallization from EtOH gave pure 3, mp 169-169.5°. UV λmax (H_2O): 276 nm, λmin: 245 nm. IR ($CHCl_3$): 2170 cm^{-1} (SCN). NMR ($CDCl_3$) δppm: 8.38 (s, 1, H-2), 7.56 (s, 1, H-6), 6.35 (d, 1, H-1', J= 4.8 Hz), 6.14 (broad s, 2, NH_2), 5.82-5.46 (m, 2, H-2',3'), 4.38 (broad s, 3, H-4',5'), 2.14, 2.11, 2.05 (s each, 9, Ac_3). Ms m/e: 449 (M^+), 259 (sugar), 191 (B+1), 139 (sugar -2AcOH). Anal. Calcd. for $C_{18}H_{19}N_5O_7S$: C, 48.10; H, 4.26; N, 15.58; S, 7.13. Found: C, 48.16; H, 4.19; N, 15.58; S, 6.97.

2',3',5'-Tri-O-acetyl-5-methylthiotubercidin (4)---- Compound 3 (5.45 g) was dissolved in 60 mL of EtOH and 2-mercaptoethanol (9.1 mL) was added to the solution. After stirring for 1 hr CH_3I (0.79 mL) and $NaHCO_3$ (1.03 g, dissolved in H_2O) were added to the solution and stirring was continued for 1 hr. The solvent was removed in vacuo and the residue was partitioned with $CHCl_3$ and H_2O and the organic layer was concentrated to leave 4.81 g (89%) of crude 4. UV λmax (H_2O): 277 nm, λmin (H_2O): 246 nm. λmax (pH 2): 288, 255, 229 nm, λmin (pH 2): 266, 248, 219 nm. NMR ($CDCl_3$) δ ppm: 8.30 (s, 1, H-2), 7.22 (s, 1, H-6), 6.40 (d, 1, H-1', J= 5.6 Hz), 5.98 (broad s, 2, NH_2), 5.69 (dd, 1, H-2'), 5.56 (m, 1, H-3'), 4.37 (broad s, 3, H-4',5'), 2.39 (s, 3, SCH_3), 2.17, 2.14, 2.06 (s each, 9, Ac_3). Ms m/e: 438 (M^+), 259 (sugar), 180 (B+1), 139 (sugar -2AcOH).

5-Methylthiotubercidin (5)---- Compound 4 (2.78 g, 6.35 mmol) and NaOMe (1.19 g) were dissolved in abs. MeOH (100 mL) and the solution was stirred overnight at room temperature. The solution was neutralized with Amberlite IR 120B (H^+ form) resin and the neutralized solution was concentrated and mixed with 15 g of silica gel. The mixture was dried in vacuo and

the residue was applied to a column of silica gel (30 g, 3.8 x 7 cm) with $CHCl_3$ as a solvent. The column was washed with $CHCl_3$ and $CHCl_3$-MeOH (100:4), and eluted with $CHCl_3$-MeOH (100:8) to give 1.33 g (67%) of 5, which was crystallized from MeOH, mp 99.5-101°. UV λmax (H_2O): 278 nm (ε, 9000), λmin: 251 nm (ε, 6400). λmax (0.1 N HCl): 283 nm (ε, 7900), 233 nm (ε, 15900), λmin (0.1 N HCl): 268 nm (ε, 7700). NMR (DMSO-d_6) δ ppm: 8.10 (s, 1, H-2), 7.60 (s, 1, H-6), 6.85 (broad s, 2, NH_2), 6.01 (d, 1, H-1'), 5.36-5.04 (m, 3, HO-2',3',5'), 4.39 (dd, 1, H-2'), 4.08 (dd, 1, H-3'), 3.89 (dd, 1, H-4'), 3.60 (broad s, 2, H-5'), 2.35 (s, 3, SCH_3). Anal. Calcd. for $C_{12}H_{16}N_4O_4S$: C, 46.14; H, 5.16; N, 17.94; S, 10.27. Found: C, 46.00; H, 5.16; N, 17.93; S, 10.52.

5-Methylsulfonyltubercidin (6)---- [A] Compound 5 (2.06g, 6.60 mmol) was dissolved in 80 mL of AcOH and 1.55 g of $KMnO_4$ was added to the ice cooled solution. After stirring the mixture for 15 min, 30% H_2O_2 was added until the mixture became colorless. The solvent was removed in vacuo and the residue was taken up in H_2O and evaporated again. The evaporation was repeated several times to remove AcOH and the final residue was dissolved in hot water. On standing 1.31 g (58%) of 6 was deposited as colorless crystals, mp 223.5-227°. UV λmax (H_2O): 274 nm (ε, 14000), λmin: 242 nm (ε, 3100). λmax (0.1 N HCl): 270 nm (ε, 12200), λmin: 244 nm (ε, 4100). NMR (DMSO-d_6) δ ppm: 8.28 (s, 1, H-6), 8.23 (s, 1, H-2), 7.24 (broad s, 2, NH_2), 6.11 (d, 1, H-1'), 5.60-4.92 (m, 3, HO-2',3',5'), 4.40 (dd, 1, H-2'), 4.14 (dd, 1, H-3'), 3.96 (dd, 1, H-4'), 3.66 (broad s, 2, H-5'), 3.28 (s, 3, SO_2CH_3, measured on addition of D_2O). Anal. Calcd. for $C_{12}H_{16}N_4O_6S \cdot 1/2\ H_2O$: C, 40.79; H, 4.85; N, 15.86; S, 9.07. Found: C, 40.59; H, 4.98; N, 15.77; S, 9.15. ----[B] Compound 7 (0.8 g) was dissolved in 30 mL of abs. MeOH containing 0.33 g of NaOMe and kept overnight at room temperature. After neutralization of the solution with Amberlite IR 120A resin (H^+ form), the solvent was evaporated to leave 0.67 g of 6.

2',3'-O-Isopropylidene-5-methylthiotubercidin (8)---- Compound 5 (0.8 g) was dissolved in 100 mL of acetone containing 0.5 mL of 70% $HClO_4$ and the solution was stirred for 5 hr.

After neutralization of the solution with K_2CO_3 powder, the precipitate was filtered off and the filtrate was concentrated, the residue was partitioned with $CHCl_3$ and H_2O, and the organic layer was concentrated to leave 4.87 g (85%) of 8 as a powder. This was used directly without purification.

2',3',5'-Tri-O-acetyl-5-methylsulfonyltubercidin (7)---- Compound 4 (2.81 g) was dissolved in 70 mL of 90% AcOH and 1.8 g of $KMnO_4$ was added to the solution under ice-cooling, and the mixture was stirred for 1.5 hr. To the mixture was added 30% H_2O_2 until it became colorless. After evaporation of the solvent the residue was taken up in $CHCl_3$-H_2O containing $NaHCO_3$ and the organic layer was separated, dried over Na_2SO_4, and evaporated to leave 2.53 g (84%) of 7 as a powder. UV λmax (H_2O): 274 nm. λmin 247 nm. NMR ($CDCl_3$)δppm: 8.34 (s, 1, H-2), 7.89 (s, 1, H-6), 6.42 (d, 1, H-1', J= 4.6 Hz), 6.40 (broad s, 2, NH_2), 5.68 (dd, 1, H-2'), 5.54 (dd, 1, H-3'), 4.41 (broad s, 3, H-4',5'), 3.20 (s, 3, SO_2CH_3), 2.22, 2.14, 2.09 (s each, 9, Ac_3). Ms m/e: 470 (M^+), 223 (B+2), 222 (B+1), 259 (sugar), 139 (sugar -2AcOH).

2',3'-O-Isopropylidene-6-bromo-5-methylthiotubercidin (9) ---- Compound 8 (0.81 g) and N-bromosuccinimide (0.36 g) were dissolved in 15 mL of CH_2Cl_2 and the solution was stirred for 1.5 hr at room temperature. The solution was washed with aqueous $NaHCO_3$, H_2O, and concentrated to a small volume. This was applied to a silica gel column and the product was eluted with $CHCl_3$-MeOH (10:1). On evaporation of the solvent 0.98 g of 9 was obtained. Ms m/e: 432, 430 (M^+), 417, 415 (M-15), 402, 400 (M-30), 260-258 (B+1), 245, 243 (B+1-15). NMR ($CDCl_3$) δppm: 8.21 (s, 1, H-2), 6.18 (d, 1, H-1', J= 5.4 Hz), 6.10 broad s, 2, NH_2), 5.36 (t, 1, H-2'), 5.10 (d, 1, H-3'), 4.50 (broad s, 1, HO-5'), 3.90 (m, 3, H-4',5'), 2.34 (s, 3,SCH_3), 1.68, 1.38 (s each, 6, CMe_2).

6,5'-O-Cyclo-6-hydroxy-2',3'-O-isopropylidene-5-methylthiotubercidin (10)---- A mixture of 9 (2.66 g, 6.17 mmol) and 60 % NaH (0.74 g) in 15 mL of dimethylformamide was kept at 110° for 30 min under stirring. The solvent was removed *in vacuo* and the residue was taken in $CHCl_3$-H_2O and neutra-

lized with dil. HCl. The organic layer was dried over Na_2SO_4 and concentrated and applied to a column of silica gel (90 g, 3.8x20 cm). The column was washed with $CHCl_3$ and the product was eluted with $CHCl_3$-MeOH (100:1). On evaporation of the solvent 1.11 g of 10 (51%) was obtained as a powder. UV λmax (H_2O): 280 nm, λmin: 250 nm. λmax (0.1 N HCl): 285 nm, λmin 255 nm (plateau). NMR ($CDCl_3$) δ ppm: 8.26 (s, 1, H-2), 6.62 (s, 1, H-1'), 5.86 (broad s, 2, NH_2), 5.09 (d, 1, H-2', J= 5.6 Hz), 4.81 (d, 1, H-3'), 4.66 (broad s, 1, H-4'), 4.56 (d, 1, H-5'a, J_{gem}= 12.2 Hz), 4.10 (d, 1, H-5'b), 2.32 (s, 3, SCH_3), 1.57, 1.37 (s each, 6, CMe_2). Ms m/e: 350 (M^+), 335 (M-15), 195 ($C_7H_7N_4OS^+$), 181 ($C_6H_5N_4OS^+$).

6,5'-O-Cyclo-6-hydroxy-5-methylthiotubercidin (11)---- Compound 10 (0.40 g) was heated in 50 % HCO_2H at 100° for 8 hr. After evaporation of the solvent the residue was applied to a silica gel column. The column was washed with $CHCl_3$ and the product was eluted with $CHCl_3$-MeOH (100:8). The solvent was removed and the residue was crystallized from EtOH to give 170 mg (41%) of 11, mp 242-246° (dec.). UV λmax (H_2O): 280 nm (ε, 11600), λmin: 249 nm (ε, 4900), λmax (0.1N HCl): 286 nm (ε, 10100), λmin: 260 nm (ε, 5800). NMR (DMSO-d_6) δ ppm: 8.10 (s, 1, H-2), 6.74 (braod s, 2, NH_2), 6.19 (s, 1, H-1'), 5.58 (d, 1, HO), 5.26 (d, 1, HO), 4.63 (dd, 1, H-5'a, $J_{5'a,4'}$ = 2.0 Hz, J_{gem}= 12.7 Hz), 4.50 (broad s, 1, H-4'), 4,44 (t, 1, H-2'), 4.25 (t, 1, H-3'), 3.87 (d, 1, H-5'b), 2.50 (s, 3, SCH_3). Anal. Calcd. for $C_{12}H_{14}N_4O_4S$: C, 46.44; H, 4.55; N, 18.06; S, 10.33. Found: C, 46.18; H, 4.42; N, 17.98; S,10.33.

6,5'-O-Cyclo-6-hydroxy-2',3'-O-isopropylidene-5-methylsulfonyltubercidin (12)---- Compound 10 (0.74 g) was oxidized with 0.64 g of $KMnO_4$ in 90% AcOH by a similar procedure as described in the preparation of 7. The product 12 was obtained as a powder (0.48 g, 62%). UV λmax (H_2O): 275 nm,λmin: 243 nm. NMR ($CDCl_3$) δ ppm: 8.31 (s, 1, H-2), 6.70 (s, 1, H-1'), 7.10-5.70 (broad, 2, NH_2), 5.09 (d, 1, H-2', J= 5.6 Hz), 4.82 (d, 1, H-3'), 4.72 (broad s, 1, H-4'), 4.65 (d, 1, H-5'a, J_{gem}= 12.0 Hz), 4.17 (d, 1, H-5'b), 3.22 (s, 3, SO_2CH_3), 1.57, 1.37 (s each, 6, CMe_2). Ms m/e: 382 (M^+), 367 (M-15), 228 ($C_7H_8N_4O_3S^+$), 227 ($C_7H_7N_4O_3S^+$).

6,5'-O-Cyclo-6-hydroxy-5-methylsulfonyltubercidin (13) ---- Compound 12 (477 mg) in 50 % HCO_2H was heated overnight at 100°. After evaporation of the solvent and purification of the residue through a silica gel column, 13 (167 mg, 39%) was obtained as crystals from EtOH, mp 149-152.5°. UV λmax (H_2O): 275 nm (ε, 16000), λmin: 242 nm (ε, 3600). λmax (0.1N HCl): 274 nm (ε, 14500), λmin: 245 nm (ε, 3600). NMR (DMSO-d_6) δ ppm: 8.19 (s, 1, H-2), 7.29 (broad s, 2, NH_2), 6.29 (broad s, 1, H-1'), 5.63 (d, 1, HO), 5.35 (d, 1, HO), 4.75 (dd, 1, H-5'a, $J_{5'a,4'}$= 2.0 Hz, J_{gem}= 12.9 Hz), 4.57 (broad s, 1, H-4'), 4.44 (t, 1, H-2'), 4.20 (t, 1, H-3'), 4.12 (d, 1, H-5'b), 3.29 (s, 3, SO_2CH_3). Anal. Calcd. for $C_{12}H_{14}N_4O_6S \cdot H_2O$: C, 40.00; H, 4.48; N, 15.55; S, 8.90. Found: C, 39.93; H, 4.30; N, 15.50; S, 8.63.

6-Propyltubercidin (14)---- Compound 3 (0.98 g) in 10 mL of EtOH was treated with 1.55 mL of 2-mercaptoethanol for 30 min at room temperature. To the solution was added an aqueous solution of 180 mg of $NaHCO_3$ and 0.21 mL of allyl bromide and the mixture was stirred for 30 min. The solvent was removed in vacuo and the residue was taken up in benzene (5 mL), which was heated under reflux for 2 hr. The solvent was evaporated and the residue was dissolved in 30 % MeOH (10 mL). Freshly prepared Raney Ni catalyst (wet 1 g) was added to the solution and the mixture was refluxed for 4 hr. After removal of the Ni catalyst by filtration the filtrate was concentrated and the residue was taken up in abs. MeOH containing NaOMe and kept at room temperature for 4 hr. The solution was neutralized with Dowex-50 (H^+ form) resin and the filtrate was concentrated. The residue was mixed with 3 g of silica gel and the mixture was dried in vacuo. The silica gel was placed on a silica gel column (10 g, 2.8x6 cm) with $CHCl_3$ and the column was eluted with $CHCl_3$-MeOH (100:4-8). On evaporation of the eluate 14 was obtained (0.42 g, 63%), which was crystallized from EtOH, mp 153.5-155.5°. UV λmax (H_2O): 279 nm (ε, 12200), λmin: 245 nm (ε, 3000); λmax (0.1 N HCl): 280 nm (ε, 11500), 233 nm (ε, 19100); λmin: 251 nm (ε, 3600), 218 nm (ε, 12300). NMR (DMSO-d_6) δ ppm: 7.95 (s, 1, H-2),

7.02 (broad s, 2, NH_2), 6.34 (s, 1, H-5), 5.67 (d, 1, H-1', J≒ 6.8 Hz), 5.26-4.78 (m, 4, H-2' and HO-2',3',5'), 4.13 (broad s, 1, H-3'), 3.96 (broad s, 1, H-4'), 3.62 (broad s, 2, H-5'), 2.69 (t, 2, CH_2-5), 1.69 (q, 2, $-CH_2-$), 0.99 (t, 3, $-CH_3$). Anal. Calcd. for $C_{14}H_{20}N_4O_4$: C, 54.53; H, 6.54; N, 18.17. Found: C, 54.23; H, 6.67; N, 18.08.

6-Cyanotubercidin (15)---- Compound 6 (300 mg) was dissolved in 5 mL of dimethylformamide and was heated with 250 mg of NaCN at 100° overnight. The solvent was removed in vacuo and the residue was taken up in H_2O, neutralized with 1 N HCl and the solvent was evaporated. The residue was taken up in EtOH, the insoluble material was filtered off, and the filtrate was mixed with 1.2 g of silica gel. The gel was dried in vacuo and was placed on a silica gel column (3 g, 1.8x 4 cm). Elution with $CHCl_3$-MeOH (100:4-8) gave 15, which was crystallized from H_2O (217 mg, 75%), mp 243-245°. UV λmax (H_2O):307 nm (ε, 16400), 296 nm (ε, 18500); λmin: 303 nm (ε, 16200), 251 nm (ε, 2700); λmax (0.1 N HCl): 298 nm (ε, 15400), 287 nm (ε, 19600), 278 nm (ε, 16800, sh); λmin: 295 nm (ε, 14900), 253 nm (ε, 4400). IR (KBr): 2230 cm^{-1} (-CN). NMR (DMSO-d_6) δ ppm:8.20 (s, 1, H-2), 7.73 (broad s, 2, NH_2), 7.56 (s, 1, H-5), 5.97 (d, 1, H-1', J= 7.1 Hz), 5.47-5.21 (m, 3, HO-2', 3',5'), 4.79 (dd, 1, H-2'), 4.10 (m, 1, H-3'), 3.97 (broad s, 1, H-4'), 3.59 (m, 2, H-5'). Anal. Calcd. for $C_{12}H_{13}N_5O_4 \cdot 1/2H_2O$: C, 47.99; H, 4.70; N, 23.32. Found: C, 47.72; H, 4.77; N, 23.18.

6-Carbamoyltubercidin (16)---- Compound 15 (97 mg) in 5 mL of MeOH and 6 mL of 0.1 N NaOH was heated at 70° for 17 hr. The solution was neutralized with Amberlite IR-120B (H^+ form) resin and evaporated. The residue in MeOH was applied to a preparative TLC plate (silica gel) and the plate was developed with iPrOH-H_2O-NH_4OH (85: 10: 1.3, v/v). The band around Rf 0.5 was collected and extracted with EtOH. The product was crystallized from aqueous EtOH to give 44 mg (43%) of 16, mp 256.5-258° (dec.). UV λmax (H_2O): 295 nm (ε, 15600), λmin: 253 nm (ε, 3400). λmax (0.1 N HCl): 286 nm (ε, 16600); λmin: 252 nm (ε, 4600). NMR (DMSO-d_6) δ ppm: 8.07 (s, 1, H-2),

8.01 (broad s, 1, $CONH_a$), 7.41 (broad s, 3, NH_2 and $CONH_b$), 7.07 (s, 1, H-5), 6.50 (d, 1, H-1', J= 6.3 Hz), 5.86 (dd, 1, HO), 5.12-4.82 (m, 3, HOx2, H-2'), 4.17 (m, 1, H-3'), 3.91 (broad s, 1, H-4'), 3.83 (m, 2, H-5'). Anal. Calcd. for $C_{12}H_{15}N_5O_5 \cdot 1/4EtOH$: C, 46.79; H, 5.17; N, 21. 83. Found: C, 46.58; H, 4.90; N, 21.79.

6-Carboxytubercidin (17)---- From the above TLC plate the band at Rf 0.2 was collected and extracted with EtOH to give 33 mg (32%) of 17. Crystallization from aqueous MeOH gave pure 17, mp 243° (dec.). UV λmax (H_2O): 289 nm (ε, 15100), λmin: 253 nm (ε, 3700). λmax (0.1 N HCl): 305 nm (ε, 12600, sh), 292 nm (ε, 17800), 231 nm (ε, 14900, sh); λmin: 255 nm (ε, 3500). λmax (0.1 N NaOH): 309 nm (ε, 10100, sh), 296 nm (ε, 15400); λmin: 253 nm (ε, 3500). NMR (DMSO-d_6) δ ppm: 8.10 (s, 1, H-2), 7.57 (broad s, 2, NH_2) 7.49 (s, 1, H-5), 6.79 (d, 1, H-1', J= 6.6 Hz), 5.73 (broad s, 1, HO), 4.99 (m, 3, HOx2, H-2'), 4.17 (broad s, 1, H-3'), 3.90 (broad s, 1, H-4'), 3.63 (broad s, 2, H-5'). Anal. Calcd. for $C_{12}H_{14}N_4O_6 \cdot 1/2H_2O$: C, 45.14; H, 4.74; N, 17.55. Found: C, 44.93; H, 4.59; N, 17.36.

REFERENCES AND NOTES

1. Part 40: K. Miura, T. Iwano, S. Tsuda, T. Ueda, F. Harada, and N. Kato, Chem. Pharm. Bull. (Tokyo), 30, in press. (1982).
2. S. Suzuki and S. Marumo, J. Antibiotics, 14A, 34 (1961).
3. For general discussions see: R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley Interscience, New York, 1970.
4. K. Ohkuma, J. Antibiotics, 14A, 343 (1961).
5. a. T. Nagamachi, P. F. Torrence, J. A. Waters, and B. Witkop, J. Chem. Soc. Chem. Commun., 1025 (1972). b. T. Nagamachi, J. -L. Fourrey, P. F. Torrence, J. A. Waters, and B. Witkop, J. Med. Chem., 17, 403 (1974).
6. T. Ueda, S. Watanabe, and A. Matsuda, J. Carbohydrates. Nucleosides. Nucleotides, 5, 523 (1878).
7. K. Anzai and M. Matsui, Bull. Chem. Soc. Japan, 47, 417 (1974).

8, D. E. Bergstrom and A. J. Brattesani, Nucleic Acids Res., 8, 6213 (1980).

9. 8,5'-O-Cyclization of 2',3'-O-isopropylidene-8-bromo-adenosine by treatment with sodium hydride has been reported: M. Ikehara, M. Kaneko, and R. Okano, J. Am. Chem Soc., 90, 497 (1968), idem., Tetrahedron, 26, 5675 (1970).

10. H. Inoue, M. Takada, M. Takahashi, and T. Ueda, Heterocycles, 8, 427 (1977).

11. H. Inoue and T. Ueda, Chem. Pharm. Bull. (Tokyo), 26, 2657 (1978), and references therein.

12. A preliminary result including the present study has appeared: S. Watanabe and T. Ueda, Nucleic Acids. Res., Symposium Series, No. 8, s21 (1980).

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