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# Tubercidin. Its Conversion into 5′-Deoxytubercidin

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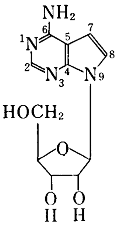
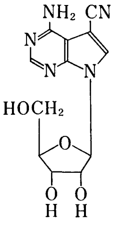
Tubercidin (I) was converted into 5′-deoxytubercidin (IV). Mesylation of 2′,3′-*O*-isopropylidenetubercidin (IX) afforded-2′,3′-*O*-isopropylidene-5′-*O*-mesyltubercidin (XVIII), which *in situ* was treated with benzoyl chloride. The product, $N^6$,$N^6$-dibenzoyl-2′,3′-*O*-isopropylidene-5′-*O*-mesyltubercidin (XXII), was converted into $N^6$,$N^6$-dibenzoyl-5′-deoxy-5′-iodo-2′,3′-*O*-isopropylidenetubercidin (XXIV). Catalytic hydrogenation of XXIV afforded $N^6$-benzoyl-5′-deoxy-2′,3′-*O*-isopropylidenetubercidin (XXVII). 5′-Deoxytubercidin (IV) was obtained on debenzoylation and deacetonation of XXVII.

Tubercidin (4-amino-7-(D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine)[1,2] (I)[3] was first isolated by Anzai *et al.*[4] from the fermentation broth of *Streptomyces tubercidicus*[5] in 1957. It has selective bactericidal activities against *Mycobacterium tuberculosis* of human and bovine types[4] as well as high activities against NF mouse sarcoma[4] and some experimental tumors.[6] Its clinical use was examined for cancer patients, and was shown to be highly toxic.

Several works[7] were directed to the total synthesis of I and related antibiotics including toyocamycin (II)[8] and sangivamycin (III),[9] both of which were also reported to have high activities against experimental tumors.



I



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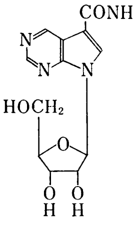

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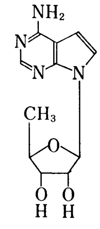

IV

In 1969 Tolman *et al.* succeeded in the synthesis of these three compounds.[10] They as well as others synthesized derivatives of tubercidin whose pyrro[2,3-*d*]-pyrimidine moiety was subjected to various substitution.[11] Phosphorylated derivatives of tubercidin, *i.e.* tubercidin-5′-phosphate,[12,15] dan tubercidin-3′,5′-cyclophosphate,[13] and the oligonucleotide analogues[14] were also synthesized, and their biological activities were investigated.[15] However, the modification of the ribose moiety has never been reported, and our investigation has been undertaken along this line. In this paper the conversion of I to 5′-deoxytubercidin (IV) will be described.

5′-Deoxyadenosine has been synthesized from adenosine[16] through a route involving catalytic reduction of $N^6$-acetyl-5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneadenosine (V) which was synthesized from 2′,3′-*O*-isopropylidene-5′-*O*-tosyladenosine (VI)[17] by treatment with sodium iodide in acetic anhydride.

We followed this experiment and found that the yield of V was moderate (58%), but that of VI was good (90%). The iodide substitution reaction of VI yielding V is likely to compete with the formation of the cyclonucleoside VII.

Synthesis of the tubercidin analogue of V was attempted. Under the same conditions as applied to

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[1] S. Suzuki and S. Marumo, *J. Antibiot.* (Tokyo), *Ser. A*, **13**, 360 (1960); *ibid.*, **14**, 34 (1961).

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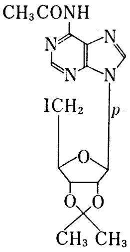
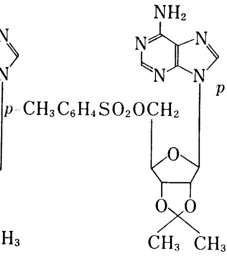
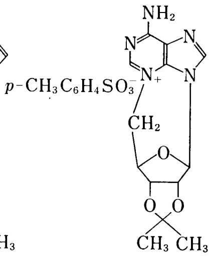


V



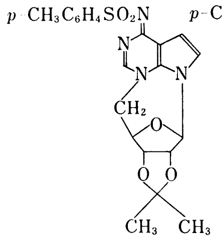
VI



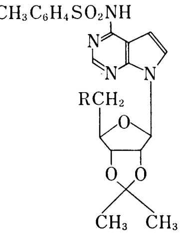
VII

the synthesis of VI 2',3'-*O*-isopropylidenetubercidin (IX)[18] was converted into 2',3'-*O*-isopropylidene-5'-*O*-tosyltubercidin (VIII) in a 76% yield.[20] However, contrary to expectation, the following treatment of VIII with sodium iodide and acetic anhydride afforded cyclonucleoside X[21] as the main product and the tosyl iodo compound XVII as a minor one. None of the acetyl iodo compound corresponding to V was obtained.

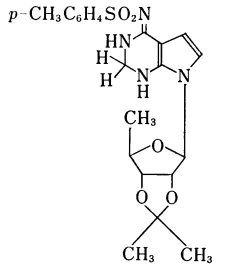
With excess tosyl chloride and at a higher temperature, cyclonucleoside XI, *N*$^6$-tosylate XII and *N*$^6$,5'-*O*-ditosylate XIII were obtained in low yields, X being still the predominant product.



XI



XII R=OH
XIII R=$p$-$CH_3C_6H_4SO_3$



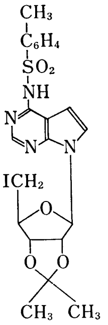
XV

Facile transformation of VIII into X was confirmed by storing VIII in a desiccator at room temperature for 2 days to give a mixture of VIII and X (28: 72), *i.e.*, VIII is transformed easily into X even in the solid state. This is in contrast with the fact that 38% of VI is transformed into VII under the same conditions.

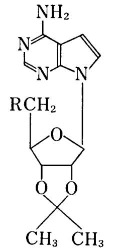
Thus, another route to IV should be explored on account of the lability of VIII.

Tosylate XIII was reduced with lithium aluminium hydride in tetrahydrofuran, XIV being expected to be produced in one step. However, the main product was a deoxydihydro derivative XV, XIV being obtained only as a minor one.

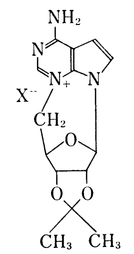
The dihydro compound XV lacks a NMR signal corresponding to the hydrogen at C-2 of tubercidin and shows a doublet at δ 1.30 assignable to the methyl group.



XVII

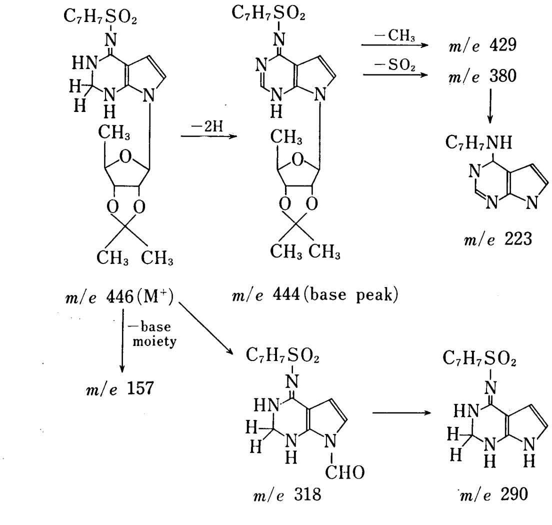


VIII R=$p$-$CH_3C_6H_4SO_3$
IX R=OH
XIV R=H
XVI R=$CH_3CO_2$
XVIII R=$CH_3SO_3$



X X=$p$-$CH_3C_6H_4SO_3$
XIX X=$CH_3SO_3$

In the mass spectrum the molecular ion was observed at *m/e* 446 and the base peak at $M^+-2$. Further fragmentations schematically shown in Chart 1 are also consistent in structure with that proposed for XV.



Treatment of XIV with dilute hydrochloric acid afforded IV, the NMR spectrum of which exhibited a three proton doublet at δ 1.28 assignable to the methyl group. The low yield of IV (1% from IX) in this procedure is, as mentioned above, attributable to the susceptibility of VIII to form X.

To prevent cyclonucleoside formation an attempt was made to protect the amino group of IX prior to *O*-tosylation. Ketal IX was treated with acetic anhydride in methanol. The reaction could not be brought to completion, and the product which was, unexpectedly, 5'-*O*-acetyl-2',3'-*O*-isopropylidenetubercidin (XVI) was obtained in a low yield.

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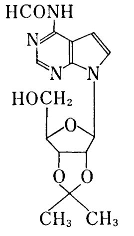
[20] Tosylation was carried out at −15°C to −20°C as reported[17] for the synthesis of VI, though Mizuno *et al.* obtained VIII at a higher temperature (0°C) in a 30% yield.

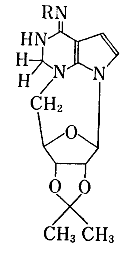
[21] Though the reported UV spectrum, $\lambda_{max}^{EtOH}$ 281 mμ (ε 11.7 × $10^3$), and that measured by the authors, $\lambda_{max}^{EtOH}$ 293 (ε 11.2 × $10^3$), 275 (shoulder, ε 10.0 × $10^3$) and 214 mμ (ε 22.7 × $10^3$) differ, analytical data and other properties show that both samples are the same.



XXVIII

An attempt to prepare the $N^6$-dimethylaminomethylene derivative[19] using dimethylformamide dimethyl acetal as a reagent was also unsuccessful, and $N^6$-formyl-2′,3′-*O*-isopropylidenetubercidin (XXVIII) was obtained in a low yield.

2′,3′-*O*-Isopropylidene-5′-*O*-mesyltubercidin (XVIII) was treated with sodium iodide and acetic anhydride. Here again the formation of the cyclonucleoside XIX was predominant and XVIII was also converted into XIX easily on being left in a desiccator. Excess mesyl chloride afforded no $N^6$-mesyl compound.



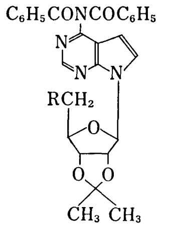
XX R=$CH_3SO_2$
XXI R=$p$-$CH_3C_6H_4SO_2$

Reduction of XIX with sodium borohydride in an aqueous solution was investigated, the nucleophilic attack at C-5′ of hydride anion being expected to afford the deoxy compound XIV. However, the product was intractable gummy material, and the dihydro cyclonucleoside XX was isolated from the reaction mixture in a low yield. The NMR spectrum of XX shows that two protons at C-5′ as well as those at C-2 are unequivalent. The mass spectrum is also consistent with the proposed structure, and the fragmentations are characterized by the cleavage of 2H, $CH_3$, CHO, -NH-$CH_2$-, -$CH_2$CHO-, and $CH_3SO_2$ from the molecular ion.

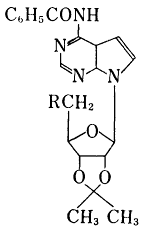
Similarly, X was reduced with sodium borohydride to give $N^6$-tosyl cyclonucleoside XXI. The close similarity of the UV spectrum of XXI to that of XV also supports the structural assignment.

Finally the conversion of tubercidin into 5′-deoxytubercidin was accomplished in the following manner. After mesylation of IX was carried out in pyridine at 0°C, excess benzoyl chloride was added to the reaction mixture without isolation of XVIII. $N^6$,$N^6$-Dibenzoyl-2′,3′-*O*-isopropylidene-5′-*O*-methanesulfonyltubercidin (XXII) was obtained along with a small amount of the tribenzoyl compound XXIII.

Compound XXII was treated with sodium iodide in methyl ethyl ketone to give three products. The main product was $N^6$,$N^6$-dibenzoyl-5′-deoxy-5′-iodo-2′,3′-*O*-isopropylidenetubercidin (XXIV), the monobenzoyl iodo compound XXV being obtained as a minor one. Another crystalline product was proved to have the molecular formula $C_{21}H_{21}N_3O_6$.[22]



XXII R=$CH_3SO$
XXIII R=$C_6H_5CO_2$
XXIV R=I
XXVI R=H



XXV R=I
XXVII R=H

Catalytic hydrogenation of XXIV afforded $N^6$,$N^6$-dibenzoyl-5′-deoxy-2′,3′-*O*-isopropylidenetubercidin (XXVI) and $N^6$-benzoyl-5′-deoxy-2′,3′-*O*-isopropylidenetubercidin (XXVII), the latter being the predominant product. Treatment of XXVII with dilute sodium hydroxide afforded 5′-deoxy-2′,3′-*O*-isopropylidenetubercidin (XIV), and the isopropylidene group of XIV was removed by heating in 80% aqueous acetic acid. Thus, tubercidin was converted into 5′-deoxytubercidin.

## Experimental

*2′,3′-O-Isopropylidene-5′-O-p-toluenesulfonyltubercidin (VIII) and its Cyclonucleoside Formation.* The reaction was carried out under the same conditions as reported by Kuhn and Jahn.[17] Tosyl chloride (500 mg) was added at once to a solution of 2′,3′-*O*-isopropylidenetubercidin (IX) (500 mg) in pyridine (3 ml) cooled at −20°C. The solution was stirred for 2 hrs and was allowed to stand overnight at −15°C to −20°C. Ice-cooled water was added to the solution and the product was extracted with chloroform. The organic layer was washed with cold 2N $H_2SO_4$ and then with water. The solvent was evaporated to dryness below 30°C, affording 598 mg of the residue (yield, 76%). Though the maximum of the UV absorption was initially found at 270 m$\mu$ which is characteristic for the pyrrolo[2,3-*d*]pyrimidine chromophore of tubercidin, it gradually shifted to a higher wavelength on prolonged standing.

The residue was kept in a desiccator for 2 days at room temperature (15—25°C) and was distributed again between chloroform and water. The organic layer was evaporated to dryness below 30°C yielding 157 mg of syrup. Evaporation of the aqueous layer to dryness afforded 433 mg of crystalline solid, which was identical with a sample of the cyclonucleoside X obtained by the method reported by Mizuno *et al.*[2]

These results show that 72% of VIII was converted into X under the above-mentioned conditions.

For comparison 2′,3′-*O*-isopropylideneadenosine (500 mg) was tosylated under the same conditions affording 706 mg of a crude sample of 2′,3′-*O*-isopropylidene-5′-*O*-*p*-toluenesulfonyladenosine (VI) (yield, 90%). The product was kept in a desiccator at room temperature for 2 days and was distributed between chloroform and water. Evaporation of the organic layer as well as the aqueous layer to dryness afforded 430 mg and 266 mg of the residue, respectively, showing that

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[22] The loss of one nitrogen atom seems to be unusual. The NMR spectrum suggests that it is a cyclonucleoside showing characteristic signals of ABX type corresponding to two unequivalent methylene protons at C-5′.

38% of VI was converted into VII.

$N^6$-p-*Toluenesulfonyl-5′-deoxy-5′-iodo-2′,3′-*O*-isopropylidenetubercidin (XVII).* A crude sample of VIII obtained from 2 g of IX was heated at 100°C in acetic anhydride (60 ml) in which sodium iodide (4 g) had been dissolved. After evaporation of acetic anhydride the residue was distributed between chloroform and an aqueous solution of sodium sulfite, and the organic layer was washed repeatedly with water. Column chromatography on silicic acid (benzene: ethyl acetate=1: 1) afforded 41 mg of XVII which was crystallized from ethyl acetate and ligroin. It decomposed gradually at 100—115°C; $\lambda_{\max}^{\text{methanol}}$ 290 m$\mu$ ($\varepsilon$ 19400).

Found: C, 44.77; H, 4.18; N, 9.83, S, 5.62%. Calcd for $C_{21}H_{23}N_4O_5SI$: C, 44.21; H, 4.06; N, 9.62; S, 5.71%.

*Tosylation of 2′,3′-*O*-Isopropylidenetubercidin (IX) at Room Temperature. Isolation of* N-*Tosylated Products.* Tosyl chloride (4.9 g) was added at once to a solution of IX (2.2 g, 0.75 mmol) in pyridine (20 ml). After the solution was left standing at room temperature for 3 days, the products were distributed between an ice-cooled aqueous solution of sodium bicarbonate and ethyl acetate. The organic layer was concentrated to dryness, and the residue was extracted with hot methanol. The methanol insoluble substance was crystallized from dimethylformamide and methanol, and was assigned as 3,5′-cyclonucleoside (XI) of $N^6$-*p*-toluenesulfonyl-2′,3′-*O*-isopropylidenetubercidin; yield, 110 mg; mp >300°C; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 313 (13800) and 220 m$\mu$ (33500); NMR (100 MHz, DMSO-*d*): 1.17, 1.42 (two isopropylidene methyls), 2.32 (s-3H, $\mathrm{C\underline{H}_3C_6H_4SO_2}$-), 4.13, 4.83 (a pair of doublet, 2H, H-2′ and H-3′, $J_{2',3'}=6$ Hz), 4.32 (broad d, 1H, $\mathrm{H_a}$-5′, $J_{\text{gem}}=15$ Hz), 4.6—5.0 (complex, 2H, H-4′ and $\mathrm{H_b}$-5′), 6.33 (s, 1H, H-1′), 6.62, 7.34 (a pair of doublet, 2H, –C$\underline{\mathrm{H}}$=C$\underline{\mathrm{H}}$–N<, $J_{7,8}=4$ Hz), 7.26, 7.80 (a pair of doublet, 4H, $\mathrm{CH_3C_6\underline{H}_4SO_2}$–, $J_{orth}=8$ Hz) and 8.24 ppm (s, 1H, –N=C$\underline{\mathrm{H}}$–N=).

Found: C, 56.95; H, 4.99; N, 12.77; S, 7.17%. Calcd for $C_{21}H_{22}N_4O_5S$: C, 57.00; H, 5.01; N, 12.67; S, 7.25%.

The methanol extract was fractionated with column chromatography on silicic acid, developed with a mixture of benzene and ethyl acetate (1: 1). Two compounds were obtained. One, moving faster and assigned as $N^6$-*p*-toluenesulfonyl-2′,3′-*O*-isopropylidenetubercidin (XII), was crystallized from benzene; yield, 275 mg; mp 106—109°C: $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 291 (16200) and 220 m$\mu$ (18600); $[\alpha]_D^{23}$ −77.5 (*c* 0.48, chloroform).

Found: C, 52.84; H, 5.45; N, 11.72; S, 7.55%. Calcd for $C_{21}H_{24}N_4O_6S \cdot H_2O$: C, 52.70; H, 5.48; N, 11.71; S, 6.70%.

The other compound, assigned as $N^6$,5′-*O*-di-*p*-toluenesulfonyl-2′,3′-*O*-isopropylidenetubercidin (XIII), could hardly be separated from XII, and to obtain an analytical sample chromatography was repeated. It crystallized from benzene; yield, 300 mg; mp 106—109°C; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 291 (19600) and 223 m$\mu$ (35900).

Found: C, 54.73; H, 4.92; N, 9.30; S, 9.72%. Calcd for $C_{28}H_{30}N_4O_8S_2$: C, 54.71; H, 4.92; N, 9.12; S, 10.43%.

Similarity of the UV spectrum of XII with that of XIII shows that XIII is not a *N*,*N*-ditosyl compound.

*Reduction of* $N^6$,*5′-*O*-di-*p*-toluenesulfonyl-2′,3′-*O*-isopropylidenetubercidin (XIII) with* $LiAlH_4$. A solution of XIII (300 mg) in tetrahydrofuran (20 ml) was refluxed with $LiAlH_4$ (300 mg) for 20 hr, and excess of the reagent was decomposed in the usual manner. The products were extracted with ethyl acetate and developed on a column of silicic acid. Two compounds were obtained. The less polar one assigned as 1,2-dihydro-$N^6$-*p*-toluenesulfonyl-5′-*O*-deoxy-2′,3′-*O*-isopropylidenetubercidin (XV) was eluted from the column with a mixture of benzene and ethyl acetate (1: 1) and was obtained as a glass melting at 75—85°C; yield, 90 mg; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 308 (6900), 252 (14200) and 222 m$\mu$ (19800); NMR (60 MHz, $CDCl_3$): 1.30 ppm (d, 3H, $J_{4',5'}=6$ MHz).

Found: C, 56.81; H, 5.74; N, 12.29; S, 6.90%. Calcd for $C_{21}H_{26}N_4O_5S$: C, 56.48; H, 5.87; N, 12.25; S, 7.18%.

The other compound, assigned as 5′-deoxy-2′,3′-*O*-isopropylidenetubercidin (XIV), was eluted from the column with a mixture of ethyl acetate and methanol (19: 1). The spectral data were identical with those of a sample obtained by alkaline hydrolysis of $N^6$-benzoyl-2′,3′-*O*-isopropylidenetubercidin (XXVII).

*Acetylation of 2′,3′-*O*-Isopropylidenetubercidin (IX) in Methanol.* Acetic anhydride was added (2 ml, each time) at 8 hr intervals (three times) at room temperature to a methanolic solution of IX (1 g/10 ml). After 24 hr the solution was poured on an ice-cooled aqueous solution of sodium bicarbonate. The product was extracted with ethyl acetate and separated from the starting material by silicic acid chromatography using a solvent system of ethyl acetate and methanol gradually increasing the content of the latter.

It was assigned as 5′-*O*-acetyl-2′,3′-*O*-isopropylidenetubercidin (XVI), the UV spectrum being similar to that of tubercidin; $\lambda_{\max}^{\text{methanol}}$ 270 m$\mu$ ($\varepsilon$ 11000); M$^+$ 348 ($C_{16}H_{20}N_4O_5$); $\nu_{\max}^{\text{KBr}}$ 1745 cm$^{-1}$ (ester).

Found: C, 55.15; H, 5.93; N, 15.55%. Calcd for $C_{16}H_{20}N_4O_5$: C, 55.16; H, 5.93; N, 16.08%.

*Reaction of 2′,3′-*O*-Isopropylidenetubercidin (IX) with Dimethylformamide Dimethylacetal.* Dimethylformamide dimethyl acetal (0.5 ml) was added at once to a solution of IX (200 mg) in dimethylformamide (5 ml). The solution was heated at 70°C for 60 hr and then left standing at room temperature for 14 hr. After the solvent was removed, the residue, which showed two distinct spots on tlc, was applied to column chromatography on silicic acid. The less polar compound, assigned as $N^6$-formyl-2′,3′-*O*-isopropylidenetubercidin (XXVIII), was eluted from the column with ethyl acetate and obtained as a syrup; yield, 40 mg: Mass: M$^+$ 334.1292 (Calcd for $C_{15}H_{18}N_4O_5$ 334.1278), M$^+$ +CO 362.1196 (Calcd for $C_{16}H_{18}N_4O_6$ 362.1226).

To obtain an analytical sample, fractional precipitation from ethyl acetate and ligroin was effective.

Found: C, 53.54; H, 5.23; N, 15.92%. Calcd for $C_{15}H_{18}N_4O_5$: C, 53.88; H, 5.43; N, 16.76%.

The other compound, eluted with a mixture of ethyl acetate and methanol (9: 1), was found to be the starting material.

*3,5′-Cyclonucleoside Methanesulfonate (XIX) of 2′,3′-*O*-Isopropylidenetubercidin.* Mesyl chloride (1.432 g, 13.2 mmol) was added dropwise to an ice-cooled and stirred solution of IX (1.224 g, 4 mmol) in pyridine (30 ml). After the solution was kept at 0°C overnight, it was poured on an ice-cooled aqueous solution of sodium bicarbonate. The product, 2′,3′-*O*-isopropylidene-5′-*O*-methanesulfonyltubercidin (XVIII), was extracted with ice-cooled chloroform and the organic layer was heated under reflux for 1 hr. The product XIX was hardly soluble in solvents and could be easily separated from XVIII by extraction with water; yield, 1.2 g. To prepare an analytical sample, fractional precipitation from methanol and benzene was effective: mp 188°C; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 293 (11200), 275 (10000) and 214 m$\mu$ (22700).

Found: C, 45.97; H, 5.23; N, 14.24; S, 8.27%. Calcd for $C_{15}H_{20}N_4O_6S \cdot 1/2H_2O$: C, 45.79; H, 5.38; N, 14.24; S, 8.15%.

*Reduction of the Cyclonucleoside XIX with Sodium Borohydride.* $NaBH_4$ (800 mg) was added at once to an aqueous solution of XIX (800 mg/10 ml), and the solution was stirred at room temperature for 6 hr. Extraction with chloroform and evaporation of the solvent afforded 400 mg of the residue, which was then purified with silicic acid chromatography. A compound showing a distinct spot on tlc was obtained. It was assigned as 3,5′-cyclonucleoside (XX) of $N^6$-methanesulfonyl-1,2-dihydro-2′,3′-*O*-isopropylidenetubercidin; syrup; yield, 35 mg; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 308 (7000), 240 (15600) and 222 m$\mu$ (18200); Mass: M$^+$ 368 ($C_{15}H_{20}N_4O_5S$), M$^+$ $-$2H 366, M$^+$ $-CH_2NH$ 260; NMR (100 MHz, $CDCl_3$): 1.34, 1.52 (two isopropylidene methyls), 3.02 (s, 3H, $CH_3SO_2N$=), 3.29 (q, 1H, $H_a$-5′, $J_{\text{gem}}=24$ Hz, $J_{5'a,4'}=3$ Hz), 4.70, 4.96 (a pair of doublet, 2H, –NH–$CH_2$–N=, $J_{\text{gem}}=6$ Hz), 5.62 (s, 1H, H-1′), 6.36 and 6.44 ppm (a pair of doublet, 2H, –CH=CH–N=, $J_{7,8}=4$ Hz).

With increase of methanol content in the developing solvent, gummy materials which tailed on tlc were eluted, and an attempt to obtain another compound failed.

*Reaction of the Cyclonucleoside X with Sodium Borohydride.* $NaBH_4$ (10 mg) was added to an aqueous solution of X (10 mg/ml), and the solution was stirred at room temperature for 1 hr. The product, 3,5′-cyclonucleoside (XXI) of 1,2-dihydro-$N^6$-*p*-toluenesulfonyl-2′,3′-*O*-isopropylidenetubercidin, was extracted with ethyl acetate, and was fractionally precipitated with ethyl acetate and ligroin to give a syrup; yield, 3 mg; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 312 (5700), 245 (shoulder, 8400) and 220 m$\mu$ (17500); Mass: M$^+$ 444.1455 (Calcd for $C_{21}H_{24}N_4O_5S$ 444.1468), M$^+$$-$H$-SO_2$ 379.1724 ($C_{21}H_{23}N_4O_3$ 379.1770), M$^+$$-C_7H_7SO_2$ 289.1289 ($C_{14}H_{17}N_4O_3$ 289.1301), M$^+$$-C_7H_7SO_2-CH_2N$ 261.1126 ($C_{13}H_{15}N_3O_3$ 261.1113).

N$^6$,N$^6$-*Dibenzoyl-2′,3′-*O*-isopropylidene-5′-*O*-methanesulfonyltubercidin (XXII).* Mesyl chloride (1.37 g, 12 mmol) was added dropwise to a stirred and ice-cooled solution of IX (3.06 g, 10 mmol) in pyridine (10 ml), and the solution was kept at 0°C overnight. Benzoyl chloride (4.2 g, 30 mmol) was added, and after the solution was left overnight in a refrigerator it was poured on an ice-cooled aqueous solution of sodium bicarbonate. The products were extracted with chloroform and separated with column chromatography on silicic acid. The less polar product, eluted with a mixture of benzene and ethyl acetate (4: 1), was crystallized from ethyl acetate and ligroin, and was assigned as $N^6$,$N^6$,5′-*O*-tribenzoyl-2′,3′-*O*-isopropylidenetubercidin (XXIII); yield, 170 mg; mp 110—113°C; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 281 (11600), 273 (12000) and 226 m$\mu$ (43000); $[\alpha]_D^{23}$ $-38.1°$ (*c* 17.0, chloroform).

Found: C, 67.37; H, 4.87; N, 8.99%. Calcd for $C_{35}N_{30}N_4O_7$: C, 67.95; H, 4.89; N, 9.06%.

The main product, $N^6$,$N^6$-dibenzoyl-2′,3′-*O*-isopropylidene-5′-*O*-methanesulfonyltubercidin (XXII), was eluted from the column with a mixture of benzene and ethyl acetate (1: 1). After being crystallized from ethyl acetate and ligroin, the yield was 3.38 g; mp 110—113°C; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 280 (shoulder, 11100), 240 (shoulder, 27200) and 222 m$\mu$ (33400); NMR (60 MHz, $CDCl_3$): 1.38, 1.63 (two isopropylidene methyls), 2.86 (s, 3H, $C\underline{H}_3SO_2$-) and 7.3—7.9 ppm (complex, 10H, $C_6\underline{H}_5CONCOC_6\underline{H}_5$); $[\alpha]_D^{25}$ $-13.4°$ (*c* 1.61, chloroform).

Found: C, 58.66; H, 4.69; N, 9.33; S, 5.39%. Calcd for $C_{29}H_{28}N_4O_8S$: C, 58.77; H, 4.76; N, 9.46; S, 5.41%.

*Reaction of* N$^6$,N$^6$-*Dibenzoyl-2′,3′-*O*-isopropylidene-5′-*O*-methanesulfonyltubercidin (XXII) with Sodium Iodide.* A solution of XXII (1.284 g, 2 mmol) and sodium iodide (1.3 g, 8 mmol) in methyl ethyl ketone (20 ml) was refluxed for 30 min. After sodium mesylate which rapidly precipitated was removed by filtration, the products were distributed between an aqueous solution of sodium sulfite and chloroform. The organic layer was taken, washed with water and concentrated to dryness. The residue (1.18 g) was applied to column chromatography on silicic acid, developed with a mixture of benzene and ethyl acetate increasing ethyl acetate content gradually. The main product, $N^6$,$N^6$-dibenzoyl-5′-deoxy-5′-iodo-2′,3′-*O*-isopropylidenetubercidin (XXIV), was eluted first and was crystallized from ethyl acetate and ligroin; yield, 784 mg; mp 105—112°C; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 280 (12000) and 223 m$\mu$ (21000); $[\alpha]_D^{23}$ $-51.2°$ (*c* 0.91, chloroform).

Found: C, 53.90; H, 3.99; N, 8.83; I, 20.42%. Calcd for $C_{28}H_{25}N_4O_5I$: C, 53.85; H, 4.04; N, 8.97; I, 20.32%.

$N^6$-Benzoyl-5′-deoxy-5′-iodo-2′,3′-*O*-isopropylidenetubercidin (XXV) was eluted next, and was purified with fractional precipitation from ethyl acetate and ligroin; syrup; yield, 77 mg; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 299 (9800), 235 (shoulder, 22000) and 223 m$\mu$ (27000); M$^+$ 520.0583 (Calcd for $C_{21}H_{21}N_4O_4I$ 520.0607).

The starting material, which moved slightly more slowly than XXV, was also isolated; yield, 48 mg. It was also found by other experiments that attempts to complete the reaction by prolonged reaction time resulted in the decrease of the yield of XXIV.

The most polar substance, eluted from the column with a mixture of benzene and ethyl acetate (1: 1) was obtained as crystals; yield, 100 mg; mp 191—193°C. The structure is still undetermined.

N$^6$,N$^6$-*Dibenzoyl-5′-deoxy-2′,3′-*O*-isopropylidenetubercidin (XXVI) and* N$^6$-*Benzoyl-5′-deoxy-2′,3′-*O*-isopropylidenetubercidin (XXVII).* The iodo compound XXIV (784 mg) was catalytically hydrogenated at room temperature for 3 hr in 95% ethanol (20 ml) containing sodium acetate (1 g) in the presence of 10% Pd-C (200 mg). Ethanol was removed by evaporation and the residue was extracted with chloroform. Tlc showed the presence of two compounds which were separated with column chromatography on silicic acid developed with a mixture of benzene and ethyl acetate (2: 1). The less polar compound, assigned as $N^6$,$N^6$-dibenzoyl-5′-deoxy-2′,3′-*O*-isopropylidenetubercidin (XXVI), was crystallized from ethyl acetate and ligroin; yield, 58 mg; mp 192—194.5°C; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 280 (shoulder, 11500) and 223 m$\mu$ (33500); $[\alpha]_D^{23}$ $-60.8°$ (*c* 0.45, chloroform); M$^+$ 498.1882 ($C_{28}H_{26}N_4O_5$ 498.1903); NMR (60 MHz, $CDCl_3$): 1.37, 1.62 (two isopropylidene methyls) and 1.39 ppm (d, 3H, $C\underline{H}_3$-$\underset{|}{C}H$–, $J_{4',5'}=7$ Hz).

Found: C, 66.93; H, 5.05; N, 11.16%. Calcd for $C_{28}H_{26}N_4O_5$: C, 67.46; H, 5.26; N, 11.24%.

The main product, $N^6$-benzoyl-5′-deoxy-2′,3′-*O*-isopropylidenetubercidin (XXVII), was eluted later and crystallized from ethyl acetate and ligroin; yield, 430 mg; mp 128.5—129.0°C; $\lambda_{\max}^{\text{methanol}}$ ($\varepsilon$) 300 (9400) and 224 m$\mu$ (26400); $[\alpha]_D^{23}$ $-43.2°$ (*c* 0.84, chloroform); M$^+$ 394.1675 (Calcd for $C_{21}H_{22}N_4O_4$ 394.1641); NMR (60 MHz, $CDCl_3$): 1.37, 1.63 (two isopropylidene methyls) and 1.38 ppm (d, 3H, $C\underline{H}_3$–$\underset{|}{C}H$–, $J_{4',5'}=6$ Hz).

Found: C, 63.98; H, 5.48; N, 14.88%. Calcd for $C_{21}H_{22}N_4O_4$: C, 63.94; H, 5.62; N, 14.21%.

*5′-Deoxy-2′,3′-*O*-isopropylidenetubercidin (XIV).* A solution of XXVII (100 mg) in 50% ethanol (10 ml) containing 0.05N NaOH was refluxed. Tlc monitoring showed that the reaction was complete after 1 hr, and the product was homogeneous. Extraction with chloroform and concentration to dryness afforded 70 mg of syrup: $\lambda_{\max}^{\text{methanol}}$ 268 m$\mu$ ($\varepsilon$ 11900); M$^+$ 290.1422 (Calcd for $C_{14}H_{18}N_4O_3$ 290.1379).

*5′-Deoxytubercidin (IV).* A solution of XIV (150 mg) in 80% acetic acid (20 ml) was refluxed for 5 hr and concentrated to dryness. The residue, which was homogeneous on tlc, was crystallized from ethanol and benzene, and assigned as 5′-deoxytubercidin (IV) containing one mole of acetic acid; yield, 110 mg; mp 203—207°C; $\lambda_{\max}^{\text{methanol}}$ 270 m$\mu$ ($\varepsilon$ 12700); NMR (100 MHz, DMSO-*d*): 1.28 (d, 3H, $C\underline{H}_3$–$\underset{|}{C}H$–, $J_{4',5'}=6$ Hz), 1.92 (s, 3H, $C\underline{H}_3COOH$), 6.03 (s, H-1,′ $J_{1',2'}=5$ Hz), 6.63, 7.27 (a pair of doublet, 2H, –C$\underline{H}$=C$\underline{H}$–N=, $J_{7,8}=3$ Hz) and 8.04 ppm (s, 1H, –N=C$\underline{H}$–N=).

Found: C, 50.03; H, 5.70; N, 18.19%. Calcd for $C_{13}H_{18}$-

$N_4O_5$: C, 50.31; H, 5.85; N, 18.06%. $[\alpha]_D^{23}$ −70.3° (*c* 0.48, DMSO).

Acetic acid was lost on heating at 100°C for 4 hr *in vacuo*. M$^+$ 250.1078 ($C_{11}H_{14}N_4O_3$ 250.1066).

Found: C, 52.28; H, 5.54; N, 22.29%. Calcd for $C_{11}H_{14}N_4O_3$: C, 52.79; H, 5.64; N, 22.39%.

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