Tubercidin

Its Conversion to 2',3'-Didehydro-2',3'-dideoxy Derivatives

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Treatment of N⁶,N⁶,5'-O-tribenzoyl-2',3'-O-isopropylidenetubercidin (VI) with aqueous acetic acid afforded N⁶,5'-O-dibenzoyltubercidin (V), which was mesylated to yield the dimesylate X. On treatment of X with sodium iodide and zinc dust, the 2',3'-unsaturated derivatives of tubercidin XI and XIII were obtained.

N⁶,5'-O-Dibenzoyltubercidin 2',3'-thionocarbonate (XIV), prepared from V by treatment with Corey-Winter reagent, was converted to the 1-methyl-2',3'-unsaturated derivative XV in refluxing trimethyl phosphite.

Conversion of tubercidin (I)¹⁾ (7-deaza-adenosine)^{2, 3)} to 5'-deoxytubercidin (II) has already been accomplished.⁴⁾ As an approach to 2'-deoxy- or 3'-deoxytubercidin, the synthesis of some 2',3'-unsaturated derivatives of tubercidin was attempted. These might also be useful as intermediates for other derivatives of tubercidin modified at C-2' or C-3'

First, the investigation was directed to the introduction of protecting groups at both N⁶ and 5'-O of tubercidin. Tritylation of I with excess trityl chloride afforded N⁶-trityl- and 5'-O-trityl-tubercidin (III and IV) in a ratio of 0.8~0.9: 1, and a ditrityl compound was not obtained.

N⁶,5'-O-Dibenzoyltubercidin (V) was obtained by heating N⁶,N⁶,5'-O-tribenzoyl-2',3'-O-isopropylidenetubercidin (VI)⁵⁾ in aqueous acetic acid. N⁶,5'-O-Dibenzoyl-2',3'-O-isopropylidene-tubercidin (VII) was also obtained as a minor product. Attempts at increasing the yield of V by prolonged heating were unpromising, because V was further debenzoylated, yielding the monobenzoate VIII.

Treatment of V with excess tosyl chloride afforded N⁶,5'-O-dibenzoyl-2',3'-O-ditosyltubercidin (IX). Similarly, the dimesylate X was prepared. Compound X was treated with zinc dust and sodium iodide in dimethylformamide, yielding three products, *i.e.*, N⁶,5'-O-dibenzoyl-2',3'-didehydro-2',3'-dideoxytuber-

cidin (XI), 5'-O-benzoyl-2',3'-O-dimesyltubercidin (XII) and 5'-O-benzoyl-2',3'-O-didehydro-2',3'-O-dideoxytubercidin (XIII).

The NMR spectra of these 2',3'-unsaturated nucleosides are noteworthy. The spectrum of XI (Fig. 1) clearly shows that the two hydrogens at C-5' exhibit different chemical shifts (δ 4.43 and δ 4.62, $J_{gem}=12\text{Hz}$), being distinct from those of ribose nucleosides where, in general, the two hydrogens at C-5' show a broad line or a doublet with a coupling constant of about 2-4Hz. It is hard to decide at present whether restricted rotation around the bond between C-4' and C-5' or conformational rigidity of the dihydrofuran ring system in XI makes them nonequivalent. Though the signal of the hydrogen at C-1' is shifted to the aromatic region and is not observable here, its position was estimated by a decoupling experiment.

Other methods for the synthesis of 2',3'-unsaturated derivatives of tubercidin were sought. Reaction of V with Corey-Winter reagent^{6, 7)} afforded the 2',3'-thionocarbonate XIV, which was then treated with trimethylphosphite. Two products were obtained, and were isolated by silicic acid chromatography.

The minor one was the expected 2',3'-unsaturated nucleoside XI, identical with a sample of XI obtained previously. The main product was assigned as N⁶,5'-O-dibenzoyl-1-

FIG. 1. The NMR Spectrum of $N^6,5'$ -O-Dibenzoyl-2',3'-didehydro-2',3'-dideoxytubercidin (XI) (100 MHz, $CDCl_3$).

methyl-2', 3'-didehydro-2',3'-dideoxytubercidin (XV). The analytical data and the mass spectrum of XV shows that a hydrogen of XI is substituted here by a methyl group. The NMR spectrum also exhibits a singlet at δ 3.78 corresponding to an N-methyl group. The position of methylation was tentatively assigned as N-1 by a fragment ion at m/e 160, corresponding to $C_6H_5CON=C=\dot{N}CH_3$.⁸⁾ An intense peak at m/e 250 corresponding to the base moiety of XV was also observed.

We are now making an effort to obtain 2',3'-unsaturated derivatives of tubercidin in better yields. Investigation of the mechanism of the methylation affording XV is also in progress.

EXPERIMENTAL

Tritylation of tubercidin. Tubercidin (2.66 g, 10 mm) was suspended in anhydrous pyridine (200 ml) and trityl chloride (5.57 g, 20 mm) was added with stirring at room temperature. Tubercidin gradually went into solution. After 18 hr an additional supply of trityl chloride (2.785 g, 10 mm) was added, and the solution was further stirred for 3 days. It was then poured into an ice-cooled solution of sodium bicarbonate, and the products were extracted with chloroform. Evaporation of the solvent to dryness afforded a solid, which was then dissolved in ethyl acetate (100 ml). On leaving the solution in a refrigerator overnight, crystals of 5'-O-trityltubercidin (IV) separated out; yield 2.0 g; mp 210~212°C; $\lambda_{\text{max}}^{\text{MeOH}}$ (ϵ) 270 (13500); $[\alpha]_{\text{D}}^{25} -23.8$ ($c=2.02$, DMSO). Found: C, 70.69; H, 5.22; N, 10.89%. Calcd. for $\text{C}_{30}\text{H}_{27}\text{N}_4\text{O}_4$: C, 70.99; H, 5.36; N, 11.04%.

After IV was collected, the mother liquor was submitted to silicic acid chromatography. Triphenylcarbinol was eluted first on developing with ethyl acetate, and the solvent was changed to a mixture of ethyl acetate and methanol (9:1). Two products were separated, the one eluted later being IV (120 mg). The less polar one was identified as N⁶-trityltubercidin (III); yield 1.63 g. Precipitation from ethyl acetate and ligroin afforded a powder melting at 137~142°C; $\lambda_{\text{max}}^{\text{MeOH}}$ (ϵ) 283 (11900); $[\alpha]_{\text{D}}^{25} -27.8$ ($c=1.60$, DMSO). Found: C, 71.14; H, 5.79; N, 10.84%. Calcd. for $\text{C}_{30}\text{H}_{27}\text{N}_4\text{O}_4$: C, 70.99; H, 5.36; N, 11.04%.

N⁶, 5'-O-Dibenzoyl-2', 3'-O-isopropylidenetubercidin (VII) and N⁶,5'-O-dibenzoyltubercidin (V). A solution of VI¹ (5.8 g) dissolved in 70% acetic acid (200 ml) was heated on a water bath for 2 hr, and the solvent was evaporated to dryness. The residue was distributed between ethyl acetate and an aqueous solution of sodium bicarbonate, the organic layer being taken. TLC showed the presence of several compounds, from which the two main products were isolated by silicic acid chromatography using ethyl acetate as a developing solvent. The less polar compound, identified as VII, was obtained as a powder melting at 145~150°C on precipitation from ethyl acetate and ligroin; yield 425 mg; $\lambda_{\text{max}}^{\text{MeOH}}$ (ϵ) 300 (10300) and 225 (40400); $\nu_{\text{max}}^{\text{KBr}}$ 1730 cm^{-1} (ester); $[\alpha]_{\text{D}}^{25} -42.6$ ($c=1.40$, CHCl_3); NMR (60 MHz, CDCl_3): 1.40 and 1.64 ppm (two singlets, 6H, two isopropylidene methyls). Found: C, 65.28; H, 5.14; N, 10.84%. Calcd. for $\text{C}_{28}\text{H}_{26}\text{N}_4\text{O}_6$: C, 65.36; H, 5.09; N, 10.89%.

The other compound, which was eluted later from the column and crystallized from ethyl acetate and benzene, was identified as V; yield 1.6 g (35%); mp 163~164°C; $\lambda_{\text{max}}^{\text{MeOH}}$ (ϵ) 300 (10400) and 225 (42200); $\nu_{\text{max}}^{\text{KBr}}$ 1730 cm^{-1} (ester); $[\alpha]_{\text{D}}^{25} -41.0$ ($c=1.77$ DMSO). Found: C, 63.00; H, 4.66; N, 11.69%. Calcd. for

$\text{C}_{28}\text{H}_{26}\text{N}_4\text{O}_6$: C, 63.28; H, 4.67; N, 11.81%.

5'-O-Benzoyltubercidin (VIII). A solution of VI (5.6 g) in 70% acetic acid (220 ml) was heated on a water bath for 8 hr, and was evaporated to dryness. The residue was dissolved in hot ethyl acetate (500 ml). On cooling the solution in a refrigerator overnight, a crystalline precipitate separated out. It was collected and distributed between ethyl acetate and an aqueous solution of sodium bicarbonate, the organic layer being taken. Evaporation of the solvent and precipitation from methanol and benzene afforded 600 mg of a powder melting at 207~210°C; $\lambda_{\text{max}}^{\text{MeOH}}$ (ϵ) 270 (13500); $\nu_{\text{max}}^{\text{KBr}}$ 1725 cm^{-1} (ester); $[\alpha]_{\text{D}}^{24} -81.7$ ($c=0.38$, DMSO). Found: C, 58.27; H, 4.78; N, 14.91%. Calcd. for $\text{C}_{18}\text{H}_{18}\text{N}_4\text{O}_5$: C, 58.37; H, 4.90; N, 15.13%.

TLC showed the presence of several compounds in the mother liquor. Of these, V was isolated by silicic acid chromatography; yield 980 mg.

N⁶,5'-O-Dibenzoyl-2',3'-O-ditosyltubercidin (IX). To an ice-cooled solution of V (308 mg, 0.65 mm) in pyridine, tosyl chloride (259 mg, 1.36 mm) was added with stirring, and, after the solution was allowed to stand at room temperature overnight, it was poured onto an ice-cooled aqueous solution of sodium bicarbonate. The product was extracted with ethyl acetate and was purified by silicic acid chromatography, developing with a mixture of benzene and ethyl acetate (2:1); glass melting at 80~85°C; $\lambda_{\text{max}}^{\text{MeOH}}$ (ϵ) 298 (10900) and 226 (54700); $[\alpha]_{\text{D}}^{24} -57.8$ ($c=5.03$, CHCl_3). Found: C, 60.18; H, 4.59; N, 6.88; S, 7.99%. Calcd. for $\text{C}_{37}\text{H}_{30}\text{N}_4\text{O}_{10}\text{S}_2$: C, 59.83; H, 4.38; N, 7.16; S, 8.19%.

N⁶,5'-O-Dibenzoyl-2',3'-O-dimesyltubercidin (X). Compound V (1.5 g, 3.2 mm) was treated with mesyl chloride (0.9 g, 7.9 mm) in an analogous manner to that described in the preceding section. The reaction mixture was poured onto an ice-cooled solution of sodium bicarbonate and the product was extracted with ethyl acetate. Evaporation of the solvent afforded a syrup, which was precipitated from benzene and ligroin; yield 1.6 g; $\lambda_{\text{max}}^{\text{MeOH}}$ $m\mu$ (ϵ) 298 (6300) and 224 (23300); NMR (60 MHz, CDCl_3): 3.00 and 3.22 ppm (two singlets, 6H, 2 CH_3SO_3^-). Found: C, 51.24; H, 4.11; N, 8.55; S, 9.87%. Calcd. for $\text{C}_{27}\text{H}_{26}\text{N}_4\text{O}_{10}\text{S}_2$: C, 51.42; H, 4.16; N, 8.89; S, 10.17%.

Treatment of X with sodium iodide and zinc dust. Compound X (1.5 g) was treated with sodium iodide (1 g) and zinc dust (0.5 g) in refluxing dimethylformamide for 8 hr. The solvent was evaporated, and the residue was distributed between water and chloroform, the organic layer being taken. TLC showed the presence of four UV absorbing compounds, which were separated by silicic acid chromatography. Using a mixture of benzene and ethyl acetate (1:1) as the developing solvent, two compounds were eluted, the less polar one being the starting material (95 mg).

The other compound was identified as N⁶,5'-O-dibenzoyl-2',3'-didehydro-2',3'-dideoxytubercidin (XI); syrup; yield 93 mg; $\lambda_{\text{max}}^{\text{MeOH}}$ (ϵ) 300 (8400), 226 (34000); $[\alpha]_{\text{D}}^{24} -67.2$ ($c=1.25$, CHCl₃); Mass m/e 440 (M⁺); NMR (100 MHz, CDCl₃): 4.43 (q, 1H, Ha-5', $J_{\text{gem}}=12$ Hz, $J_{4',5'a}=4$ Hz), 4.63 (q, 1H, H_b-5', $J_{\text{gem}}=12$ Hz, $J_{4',5'b}=4$ Hz), 5.22 (broad, 1H, H-4'), 6.07 (dq, 1H, H-3', $J_{2',3'}=6$ Hz, $J_{3',4'}=2.5$ Hz, $J_{1',3'}=1$ Hz), 6.43 (dt, 1H, H-2', $J_{2',3'}=6$ Hz, $J_{1',2'}=2$ Hz, $J_{2',4'}=2$ Hz), 6.19, 6.98 (two doublets, 2H, -HC=CH-N<, $J_{7,8}=3.5$ Hz), 7.4~8.1 (complex, 11H, H-1' and aromatic protons) and 8.50 ppm (s, 1H, -N=CH-N<).

An analytical sample was prepared by fractional precipitation from benzene and ligroin. Found: C, 67.74; H, 4.58; N, 12.48%. Calcd. for C₂₆H₂₀N₄O₄: C, 68.17; H, 4.58; N, 12.72%.

The developing solvent was changed to ethyl acetate and then to a mixture of ethyl acetate and methanol (9:1). Two other compounds were eluted. The less polar one was identified as 5'-O-benzoyl-2',3'-O-dimesyltubercidin (XII); syrup; yield 290 mg; $\lambda_{\text{max}}^{\text{MeOH}}$ $m\mu$ (ϵ) 267 (12200); $[\alpha]_{\text{D}}^{24} -43.3$ ($c=0.85$ CHCl₃); Mass m/e 526 (M⁺); NMR (100 MHz, CDCl₃): 3.02 and 3.22 ppm (two singlets, 6H, 2CH₃SO₃-).

An analytical sample was prepared by fractional precipitation from ethyl acetate and ligroin. Found: C, 45.61; H, 4.14; N, 10.42; S, 11.85%. Calcd. for C₂₀H₂₂N₄O₈S₂: C, 45.62; H, 4.21; N, 10.64; S, 12.18%.

The most polar compound among these products was identified as 5'-O-benzoyl-2',3'-didehydro-2',3'-dideoxytubercidin (XIII); syrup; yield 207 mg; $\lambda_{\text{max}}^{\text{MeOH}}$ $m\mu$ (ϵ) 270 (12100); $[\alpha]_{\text{D}}^{24} -148$ ($c=1.33$ CHCl₃); Mass m/e 336 (M⁺); NMR (100 MHz, CHCl₃): 4.43 (q, 1H, Ha-5', $J_{\text{gem}}=12$ Hz, $J_{4',5'a}=4$ Hz), 4.58 (q, 1H, H_b-5', $J_{\text{gem}}=12$ Hz, $J_{4',5'b}=5$ Hz), 5.20 (broad, 1H, H-4'), 6.05 (dq, 1H, H-3', $J_{2',3'}=6$ Hz, $J_{3',4'}=2.5$ Hz, $J_{1',3'}=1$ Hz), 6.36 (dt, 1H, H-2', $J_{2',3'}=6$ Hz, $J_{1',2'}=2$ Hz, $J_{2',4'}=2$ Hz), 6.30, 6.98 (two doublets, 2H, -HC=CH-N<, $J_{7,8}=3.5$ Hz), 7.3~8.1 (complex, 6H, H-1' and aromatic protons) and 8.32 ppm (s, 1H, -N=CH-N<).

An analytical sample was prepared by fractional precipitation from ethyl acetate and ligroin. Found: C, 62.69; H, 5.04; N, 15.98%. Calcd. for C₁₈H₁₆N₄O₈·1/2H₂O: C, 62.60; H, 4.96; N, 16.23%.

Water was scarcely excluded even on prolonged heating.

N⁶,5'-O-Dibenzoyltubercidin 2',3'-thionocarbonate (XIV). Bis(imidazol-1-yl)thione (Corey-Winter reagent)⁶ was prepared from imidazole and thiophosgene in dichloromethane according to the method of Horton and Turner,⁷ and the reagent was used *in situ* after imidazole hydrochloride, which was also formed, was removed by filtration. A solution of Corey-Winter reagent corresponding to 1 g of the reagent was added to a solution of V (474 mg) dissolved in acetone (30 ml).

On refluxing for 4 hr, crystals of imidazole hydrochloride precipitated, and were removed by filtration. The filtrate was concentrated and charged onto a column of silicic acid. Elution with a mixture of benzene and ethyl acetate (2:1) afforded a syrup, which was shown to be homogeneous on TLC; yield 300 mg; $\lambda_{\text{max}}^{\text{MeOH}}$ $m\mu$ (ϵ) 298 (14300) and 235 (28000); $[\alpha]_{\text{D}}^{25} -41.1$ ($c=1.55$, CHCl₃); Mass m/e 682 (M⁺). Found: C, 60.44; H, 3.70; N, 10.81; S, 5.92%. Calcd. for C₃₆H₃₄N₄O₈S: C, 60.45; H, 3.95; N, 10.85; S, 6.21%.

N⁶,5'-O-Dibenzoyl-1-methyl-2',3'-didehydro-2',3'-dideoxytubercidin (XV). A solution of XIV (400 mg) dissolved in trimethyl phosphite (5 ml) was refluxed for 4 hr, and was evaporated to dryness. Water was added and the products were extracted with ethyl acetate. The organic layer was washed with an aqueous solution of sodium bicarbonate and then with water. TLC showed the presence of two UV absorbing compounds, which were separated by silicic acid chromatography, developing with a mixture of benzene and ethyl acetate (1:1) and then with ethyl acetate. The less polar one (67 mg) was identical with a sample of XI obtained previously.

The main product was obtained as a syrup; yield 107 mg; $\lambda_{\text{max}}^{\text{MeOH}}$ $m\mu$ (ϵ) 330 (15700) and 227 (31200); $[\alpha]_{\text{D}}^{25} -52.0$ ($c=0.66$, CHCl₃); Mass m/e 454.1591 (M⁺, C₂₆H₂₂N₄O₄ gives 454.1641); NMR (100 MHz, CDCl₃): 3.78 (s, 3H, >N-CH₃), 4.38 (q, 1H, Ha-5', $J_{\text{gem}}=12$ Hz, $J_{4',5'a}=4$ Hz), 4.56 (q, 1H, H_b-5', $J_{\text{gem}}=12$ Hz, $J_{4',5'b}=5$ Hz), 5.18 (broad, 1H, H-4'), 6.00 (broad d, 1H, H-3', $J_{2',3'}=5$ Hz), 6.38 (broad d, 1H, H-2', $J_{2',3'}=5$ Hz), 6.54, 6.86 (two doublets, 2H, -HC=CH-N<, $J_{7,8}=3.5$ Hz), 7.20 (broad, 1H, H-1'), 7.3~8.4 (complex, 10H, aromatic protons) and 8.00 ppm (s, 1H, >N-CH₃-N-). Found: C, 68.32; H, 4.98; N, 12.16%. Calcd. for C₂₆H₂₂N₄O₄: C, 68.71; H, 4.88; N, 12.33%.

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