

ALKYL AND ACYL DERIVATIVES OF TUBERCIDIN

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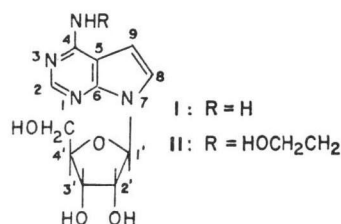
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A series of acyl and N-alkyl derivatives of tubercidin has been prepared. *In vitro* and *in vivo* activities are reported.

For many years the antibiotic tubercidin (1)¹⁾ has been of interest as an antitumor agent. A large number of tubercidin derivatives and analogs²⁻⁷⁾ have been prepared in order to study structure-activity relationships and in efforts to find compounds having the antitumor activity of tubercidin but being more readily absorbed orally or more effective

in passing through cell walls. The present paper extends this series and reports the synthesis of a group of compounds derived by esterification of the hydroxyl groups, primarily the 5'-hydroxyl, of tubercidin as well as some N-acylated and N-alkylated derivatives. The purpose of the investigation was to prepare compounds which, while not necessarily antitumor agents in themselves, would be more readily absorbable orally or might pass cell walls more readily and eventually be metabolized *in vivo* to tubercidin.



Chemistry

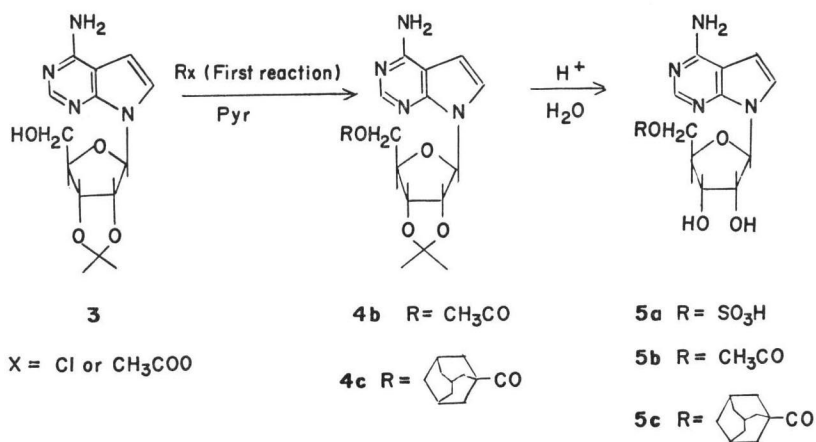
A series of three 5'-O-monoesters of tubercidin (**5a**, **5b** and **5c**) was prepared. Initial attempts to prepare the esters of organic acids was by acylation of tubercidin hydrochloride (**2**) using the procedure of GISH *et al.*,⁸⁾ but the attempts failed. Subsequently success was achieved by treatment of 2',3'-O-isopropylidene-tubercidin (**3**)²⁾ with chlorosulfonic acid or an acyl chloride or anhydride followed by mild hydrolysis to remove the isopropylidene group selectively (Scheme 1).

The structures of the five compounds isolated were established by method of preparation, analysis and spectral data all of which are reported in the Experimental. Somewhat surprisingly neither the hydrochloride **2** nor the 5'-O-sulfate **5a** had ultraviolet spectra in water characteristic of tubercidin in strongly acidic solvents as these two compounds must be protonated even in neutral solutions.

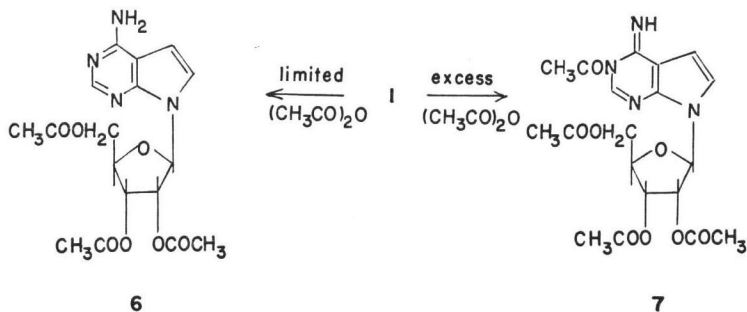
Acetylation of tubercidin using acetic anhydride in pyridine gave the tri-O-acetyl derivative **6** when three moles of acetic anhydride per mole of tubercidin were employed. When a large excess of acetic anhydride was used, the product was the tri-O-acetyl-N-acetyl derivative **7**

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Scheme 1



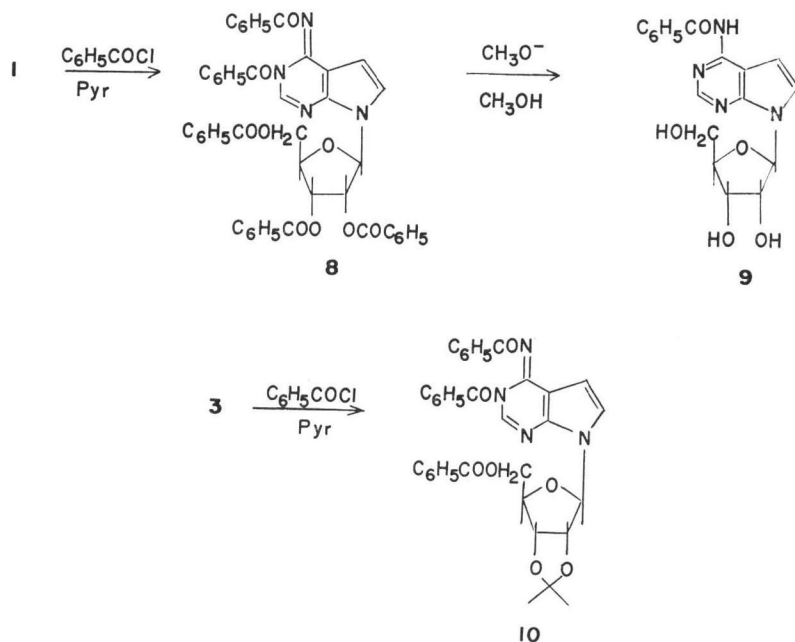
Scheme 2



which has already been reported but not characterized⁹⁾ (Scheme 2). Neither **6** nor **7** were crystalline and both were unstable at room temperature but stable at -15°C . The structure of **6** is largely based on spectral data as the analysis gave values slightly outside the permitted limits. However, the high resolution mass spectrum conclusively established the proposed molecular formula. The only previously reported characterization of **7** was a KUHN-ROTH acetyl determination indicating a tetraacetate.⁹⁾ The analytical data and nmr spectrum derived from **7** quite conclusively demonstrate that it is a tetraacetyl compound and also indicate that three acetyl groups are attached to oxygen. Compound **7** shows maxima in its ultraviolet spectrum in neutral solution at 220 and 286 nm. GERSTER *et al.*⁵⁾ have shown that treatment of tubercidin with methyl iodide forms the N³-methyl hydroiodide. The compound thus prepared has two maxima (228 nm and 274 nm) in its ultraviolet spectrum in neutral solution.³⁾ This is in contrast with tubercidin and tubercidin substituted by alkyl groups on N⁴ which have a single maximum in neutral or basic solutions and two maxima in strongly acidic solutions.^{8, 5)} Therefore the double maxima must indicate the presence of an imino form such as **7** which must then have an acetyl group at N³.

Benzoylation of tubercidin with an excess of benzoyl chloride under mild conditions resulted in a pentabenzoyl derivative **8**. Similar treatment of 2',3'-O-isopropylidene tubercidin formed a tribenzoyl derivative **10** (Scheme 3). The number of benzoyl groups introduced was readily

Scheme 3



established by analysis, and in the case of **10** by its nmr spectrum. Treatment of **8** with sodium methoxide formed N-benzoyltubercidin (**9**). Since spectral and analytical data derived from **8** and **10** indicate clearly that they are, respectively, pentabenzoyl and tribenzoyl derivatives of tubercidin then two of the benzoyl groups in each case must be attached to the 7-deazaadenine portion of the molecule. The structures proposed were arrived at on the basis of analogy with the proposal of SMITH *et al.*¹⁰⁾ that a tetrabenzoyladenosine prepared by them had benzoyl groups at N^1 and N^6 , and the proposal of BENTLEY, CUNNINGHAM and SPRING¹¹⁾ of a similar structure for tetrabenzoylcordycepin. It is not completely established that N-benzoyltubercidin has structure **9**, but, again by analogy with the conversion of N^3 -methyltubercidin to 4-methylaminotubercidin,^{3,5)} that structure seems more probable than does the alternative N^3 -benzoyl-4-imino structure analogous to **8**.

N^4 -Hydroxyethyltubercidin (**11**)⁴⁾ was prepared by the procedure of WINDMUELLER and KAPLAN¹²⁾ which involves treatment with ethylene oxide followed by base.

Biological

These compounds (**2** through **11** with the exception of **8** and **9**) were tested *in vitro* against L1210, a leukemia cell line. The results are given in Table 1 and are expressed as $\mu\text{g}/\text{ml}$ inhibiting growth of the cells by 50% and 90%. As can be seen by comparison of **2** with the other compounds any sort of derivatization reduces activity in this assay although the monoacylates (**5b** and **5c**) are moderately active. Only compounds **5b**, **5c**, **6** and **7** were tested *in vivo*. The first two were tested against PS 388 in mice by i.p. administration. Compound **5c** was inactive, but **5b** had a T/C of 1.52 when given at a dose of 1 mg/kg. This is in the range of tubercidin itself. Compounds **6** and **7** were inactive against L1210 in mice and WALKER carcinoma 256 in rats. Other biological data will be reported elsewhere.

Table 1. Cytotoxicity.

Compound	ID ₅₀	ID ₉₀
2	0.0040	0.012
4b	>10	>10
4c	>10	>10
5a	0.32	0.94
5b	0.043	0.12
5c	0.024	0.069
6	0.40	2.2
7	0.14	0.33
10	>10	>10
11	3.5	>10

(ϵ 22,080) and 270 nm (ϵ 10,430); nmr (D₂O) δ 3.78 (d, 2, CH₂O), δ 4.03~4.50 (m, 3, CHO), δ 4.56 (s, exch. H), δ 6.08 (d, 1, anomeric H), δ 6.66 (d, 1, CH=), δ 7.47 (d, 1, NCH=), δ 8.12 (s, 1, NCH=N).

Anal. Calcd. for C₁₁H₁₃ClN₄O₄: C, 43.65; H, 5.00; N, 18.51; Cl, 11.71.

Found: C, 43.49; H, 5.20; N, 18.33; Cl, 11.76.

4-Amino-7- $[\beta$ -D-ribofuranosyl-5'-sulfate]-7H-pyrrolo-[2,3-d]-pyrimidine (5a)

A mixture of 2 g (6.5 mmoles) of 2',3'-O-isopropylidene-tubercidin and 20 ml of dry pyridine was stirred at -10°C while adding dropwise a solution of 1.51 g (13 mmoles) of ClSO₃H in 6 ml of dry CHCl₃. The reaction mixture was allowed to warm slowly to room temperature followed by heating at 50°C for 15 minutes. The mixture was evaporated under reduced pressure to a syrup which was chromatographed on 100 ml of Dowex 1×8 (HCOO⁻). The column was eluted with 500 ml each of 0.01 N, 0.02 N and 0.04 N HCOOH and 500 ml each of 0.01 N, 0.02 N, 0.04 N, 0.1 N, 0.5 N and 1 N HCl collecting 10-ml fractions. Fractions 309~368 were combined and concentrated to a volume of about 3 ml under reduced pressure. Refrigeration of the residue followed by filtration and washing of the filter cake with ethanol gave 290 mg of crystalline solid, mp 245°C (dec.). Two recrystallizations of 100 mg from water gave a white solid, mp 265~267°C (dec.); ir (Nujol) 3310, 3120 (NH/OH), 1660, 1580, 1530, 1490, 1445, 1365, 1255, 1220, 1205, 1100, 1055, 995, 950, 900, 865, 815, 790 and 728 cm⁻¹. UV (H₂O) max 272 nm (ϵ 10,035), nmr (D₂O) δ 4.38 (s, 2, CH₂O), δ 4.3~4.70 (m, 3, CHO), δ 4.73 (s, exch. H), δ 6.22 (d, 1, anomeric H), δ 6.57 (d, 1, CH=), δ 7.42 (d, NCH=), δ 8.05 (s, 1, NCH=N). pKa' (H₂O) 5.2.

Anal. Calcd. for C₁₁H₁₄N₄O₇S: C, 38.16; H, 4.08; N, 16.18; S, 9.27; eq. wt. 346.

Found: C, 38.63; H, 4.17; N, 15.97; S, 9.47; eq. wt. 337.

4-Amino-7-[5'-O-acetyl-2',3'-O-isopropylidene- β -D-ribofuranosyl]-7H-pyrrolo-[2,3-d]-pyrimidine (4b).

A solution of 5.35 g (17 mmoles) of 2',3'-O-isopropylidene-tubercidin in 65 ml of dry pyridine was cooled in an ice-bath and stirred while a solution of 1.74 g (1.6 ml; 17 mmoles) of acetic anhydride was added dropwise. The reaction mixture was stirred at 0°C for 2 hours followed by stirring at room temperature overnight. The solution was poured into 750 ml of water, and the aqueous mixture was extracted with four 400-ml portions of chloroform. The combined extracts were dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was dissolved in 30 ml of chloroform and 250 ml of Skellysolve B was added. The resulting ppt was purified further by two more precipitations in the same fashion. The final product was chromatographed on 250 g of silica gel using a solvent system consisting of ethyl acetate - 95% ethanol - water (94:4:2) and collecting 5-ml fractions. Fractions 144~205 were combined and evaporated to dryness under reduced pressure. The residue was triturated with

Experimental

4-Amino-7- β -D-ribofuranosyl-7H-pyrrolo-[2,3-d]-pyrimidine hydrochloride (2)

One gram of tubercidin was dissolved in 4 ml of 1 N HCl. The solution was refrigerated, filtered and the filter cake was washed with ethanol. Yield 0.73 g, mp 250~252°C (dec.). Two recrystallizations from 85% and 80% ethanol successively caused no change in the mp: ir (Nujol) 3350~3120, 1685, 1650 (sh), 1520, 1505, 1460, 1380, 1350, 1330, 1240, 1125, 1115, 1080, 1055, 1035, 1000, 910, 900, 870, 750 and 705 cm⁻¹. UV (H₂O, pH 7) max 270 nm (ϵ 10,950), (H₂O, pH 2) max 226 nm

hexane and filtered. Yield 3.1 g, 58 %, mp 47~49°C: ir (Nujol) 3420 (NH), 1740 (ester), 1620, 1580, 1510, 1465, 1375, 1210, 1180, 1150~1050 cm^{-1} ; nmr (CDCl_3) δ 1.42 (s, 3, CH_3C), δ 1.63 (s, 3, CH_3C), δ 2.04 (s, 3, CH_3CO), δ 4.38 (m, 3, CHO), δ 5.0~5.85 (m, 4H, CHO and NH_2), δ 6.24 (d, 1, anomeric H), δ 6.42 (d, 1, $\text{HC}=\text{C}$), δ 7.08 (d, 1, $\text{NHCH}=\text{C}$), δ 8.35 (s, 1, $\text{NCH}=\text{N}$); mass spectrum [m/e , (relative intensity)] 348 (151), 217 (538), 163 (651), 135 (217), 134 (469), 107 (237), 69 (143), 55 (123), 43 (999).

Anal. Calcd. for $\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_5$: C, 55.22; H, 5.79; N, 16.10.

Found: C, 55.10; H, 5.93; N, 15.97.

4-Amino-7-[5'-O-acetyl- β -D-ribofuranosyl]-7H-pyrrolo-[2,3-d]-pyrimidine (5b).

A solution of 9.01 g (26 mmoles) of 5'-O-acetyl-2',3'-O-isopropylidene-tubercidin in 90 ml of trifluoroacetic acid was allowed to stand at room temperature for 10 minutes. The solvent was removed by evaporation under reduced pressure followed by repeated addition of methanol and reduced pressure evaporation. The residue was dissolved in 200 ml of water, and the mixture was filtered. The filtrate was adjusted to pH 6.0 with saturated NaHCO_3 solution. After the mixture had been refrigerated, the crystals were collected, yield 3.85 g, 48 %. Recrystallization from methanol gave 2.48 g, mp 198~200°C: ir (Nujol) 3400, 3330, 3180 (NH/OH), 1745 (ester), 1640, 1595, 1560, 1510, 1485, 1340, 1320, 1275, 1250, 1225, 1130, 995 and 750 cm^{-1} . UV (EtOH) max 269 nm (ϵ 11,710), (pH 2) max 227 nm (ϵ 23,240), 270 nm (ϵ 11,125); nmr ($\text{DMSO}-d_6$) δ 2.05 (s, 3, CH_3CO), δ 4.0~4.6 (m, 3H, CHO), δ 5.2~5.6 (m, 2H, CHO), δ 6.08 (d, 1, anomeric H), δ 6.6 (d, 1, $\text{HC}=\text{C}$), δ 7.3 (d, 1, $\text{NCH}=\text{C}$), δ 8.1 (s, 1, $\text{NCH}=\text{N}$); mass spectrum [m/e (relative intensity)] 308 (86), 177 (84), 163 (763), 147 (75), 135 (423), 134 (999), 107 (262), 43 (226); tlc (CHCl_3 - MeOH - H_2O ; 78:20:2) Rf 0.37.

Anal. Calcd. for $\text{C}_{18}\text{H}_{18}\text{N}_4\text{O}_5$: C, 50.69; H, 5.24; N, 18.19.

Found: C, 50.59; H, 5.32; N, 17.87.

4-Amino-7-[5'-O-adamantane-1-carbonyl-2', 3'-O-isopropylidene- β -D-ribofuranosyl]-7H-pyrrolo-[2,3-d]-pyrimidine (4c).

Eight and two-tenths grams (27 mmoles) of 2',3'-O-isopropylidene-tubercidin was dissolved in 125 ml of dry pyridine. The solution was stirred while adding dropwise a solution of adamantane-1-carbonyl chloride [prepared from 5.2 g (29 mmoles) of adamantane-1-carboxylic acid and thionyl chloride] in 25 ml of dry pyridine. After the reaction mixture had been stirred overnight at room temperature, it was heated under reflux for 3 hours. The pyridine was removed by evaporation at room temperature under reduced pressure. The residue was partitioned between 165 ml of chloroform and 165 ml of water. The water layer was removed and extracted with three 82-ml portions of chloroform. The combined chloroform extracts were washed with 82 ml of saturated NaHCO_3 solution followed by washing with two 82-ml portions of water. The chloroform solution was dried (MgSO_4), filtered and concentrated under reduced pressure to a gummy solid, weight 12 g. The crude product was chromatographed on 820 g of silica gel using the solvent system chloroform - methanol (95:5) and collecting 175 twenty-ml fractions. Fractions 128~163 were combined and evaporated to dryness under reduced pressure leaving 9.2 g of residue which was dissolved in 55 ml of methanol and treated with charcoal. Slow addition of 18 ml of water to the hot filtrate followed by refrigeration gave 6.8 g (54 %) of crystalline product, m.p. 155~158°C. Repeated recrystallization from ethanol gave m.p. 163~165°C: ir (Nujol) 3505, 3365, 3245, 3135, 1695 (ester), 1660, 1610 and 1570 cm^{-1} ; UV (MeOH) max 268 nm (ϵ 12,450); nmr (CDCl_3) δ 1.41 (s, 3, CH_3), δ 1.63 (s, 3, CH_3), δ 1.72~1.91 (m, 15, CH_2 and CH), δ 4.18 (m, 3, CHO), δ 4.98 (m, 1, CHO), δ 5.25~5.50 (m, 3H, CHO and NH_2), δ 6.22 (d, 1, anomeric H), δ 6.38 (d, 1, $\text{CH}=\text{C}$), δ 7.08 (d, 1, $\text{NCH}=\text{C}$), δ 8.34 (s, 1, $\text{NCH}=\text{N}$).

Anal. Calcd. for $\text{C}_{25}\text{H}_{32}\text{N}_4\text{O}_5$: C, 64.08; H, 6.88; N, 11.96; mass spectrum 468.2393.

Found: C, 62.80; H, 7.04; N, 11.63; mass spectrum 468.2372.

4-Amino-7-[5'-O-adamantane-1-carbonyl- β -D-ribofuranosyl]-7H-pyrrolo-[2,3-d]-pyrimidine (5c).

A solution of 1 g of 5'-O-adamantane-1-carbonyl-2',3'-O-isopropylidenedetubercidin in 100 ml of 0.1 N HCl was boiled for 0.5 hour. The cooled reaction mixture was filtered through filter aid, and the filtrate was adjusted to pH 6 with 1 N NaOH. After the aqueous mixture was refrigerated, the precipitate was removed by filtration to give 0.76 g of white solid. Two hundred mg of the product was chromatographed on 10 g of silica gel using the solvent system chloroform - methanol (98:2) until ninety-five 5-ml fractions had been collected. Elution was continued using the same solvent system in a ratio of 19:1 until a total of two hundred 5-ml fractions had been collected. Fractions 101~120 were combined and evaporated to dryness under reduced pressure. The residue (146 mg) was crystallized from a mixture of chloroform and methanol at -15°C , mp $235\sim 237^{\circ}\text{C}$, yield 78 mg: ir (Nujol) 3390, 3290 (NH/OH), 1710 (ester), 1645, 1600, 1555, 1490, 1475, 1465, 1385, 1325, 1290, 1265, 1240, 1145, 1115, 1105, 1085, 1070, 1025, 965, 908, 895, 857, 828, 772, 726 and 702 cm^{-1} ; UV (0.1 N HCl) max 226 nm (ϵ 21,640), max 270 nm (ϵ 10,700); nmr (d_7 DMF) δ 1.7 and 1.86 (d, 15, adamantanyl), δ 4.1~4.7 (m, 7, CHO and OH), δ 6.25 (d, 1, anomeric H), δ 6.72 (d, 1, CH=), δ 6.96 (s, 2, NH₂), δ 7.32 (d, 1, NCH=), δ 8.13 (s, 1, N=CHN).

Anal. Calcd. for C₂₂H₂₃N₄O₅: C, 61.67; H, 6.59; N, 13.08.

Found: C, 61.44; H, 6.59; N, 13.62.

4-Amino-7-[2',3',5'-tri-O-acetyl- β -D-ribofuranosyl]-7H-pyrrolo-[2,3-d]-pyrimidine (6).

A mixture of 2 g (7.5 mmoles) of tubercidin and 14 ml of dry pyridine was cooled in an ice-bath and stirred while adding dropwise a solution of 2.6 g (2.4 ml, 26 mmoles) of acetic anhydride in 7 ml of dry pyridine. The mixture was cooled and stirred an additional 2 hours and finally stirred overnight at room temperature. As stirring proceeded the solid present gradually dissolved. The reaction mixture was poured into 200 ml of water, and the resulting mixture was extracted with four 50-ml portions of chloroform. The combined extracts were dried (MgSO₄), filtered and concentrated under reduced pressure finally keeping at 0.5 mm for 16 hours. The residue was dissolved in 10 ml of chloroform and 75 ml of Skellysolve B was added. After the supernatant had become clear, it was decanted, and the residual solvent was removed by evaporation under reduced pressure (0.5 mm). The solution and precipitation procedure was repeated, yield 2.3 g.

One gram was chromatographed on 50 g of silica gel using chloroform - methanol (98:2) and collecting two hundred and fifty 5-ml fractions. Fractions 80~105 were combined and evaporated to dryness under reduced pressure, yield 486 mg, mp $56\sim 80^{\circ}\text{C}$; tlc (silica gel; cyclohexane - ethyl acetate - 95 % ethanol; 5:3:2) R_f 0.25; ir (Nujol) 3440, 3340, 3160, 1740, 1635, 1585, 1560, 1510, 1235, 1095 and 1040 cm^{-1} ; UV (EtOH) max 268 nm (ϵ 11,150); nmr (CDCl₃) δ 2.03 (s, 3, CH₃CO), δ 2.13 (s, 6, CH₃CO), δ 4.39 (d, 2, CH₂O), δ 5.3~5.9 (m, 3, CHO), δ 6.45 (m, 2, anomeric and CH=), δ 7.12 (d, 1, NCH=), δ 8.34 (s, 1, N=CHN).

Anal. Calcd. for C₁₇H₂₀N₄O₇: C, 52.04; H, 5.14; N, 14.28; mass spectrum 392.1332.

Found: C, 51.40; H, 5.21; N, 13.48; mass spectrum 392.1345.

4-Imino-3-acetyl-7-[2',3',5'-tri-O-acetyl- β -D-ribofuranosyl]-7H-pyrrolo[2,3-d]-pyrimidine (7).

A mixture of 1 g of tubercidin, 10 ml of acetic anhydride and 30 ml of dry pyridine was stirred at room temperature until the solid had dissolved and was then allowed to stand for 2 days. The solution was poured into 400 ml of water, and the mixture was extracted with five 50-ml portions of chloroform. The combined extracts were dried (MgSO₄), the mixture was filtered and the filtrate was evaporated to dryness under reduced pressure at 35°C . The residual gum was chromatographed on 50 g of silica gel using chloroform as the eluent until one hundred and thirty 5-ml fractions had been collected. Elution was continued with a 98:2 mixture of chloroform - methanol until a total of 200 fractions had been collected. Fractions 148~189 were combined and concentrated under reduced pressure to give 1.31 g of a gummy, colorless residue. Solution of the residue in 5 ml of chloroform and precipitation with Skellysolve B gave 0.90 g of amorphous solid, mp $60\sim 80^{\circ}\text{C}$: ir (Nujol) 3200 (NH), 1730 (ester),

1680 (sh, amide), 1590, 1550, 1490, 1445, 1360, 1295, 1230, 1090, 1040, 890 and 740 cm^{-1} ; UV (EtOH) max 220 nm (ϵ 28,850), sh 230 nm (ϵ 13,900), max 286 nm (ϵ 7,600), (EtOH, HCl) max 242 nm (ϵ 38,500), max 287 nm (ϵ 11,525); nmr (CDCl_3) δ 2.05 (s, 3, CH_3COO), δ 2.15 (s, 6, CH_3COO), δ 2.37 (s, 3, CH_3CON), δ 4.42 (m, 3, CHO), δ 5.55~5.90 (m, 2, CHO), δ 6.53 (d, 1, anomeric H), δ 7.08 (d, 1, $\text{CH}=\text{}$), δ 7.32 (d, 1, $\text{NCH}=\text{}$), δ 8.57 (s, 1, $\text{NCH}=\text{N}$).

Anal. Calcd. for $\text{C}_{10}\text{H}_{22}\text{N}_4\text{O}_3$: C, 52.53; H, 5.11; N, 12.90.

Found: C, 52.27; H, 5.36; N, 13.17.

4-Benzamido-7-(β -D-ribofuranosyl)-pyrrolo-[2,3-d]-pyrimidine (9).

A mixture of 1.25 g (4.7 mmoles) of tubercidin and 25 ml of dry pyridine was stirred and cooled in an ice-bath while adding 3 g (3.8 ml; 21 mmoles) of benzoyl chloride. The ice-bath was removed, and the reaction mixture was stirred at room temperature for 40 hours. The mixture was poured with constant stirring into a mixture of ice and water, and stirring was continued until the precipitate was crystalline. The solid was removed by filtration and recrystallized twice by dissolving in hot acetone and adding about 10 % water, yield 2.37 g (64 %), m.p. 187~188°C, tlc (silica gel; ethyl acetate-cyclohexane, 1:1) Rf 0.61.

Anal. Calcd. for $\text{C}_{46}\text{H}_{84}\text{N}_4\text{O}_9$ (pentabenzoyl): C, 70.22; H, 4.36; N, 7.12.

Found: C, 70.40; H, 4.46; N, 7.40.

One-half gram (0.63 mmole) of the above product in a mixture of 25 ml of absolute methanol and 25 ml of anhydrous tetrahydrofuran was cooled in an ice-bath and 0.5 ml of 25 % methanolic sodium methoxide solution was added. After the reaction had proceeded for eight hours, the reaction mixture was neutralized with an excess of Dowex 50 \times 8 (pyridinium form). The mixture was filtered, and the filtrate was concentrated to a syrup under reduced pressure. The residue was crystallized twice from isopropanol and once from a 1:1 mixture of methanol-isopropanol, yield 65 mg (29 %), m.p. 181~182°C: ir (Nujol) 3560, 3300, 3260, 1680 (amide), 1605, 1565, 1515, 1510, 1490, 1255, 1120, 1115, 1050, 1030, 735 and 720 cm^{-1} ; UV (EtOH) max 225 nm (ϵ 27,100), sh 238 nm (ϵ 22,950), max 305 nm (ϵ 9,200).

Anal. Calcd. for $\text{C}_{13}\text{H}_{13}\text{N}_4\text{O}_5$: C, 58.37; H, 4.90; N, 15.13.

Found: C, 58.42; H, 4.96; N, 14.96.

3-Benzoyl-4-benzimido-7-[5'-O-benzoyl-2',3'-O-isopropylidene- β -D-ribofuranosyl]-7H-pyrrolo-[2,3-d]-pyrimidine (10).

A solution of 1.74 g (6 mmoles) of 2',3'-O-isopropylidene-tubercidin in 50 ml of dry pyridine was stirred in an ice-bath and 4.35 ml (5.35 g; 37 mmoles) of benzoyl chloride was added. After the reaction mixture had stood for 1½ hours, the solution was poured into a mixture of ice and water which was then made slightly acidic by addition of 2 N HCl. The resulting precipitate was removed by filtration. The product was recrystallized from acetone-water to give 3.28 g, m.p. 178~180°C, in two crops, yield 89 %. Recrystallization from the same solvent gave 2.4 g, m.p. 181.5~183°C: ir (Nujol) 1718 (ester), 1690 (amide), 1585, 1575, 1543, 1440, 1362, 1255, 1245, 1070, 850, 715 and 690 cm^{-1} ; UV (EtOH) max 225 nm (ϵ 36,640), sh 275 (ϵ 10,500); nmr (CDCl_3) δ 1.44 (s, 3, CH_3), δ 2.0 (s, 3, CH_3), δ 4.75 (s, 3, CHO), δ 5.0~5.5 (m, 2, CHO), δ 6.35 (m, 2, anomeric and $\text{CH}=\text{}$), δ 7.15~8.2 (m, 16H, aromatic and $\text{NCH}=\text{}$), δ 8.75 (s, 1, $\text{NCH}=\text{N}$).

Anal. Calcd. for $\text{C}_{30}\text{H}_{30}\text{N}_4\text{O}_7$: C, 67.93; H, 4.89; N, 9.06; mass spectrum 618.2114.

Found: C, 67.31; H, 5.04; N, 9.13; mass spectrum 618.2108.

4-(2-Hydroxyethyl) amino-7- β -D-ribofuranosyl-7H-pyrrolo-[2,3-d]-pyrimidine (11).

A mixture of 1 g of tubercidin and 15 ml of water saturated with ethylene oxide was stirred while ethylene oxide was bubbled through for five days. The solution was maintained at pH 6.5 by occasional addition of 1 % perchloric acid. After the reaction was complete the pH was lowered to 5.0 with perchloric acid, and the mixture was filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 40 ml of water, and 1 N NaOH was added until the pH was 11.0. The mixture was kept at 60°C for 24 hours

followed by concentration to dryness under reduced pressure. The residue was triturated with water, refrigerated and filtered with thorough washing. The product was recrystallized by dissolving it in acid and making the solution basic, yield 0.25 g. Repeated recrystallization gave a mp of 210~210.5°C; ir (Nujol) 3370, 3320, 3180, 1620, 1565, 1530 and 1505 cm^{-1} ; UV (0.01 N H_2SO_4) max 230 nm (ϵ 22,940), max 274 nm (ϵ 17,670); nmr ($d_7\text{DMF}$) δ 3.78 (m, 4, $\text{OCH}_2\text{CH}_2\text{N}$), δ 4.10~4.70 (m, 5, CHO), δ 6.11 (d, 1, anomeric H), δ 6.70 (d, 1, $\text{CH}=\text{N}$), δ 7.40 (d, 1, $\text{NCH}=\text{N}$), δ 8.18 (s, 1, $\text{NCH}=\text{N}$).

Anal. Calcd. for $\text{C}_{13}\text{H}_{13}\text{N}_4\text{O}_5$: C, 50.31; H, 5.84; N, 18.06.

Found: C, 50.13; H, 5.83; N, 18.11.

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