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Structural Elucidation of Tubercidin[1]

The nucleoside-type antibiotic, tubercidin, was isolated from *Streptomyces tubercidicus* by Anzai and Marumo[2] and has been assigned the 4-amino-7-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine structure (I).[3] The assignment of β-configuration to the glycosyl linkage is ambiguous. The authors are now completing an unambiguous structural elucidation of this nucleoside, employing a new method for the preparation of 7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidines.

Reaction of heavy metal salts of 4-chloro- or 4-aminopyrrolo[2,3-*d*]pyrimidine[4] with poly-O-acyl-D-ribofuranosyl chloride, a method which has been employed successfully for nucleoside synthesis[5] gave an intractable resinous mixture. Another approach would be to employ a series of reactions as shown in Chart 1: condensation of 4-amino-5-(2,2-diethoxyethyl)-6-hydroxy-pyrimidine(III)[4] with 2,3,4-tri-O-acetyl-5-O-trityl-D-ribose (IV),[6] should yield V which, after removal of the acyl blocking groups to give VI, should form the hemiacetal structures (VIa) and (VIb). Subsequent ring closure (pyrrole ring formation) followed by de-tritylation should yield Xa or Xb. Replacement of the amino group of tubercidin (I) by hydroxyl should yield Xb if, as Suzuki and Marumo proposed,[3] tubercidin is a *beta* nucleoside.

III (prepared by Traube type condensation[7] of ethyl 2,2-diethoxyethylcyanoacetate with formamidine acetate,[8] m.p. 196~197° (recrystallized from ethanol[9]) lit.,[4] 185~186°; UV : λ_{max}^{EtOH} 261 mμ; Rf : 0.66 (BuOH-H_2O=84:16)[10]; *Anal.* Calcd. for $C_{10}H_{17}O_3N_3$: C, 52.58; H, 7.54; N, 18.49. Found : C, 53.12; H, 7.79; N, 18.54), was treated with IV (m.p. 56~60°) in refluxing ethanol in the presence of a trace of ammonium chloride for 5 hours[11] to give V (Rf : 0.48 (BuOH-H_2O=84:16); UV : λ_{max}^{EtOH} 257 mμ) along with 4-hydroxy-1*H*-pyrrolo[2,3-*d*]pyrimidine[12] as needles which remained undissolved after chloroform extraction. The chloroform solution of V, after removal of the solvent gave a residue which was, without further purification, treated with methanol saturated with ammonia at 0° for two days. Removal of the solvent gave VI which was acetylated with acetic anhydride and pyridine at 0°. Excess acetic anhydride was removed by distillation *in vacuo* with ethanol to afford VII. VII in chloroform was applied to an alumina column. The first fraction eluted by benzene contained ethyl trityl ether, m.p. 81~82°. *Anal.* Calcd. for $C_{21}H_{20}O$: C,87. 50; H, 6.92. Found: C, 87.38; H, 6.92; the second fraction eluted by benzene contained nitrogen-free sugar derivative (s) ; the third fraction eluted by chloro-

1) Synthetic Studies of Potential Antimetabolites (VIII). For preceding paper in this series, see ref. 18.
2) K. Anzai, S. Marumo : J. Antibiotics, Ser., A, **10**, 20 (1957).
3) S. Suzuki, S. Marumo : *Ibid.*, **14**, 34 (1961)
4) J. Davoll : J. Chem. Soc., **1960**, 131.
5) For a leading literature, J. Davoll, B.A. Lowy : J. Am. Chem. Soc., **73**, 1650 (1951).
6) H. Zinner : Chem. Ber., **86**, 317 (1953).
7) W. Traube : Ber., **26**, 2551 (1893); W. Traube : *Ibid.*, **37**, 4544 (1904).
8) E.C. Taylor, W.A. Ehrhart : J. Am. Chem. Soc., **82**, 3138 (1960).
9) Occassionaly III melted at 185~186°.
10) Paper chromatography was performed using ascending technique; solvent systems employed : BuOH-H_2O (84 : 16); water adjusted to pH 10 with ammonia; EtOH-NH_4OH-H_2O (80 : 4 : 16).
11) The condition is similar to that used by Kenner, *et al.* for the condensation of 4,6-diamino-2-methylmercarptopyrimidine with 2,3,4-tri-O-acetyl-5-O-benzyl-D-ribose. G.W. Kenner, C.W. Taylor, A.R. Todd : J. Chem. Soc., **1949**, 1620.
12) Mode of the formation of 4-hydroxy-1*H*-pyrrolo[2,3-*d*]pyrimidine is not clear, but presumably it may come from 4-amino-5-formylmethyl-6-hydroxypyrimidine resulting from acid catalyzed acetal exchange between III and IV.

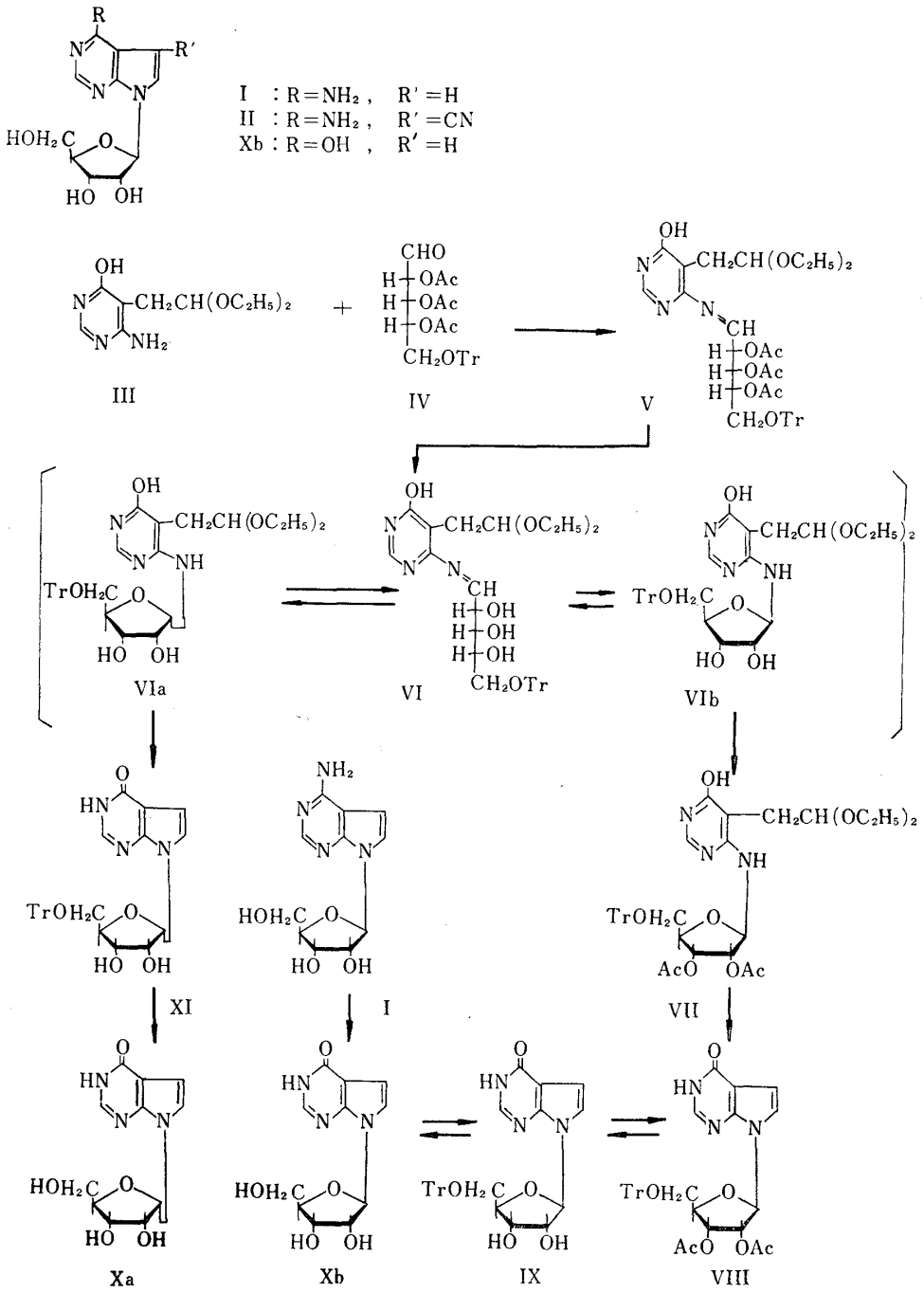
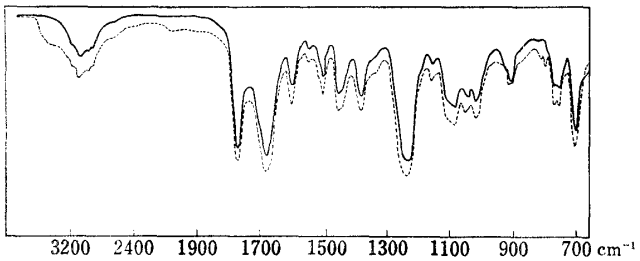
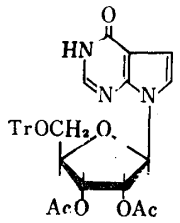


Chart 1.

form gave after removal of the solvent *in vacuo* purified Ⅶ (21% yield on the basis of Ⅲ), m.p. 231~232° (recrystallized from ethanol-petr. ether), *Anal.* Calcd. for $C_{38}H_{43}O_9N_3$: C, 66.56; H, 6.27; N, 6.13. Found : C, 66.80; H, 6.42; N, 6.57. IR ν_{max}^{KBr} cm^{-1} : 3330 (imino), 1770 (acetyl), 1500 (heteroaromatic ring), 770, 750, 700 (monosubstituted benzene) ; UV : λ_{max}^{EtOH} 260 mμ. Ⅶ was treated with a mixture (pH 2.8) of dioxane and 80% aqueous acetic acid for 2 hours at room temperature to cause pyrrole ring closure to Ⅷ which was purified by repeated reprecipitation (ethanol and petr. ether) to give a glass, *Anal.* Calcd. for $C_{34}H_{31}O_7N_3$: C, 68.80; H, 5.23; N, 7.08. Found : C, 68.73; H, 5.20; N, 7.26; $[\alpha]_D^{19.5}$ +22.2° (c=0.45, MeOH); UV : λ_{max}^{MeOH} 260 mμ. IR in KBr is given in Fig. 1, along with that of





VIII

Fig. 1.

—— VIII (of natural origin)

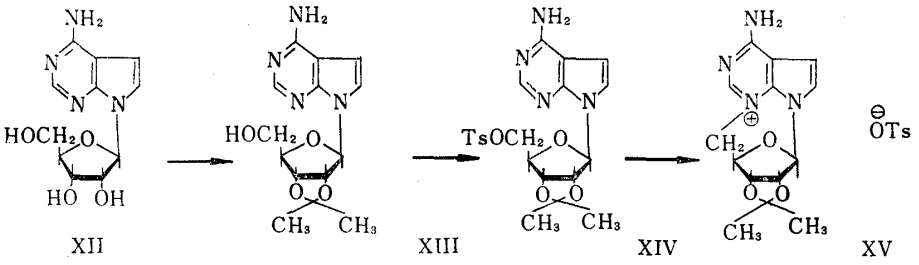
------ *de novo* synthetic VIII

a sample (VIII)[13] derived from tubercidin *via* Xb and IX, by acetylation of IX with acetic anhydride and pyridine in the presence of sodium acetate which was in turn prepared by tritylation of Xb.[3] *Anal.* Calcd. for $C_{34}H_{31}O_7N_3$ (natural origin); C, 68.80; H, 5.23; N, 7.08. Found : C, 69.12; H, 5.57; N, 6.98; $[\alpha]_D^{19.5}$ +23.6° (c=0.55, MeOH); UV : λ_{max}^{MeOH} 259 mμ (ε 8.5×10^3). The *de novo* synthetic VIII was identical with the sample of natural origin on the basis of criteria of infrared and ultraviolet spectral comparison and specific rotation. The *de novo* synthetic VIII was treated with methanol saturated with ammonia at 0° for two days at room temperature. After removal of the solvent *in vacuo* IX was obtained as a glass, $[\alpha]_D^{18}$ +19.91° (c=0.57, EtOH) which was in turn treated with boiling aqueous acetic acid (80%) for 15 min. to give Xb (4-deaminohydroxytubercidin, 7-deazainosine), m.p. 242~243° (decomp.); Rf : 0.19 (BuOH-H_2O=84:16 v/v); 0.62 (H_2O adjusted to pH 10 with NH_4OH); UV: $\lambda_{max}^{H_2O}$ 259 mμ (ε 8.5×10^3); $[\alpha]_D^{18.5}$ −6.7 (c=0.89, H_2O). *Anal.* Calcd. for $C_{11}H_{13}O_5N_3$: C, 49.43; H, 4.86, N, 15.72. Found : C, 49.40; H, 4.92; N, 15.57.

VI, without acetylation, was subjected to pyrrole ring closure at pH 2.8~3.0 with acetic acid to XI which was, without purification, treated with boiling aqueous acetic acid to afford Xa which was purified by ion exchange chromatography (Amberlite IRA 400 OH^- form) and subsequent carbon treatment essentially according to Suzuki and Marumo.[3] Xa was obtained as a glass, $[\alpha]_D^{20}$ +70° (c=0.2, H_2O); Rf: 0.89 (H_2O adjusted to pH 10 with NH_4OH). *Anal.* Calcd. for $C_{11}H_{13}O_5N_3$: C, 49.43; H, 4.86; N, 15.72. Found : C, 49.00; H, 5.02; N, 15.48. Elementary analyses and ultraviolet absorption spectral comparison failed to differentiate between Xa and Xb (Table I).

Table I.

	Rf (H_2O, pH 10)	$[\alpha]_D$	m.p. (°C)	λ_{max} mμ
Xb (*de novo* synthetic)	0.62	−6.72	242~243	259
Xb (of natural origin)	0.62	−5.20	242~243	259
Xa	0.89	+70.0	glass	259



XII XIII XIV XV

Chart 2.

13) The reaction condition of the preparation of this sample is similar to that employed by H. Zinner for the preparation of 2,3,4-tri-O-acetyl-5-O-trityl-D-ribose diethyl dithioacetal from D-ribose diethyl dithioacetal.[14] Overal yield of VIII (of natural origin) from Xb was 91.1%.

14) H. Zinner : Chem. Ber., **86**, 496 (1953).

These facts suggest that Xa possesses α-glycosyl linkage, while Xb and accordingly tubercidin have the β-configuration. A definite decision about the glycosyl linkage of tubercidin could be reached from the series of reactions shown in Chart 2 and Table II.

Table II.

	Rf (EtOH-NH_3-H_2O = 80 : 4 : 16	λ_{max}^{MeOH} mμ
2′,3′-O-Isopropylidene-5′-O-tosyladenosine (XVI)[15]	0.78	260
Quaternized XVI (XVII)[15]	0.53	271
2′,3′-O-Isopropylidene-5′-O-tosyltubercidin (XIV)	0.76	272
Quaternized XIV (XV)	0.51	281

Tubercidin (I) was converted to 2′,3′-O-isopropylidene derivative (XIII) by a reported precedure.[16] Rf : 0.43 (H_2O, pH 10); 0.38 (EtOH-NH_3-H_2O=80:4:16). These ultraviolet absorbing spots failed to give a positive test with periodate spray reagent. The ultraviolet spectrum of XIII was similar with that of I. XIII was then converted to 5′-O-tosylate (XIV), UV: λ_{max}^{MeOH} 272 mμ (ε 9.6×10^3). Paper chromatography of XIV gave a single spot; Rf : 0.76 (EtOH-NH_3-H_2O=80:4:16). Treatment of XIV with boiling acetone gave rise to XV whose ultraviolet absorption spectrum showed bathochromic shift (by 11 mμ) characteristic of intramolecularly quaternized nucleosides.[17,18] Rf : 0.51 (EtOH-NH_3-H_2O =80:4:16). Chromatographic behavior of XV was also very similar to that of intramolecularly quaternized derivative of 2′,3′-O-isopropylidene derivative (XVIII) (Table II). This type of intramolecular quaternization is feasible only with 5′-O-tosylate of the nucleoside having β-glycosyl linkage.[17,18]

These reactions established the configuration at the glycosyl center of tubercidin (I) as *beta*. The total syntheses of toyocamycin (II)[19] as well as tubercidin (I) are in progress in our laboratory.

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15) XVI and XVII were prepared according to the method reported by V.M. Clark, *et al.*[17]
16) A. Hampton, D.I. Magrath : J. Am. Chem. Soc., **79**, 3250 (1957).
17) V.M. Clark, A.R. Todd, J. Zussman : J. Chem. Soc., **1951**, 2952.
18) Y. Mizuno, M. Ikehara, T. Itoh, K. Saito : This Bulletin., **11**, 265 (1963).
19) K. Ohkuma : J. Antibiotics, Ser., A, **14**, 343 (1961).