9-β-D-Ribopyranosylhypoxanthine, A Minor Component Produced by *Streptomyces Antibioticus*

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9-β-D-Ribopyranosylhypoxanthine was isolated from fermentation broths of *Streptomyces antibioticus*. Its structure was proved by synthesis.

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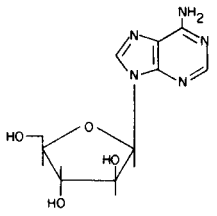
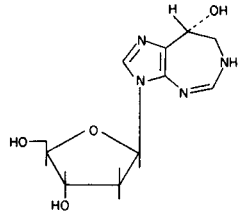
Soon after the discovery that various strains of *Streptomyces antibioticus* produce 9-β-D-arabinofuranosyladenine (Vira-A®, vidarabine) (**1**), we detected in the same fermentation beers the presence of a powerful inhibitor of adenosine deaminase (adenosine aminohydrolase). Initial efforts to isolate this inhibitor, which is named pentostatin (formerly called covidarabine), afforded very low recoveries of pentostatin (2). However, sufficient crystalline material was obtained to establish its structure as (*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (**2**). This nucleoside possesses an unusual heterocyclic moiety which is also present in the closely related adenosine deaminase inhibitor, coformycin, the D-ribofuranosyl analog of pentostatin. The structures of pentostatin and coformycin were independently elucidated at nearly the same time by Woo, *et al.*, (3) and Nakamura, *et al.*, (4), respectively.

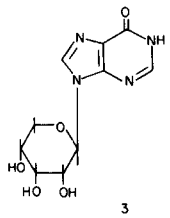


Vidarabine 1



Pentostatin 2

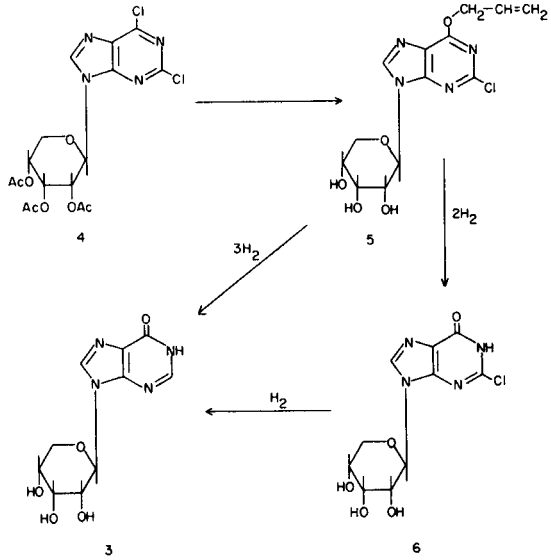
During the final steps of the original procedure (2) used to isolate pentostatin, another crystalline compound was encountered. This latter material was separated from **2** by fractional crystallization and, as described below, identified as 9-β-D-ribopyranosylhypoxanthine (**3**).



3

The molecular formula established for **3** shows it is isomeric with inosine. Although their ultraviolet properties are identical, **3** was clearly differentiated from inosine by its optical rotation and a comparison of respective ir and pmr spectra. The large coupling constant (J = 10 Hz) displayed by the anomeric proton of **3** at δ 5.75 was significant and suggested the presence of a pyranosylpentose moiety having axial protons at C-1′ and C-2′; e.g., $J_{1',2'}$ = 10 Hz in α-L-lyxopyranosylhypoxanthine (5). Inspection of the literature revealed that the pmr spectral properties reported by Townsend, *et al.*, (6) for 9-β-D-ribopyranosyladenine were similar to those exhibited by **3**. The detection of ribose and hypoxanthine in acidic hydrolyzates of **3** confirmed its nucleosidic character. The above data strongly suggested that **3** is D- or L-9-β-ribopyranosylhypoxanthine. To establish beyond doubt the structure of **3** which, to our knowledge, is the first reported pentopyranosylpurine of microbial origin we prepared 9-β-D-ribopyranosylhypoxanthine (1,7-dihydro-9-β-D-ribopyranosyl-6*H*-purin-6-one) by an unequivocal synthesis.

2,6-Dichloro-9-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)-9*H*-purine (**4**) was prepared from 1,2,3,4-tetra-*O*-acetyl-D-ribopyranose and hypoxanthine as reported by Townsend, *et al.*, (6). Treatment of **4** with sodium allyl oxide in allyl alcohol replaced the chlorine at position 6 with an allyl group, and removed the acetyl groups to yield 2-chloro-6-(2-propenyloxy)-9-β-D-ribopyranosyl-9*H*-purine



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(**5**) in 88% yield. The use of sodium allyl oxide in the relatively low boiling allyl alcohol (b.p. 97°) rather than the usual procedure of sodium benzyl oxide in benzyl alcohol (b.p. 206°) allowed the use of milder workup conditions. Catalytic hydrogenolysis of **5** using palladium/carbon caused the rapid uptake of two equivalents of hydrogen (involving deallylation and apparently subsequent hydrogenation of the liberated propene to propane) and afforded 2-chloro-9-β-D-ribopyranosylhypoxanthine (**6**). Addition of fresh catalyst allowed the slow uptake of another equivalent of hydrogen to provide **3** in excellent overall yield. Catalytic hydrogenolysis of a sample of **6** also provided **3**.

An undepressed mixed melting point, comparisons of the ir and pmr spectra and the optical rotations of **3** isolated from *Streptomyces antibioticus* beers and the final product (**3**) obtained from the above synthesis showed that the two compounds were identical. Compound **3** is devoid of significant *in vitro* antibacterial, antitumor, and antiviral activity.

EXPERIMENTAL (7)

Isolation of Pentostatin (**2**) and 9-β-D-Ribopyranosylhypoxanthine (**3**).

The preparation and fractionation of fermentation beers from *Streptomyces antibioticus* have been described (2). The crude lyophilyzed concentrate (193 g.) obtained from the final Sephadex G-10 step (described in column 13 of reference 2a) was dissolved in hot methanol (400 ml.). The resulting solution was concentrated to 300 ml. and after standing at 5° yielded three crops of crystals (11.5 g., 28.5 g., and 1.8 g.). The first crop (11.5 g.) was shown to be nearly pure **3** by thin-layer chromatography (silica gel, chloroform-methanol, 1:1), m.p., and uv and pmr spectra. The third crystal crop (1.8 g.) was shown to be nearly pure **2** by the same criteria. A final analytical sample of **3** was obtained by recrystallizing 1.0 g. from 200 ml. of methanol containing 20 ml. of water: m.p. 245-246° dec.; uv λ max (*p*H 1): 248 nm (ε 12450); λ max (*p*H 13): 252 (ε 13150); $[\alpha]_D^{23}$ -37° (c 1.03, 0.1*N* sodium hydroxide); pK_a' (water): 8.7 (equiv. wt. = 270 ± 10); pmr (deuterium oxide): δ 8.30 (s, 1, H_8), 8.15 (s, 1, H_2), 5.75 (d, 1, J = 10 Hz, H_1'), ~ 4.35 (m, 2, H_2', H_3'), ~ 3.95 (m, 3, H_4', H_5'ae).

Anal. Calcd. for $C_{10}H_{12}N_4O_5$ (268.33): C, 44.78; H, 4.51; N, 20.89. Found: C, 44.66; H, 4.57; N, 20.72; mol. ion, 268 (parent ion, 136 $[C_5H_4N_4O]^+$).

2-Chloro-6-(2-propenyloxy)-9-β-D-ribopyranosyl-9*H*-purine (**5**).

A solution of **4** (6) (2.24 g., 5.0 mmoles), sodium hydride (126 mg., 5.25 mmoles, from 252 mg. of 50% sodium hydride-oil dispersion, washed twice with hexane) in dry allyl alcohol (10 ml.), and dry allyl alcohol (25 ml.) was rapidly heated to reflux, cooled at 0° for several hours, and filtered to remove precipitated sodium chloride. The filtrate was treated with Dowex 50 x 8 (H^+) (*ca.* 20 ml.), filtered, and the filtrate evaporated *in vacuo* to furnish a white foam. The foam was co-evaporated twice *in vacuo* with ethanol and the residue was recrystallized from ethanol-water to provide **5** (1.5 g., 88%) as white crystals, m.p. 197-198° dec. (after drying at 100° for two hours); $[\alpha]_D^{23}$ +9.4° (c 1.02, DMF); λ max (*p*H 1, 7, and 12): 258 nm (ε 12500) sh 267; pmr (DMSO-d_6-deuterium oxide): δ 3.7 (m, 3, H_4' + H_5'a,e) 4.15 (m, 2, H_2' + H_3'), 5.05 (d, 2, O-CH_2-CH = CH_2, J = 5 Hz), 5.2 - 5.5 (m, 2, O-CH_2- CH = CH_2), 5.65 (d, 1, H_1', J = 9 Hz), 6.10 (m, 1, OCH$_2$C*H* = CH_2), 8.58 (s, 1, H_8).

Anal. Calcd. for $C_{13}H_{15}ClN_4O_5$ (342.74): C, 45.55; H, 4.38; N, 16.35. Found: C, 45.65; H, 4.69; N, 16.38.

2-Chloro-9-β-D-ribopyranosylhypoxanthine (**6**).

A mixture of **5** (3.7 g., 10.8 mmoles), 10% palladium on charcoal (500 mg.), triethylamine (1.10 g., 10.86 mmoles), and methanol (150 ml.) was stirred in an atmospheric hydrogenator for 35 minutes, at which time two equivalents of hydrogen had been consumed and uptake had essentially ceased. The mixture was filtered and the filtrate was absorbed on silica gel (10 g.) and placed on a silica gel column (150 g., packed in chloroform-methanol, 1:1). Elution with chloroform-methanol (1:1) and evaporation *in vacuo* of the fractions containing the product provided 2.8 g. (86%) of pure **6**. A portion was recrystallized from ethanol to furnish an analytical sample, m.p. 218° dec. (after drying at 100° for two hours); $[\alpha]_D^{23}$ -22.20° (c 1, water); uv λ max (*p*H 1): sh 247 nm (ε 11700) 251 (ε 11900); λ max (*p*H 7): 255 (ε 13100); λ max (*p*H 12) 254 (ε 13200); pmr (DMSO-d_6); δ 3.62 (m, 3); δ 4.05 (m, 2); δ 5.15 (bs, 3, OH); δ 5.48 (d, 1, J = 9 Hz, H_1'); δ 8.20 (s, 1, H_8).

Anal. Calcd. for $C_{10}H_{11}ClN_4O_5$ (302.68): C, 39.68; H, 3.67; N, 18.51. Found: C, 39.35; H, 3.82; N, 18.31.

9-β-D-Ribopyranosylhypoxanthine (**3**).

A. A mixture of **6** (2.0 g., 6.6 mmoles), 10% palladium on charcoal (500 mg.), and 0.2 *N* sodium hydroxide solution (66 ml., 13.2 mmoles) was stirred in an atmospheric hydrogenator for 0.5 hour. Approximately one equivalent of hydrogen had been consumed and uptake had ceased at this point. The mixture was filtered and the filtrate was evaporated *in vacuo* to *ca.* 20 ml. and was treated with acetic acid (0.38 ml., 6.6 mmoles). Crystallization proceeded *ca.* three hours later and was allowed to continue for *ca.* 24 hours. The product was collected, washed with cold 50% aqueous ethanol, and dried (100°, 0.5 torr for two hours) to provide 1.61 g. (91%) of **3**, m.p. 242-243° dec. with prior browning (a mixed m.p. with **3** isolated above was 242-243° dec.); uv λ max (*p*H 1): 248 (ε 12200), λ max (*p*H 13): 253 (ε 13400); $[\alpha]_D^{23}$ -37.8° (c 1.01, 0.1*N* sodium hydroxide). The ir and pmr spectra of this compound are identical to the corresponding spectra of the product **3** isolated from *S. antibioticus* beers.

Anal. Calcd. for $C_{10}H_{12}N_4O_5$ (268.24): C, 44.77; H, 4.51; N, 20.88. Found: C, 44.37; H, 4.46; N, 20.88.

B. A mixture of **5** (2.0 g., 5.8 mmoles), 10% palladium on charcoal (270 mg.), triethylamine (596 mg., 5.9 mmoles), and methanol (150 ml.) was stirred in an atmospheric hydrogenator for 40 minutes. Approximately two equivalents of hydrogen had been consumed and uptake had essentially ceased at this point. Tlc (silica gel, chloroform-methanol, 4:1) indicated a clean conversion of **5** to **6**. An additional 270 mg. of 10% palladium on charcoal was added and stirring in the hydrogen atmosphere was continued until uptake had ceased. This required *ca.* 1.5 hours and amounted to *ca.* one additional equivalent of hydrogen. The mixture was filtered and the filtrate was adsorbed on silica gel (5 g.) and placed on a column of silica gel (75 g., packed in chloroform-methanol, 1:1). Elution with chloroform-methanol (4:1) and evaporation of the fractions containing the product furnished 1.4 g. (90%) of **3**. This material was recrystallized from ethanol and dried to provide 1.26 g. (81%) of **3** from two crops, m.p. dec. *ca.* 250° with prior

browning. This material was identical to **3** prepared in A.

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