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NUCLEOSIDES—XCV

TOTAL SYNTHESIS OF PENTOPYRANAMINE D, THE NUCLEOSIDE MOIETY OF BLASTICIDIN H[a,b]

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Abstract—1-(4-Amino-3,4-dideoxy-β-D-*ribo*hexopyranosyluronic acid)cytosine was synthesized and its identity with the natural product pentopyranamine D was established.

Recently, Seto[1,2] isolated several cytosine nucleosides (pentopyranines (A → F) from the fermentation broth of *Streptomyces griseochromogenes*. The proposed[1] structures of pentopyranine A and C as 1 - (2,3 - dideoxy - α - L - *glycero*pentopyranosyl) - cytosine and 1 - (3 - deoxy - α - L - *threo*pentopyranosyl)cytosine, respectively, were confirmed[3,4] by total syntheses. Pentopyranine E and F were assigned[2] structures of 1 - (α - L - arabinopyranosyl)cytosine and 1 - (β - D-xylopyranosyl)cytosine, respectively by direct comparison to known[5] nucleosides. Seto *et al.* also isolated two new nucleosidic products, pentopyranic acid[6] and blasticidin H,[7] from the culture broth of *S. griseochromogenes*. The structure of the former compound was firmly established as 1-(β-D-glucopyranosyluronic acid)cytosine by chemical synthesis.[8] The latter product is an immediate biogenetic precursor of blasticidin S. Pentopyranamine D, the nucleoside moiety of blasticidin H, was obtained by hydrolysis and assigned the structure **9** [1 - (4 - amino - 3,4 - dideoxy - β - D - *ribo*hexopyranosyluronic acid)cytosine] on the basis of physico-chemical studies.[7] From these structural assignments, Seto *et al.*[6] proposed a reaction sequence for the biosynthesis of the important nucleoside antibiotic, blasticidin S. It is noteworthy that the proposed structure of pentopyranamine D resembles closely that of C substance 1 - (4 - amino - 4 - deoxy - β - D - glucopyranosyluronic acid)cytosine,[9] a degradation product of the antibiotic gougerotin. We report herein the total synthesis of 1 - (4 - amino - 3,4 - dideoxy - β - D - *ribo*hexopyranosyluronic acid)cytosine (**9**) and its identity with the natural product, pentopyranamine D.

The known[10] 1 - (3 - deoxy - β - D - *xylo*hexopyranosyl)cytosine (**1**) was used as starting material. Treatment of **1** with triethylorthoformate in DMF containing dry hydrogen chloride according to the procedure of Zemlicka[11] afforded an epimeric mixture of orthoesters which were benzoylated and hydrolyzed to give the dibenzoylated **3**. Reaction of **3** with bis(4-methoxyphenyl)phenylchloromethane (MMTrCl) in pyridine followed by treatment with mesyl chloride yielded the crystalline 4'mesylate (**4**) in ~50% yield. Treatment of **4** with sodium azide in hexamethylphosphoric triamide (HMPT) gave the 4'-azido derivative **5** which without purification was detritylated with 80% aqueous acetic acid to crystalline **6**. Compound **6** was oxidized with chromic anhydride in aqueous acetic acid[12] to **7** which was debenzoylated directly to the free 4'-azido nucleoside **8**. Reduction of the 4'-azido substituent of **8** by hydrogenation with palladium-charcoal catalyst afforded 1 - (4 - amino - 3,4 - dideoxy - β - D - *ribo* - hexopyranosyluronic acid)cytosine **9**. The PMR spectrum of **9** was consistent with structure **9** (Fig. 1). The IR spectrum of the dihydrochloride of **9** was identical with that of pentopyranamine D dihydrochloride (Fig. 2). The optical rotation of the dihydrochloride of pentopyranamine D ($[\alpha]_D^{26}$ - 24° ± 3°, H_2O, $C = 0.08$) and of the synthetic material **9** ($[\alpha]_D^{26}$ – 27° ± 3, H_2O, $c = 0.09$) was determined.† These values establish the D-configuration for the natural product.

EXPERIMENTAL

M.ps were determined on a Thomas-Hoover capillary apparatus and were corrected. PMR spectra were obtained on a J.E.O.L.-JIM-PET-100 spectrometer with TMS as reference unless specified otherwise. Chemical shifts are reported in ppm (δ) and signals are described as s (singlet), d (doublet), t (triplet) and q (quartet). Values given for coupling constants are first order. IR spectra were recorded on a Perkin-Elmer Infracord using pressed KBr pellets. TLC was performed on microscope slides coated with silica gel GF_{254} (Merck) and spots were detected by UV absorbance or by spraying with 20% v/v H_2SO_4–EtOH and heating. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. and by Spang Microanalytical Laboratory, Ann Arbor, Mich.

N⁴ - *Benzoyl* - 1 - (2 - O - *benzoyl* - 3 - *deoxy* - β - D - *xylohexopyranosyl*)*cytosine* (**3**). **1**[10] (16 g, 0.055 mol) was dissolved in DMF (80 ml). To the soln was added triethylorthoformate (32 ml) followed by 10 M HCl in DMF (8 ml). After 20 min, solid $NaHCO_3$ (16 g) was added and the mixture was stirred overnight and then filtered through a Celite pad which was thoroughly washed with pyridine. The combined filtrate and washings were evaporated to dryness below 45° using a mechanical pump. The residue, was triturated with ether (100 ml) and the ether was discarded.

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[b]Presented in part to the Symposium on Antibiotic Sugars, Division of Carbohydrate Chemistry, American Chemical Society, 169th National Meeting, Philadelphia, Pennsylvania, U.S.A. April 1975, Abstract. No. 21.

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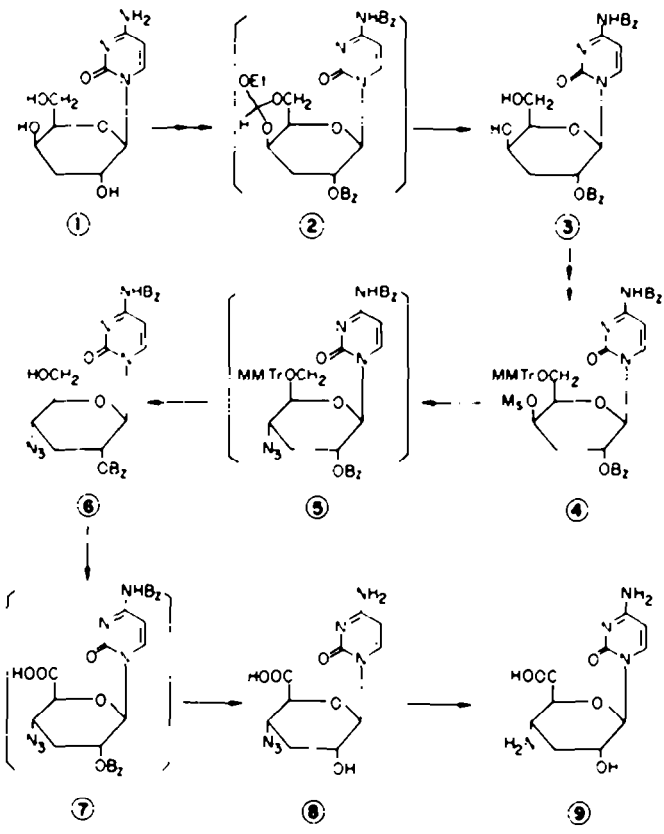

The colorless, sticky syrup (a single spot on TLC benzene–ethanol 9:1) was dissolved in 100 ml pyridine. After cooling the solution to 0°, benzoyl chloride (20 g, 0.14 mol) was added dropwise and the mixture was stirred for 30 min and then poured into ice water (800 ml). The ppt was collected by decantation of the supernatant. The residue was triturated with water (800 ml × 3), dissolved in chloroform (250 ml), dried over Na_2SO_4, and then evaporated to dryness to a syrup (2) which could not be crystallized from several solvents or mixtures of solvents.

The above syrup (2) was dissolved in methylene chloride (100 ml) and the soln was diluted with EtOH (90 ml) and water (10 ml). The mixture was acidified with conc. HCl (2 ml). After 20 min, the mixture was evaporated to dryness. Crystallization of the resulting colorless solid from EtOH afforded 3 as fine needles (18 g, 70.5%), m.p. 214° (sintered), 225–228° (eff). (Found: C, 61.85; H, 5.02; N, 9.00. Calc. for $C_{74}H_{71}N_5O_{17}$: C, 61.9; H, 4.98; N, 9.03).

N⁴ - *Benzoyl* - 1 - (2 - O - *benzoyl* - 3 - *deoxy* - 6 - O - *di* - p - *methoxytrityl* - 4 - O - *mesyl* - β - D - xylo*hexopyranosyl*)*cytosine*

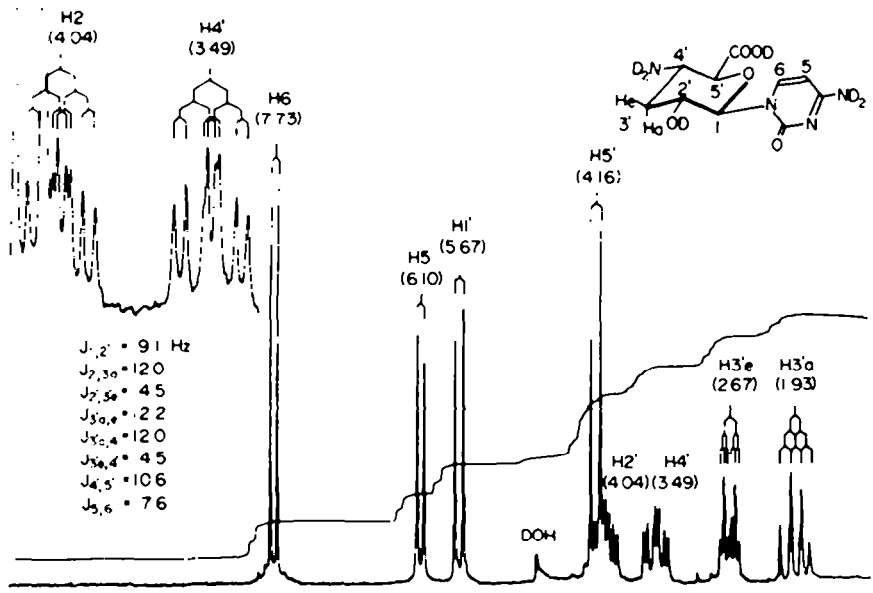

Fig. 1. PMR spectrum of synthetic pentopyranamine D (9) (D_2O). Chemical shifts are given in parentheses in parts per million (δ). DSS as the external standard.

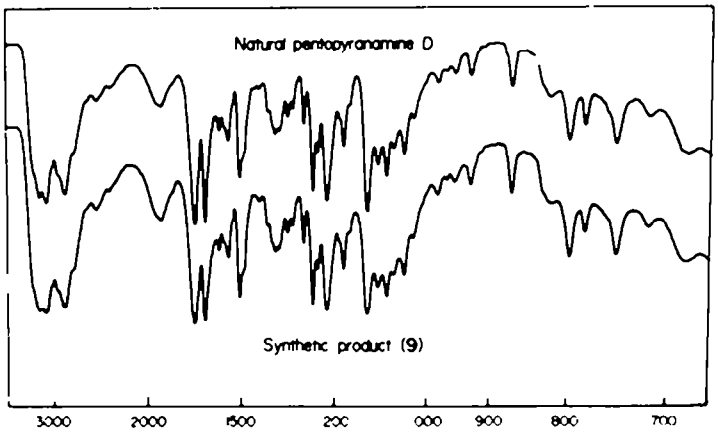

Fig. 2. IR spectra of dihydrochlorides of natural and synthetic pentopyranamine D (KBr).

(4). A mixture of 3 (13 g, 0.028 mol) and MMTrCl (10·1 g, 0.030 mol) in pyridine (150 ml) was stirred overnight at 45° and then heated to 60° for 4 hr. The mixture was cooled to 0° and mesyl chloride (4 ml, 0.05 mol) was added. After 4 hr at room temp. the mixture was poured into an ice-water mixture (800 ml). The solid ppt was collected by filtration, washed with water, dissolved in chloroform (200 ml), dried (Na_2SO_4), and evaporated to dryness. The solid residue was recrystallized from EtOH. Compound 4 (12 g, 51%) was obtained as colorless, fine needles, m.p. 194–200° (to a red liquid). PMR (DMSO-d_6) δ 2.53 (4H, mesyl CH_3 and H3'a), 3.74 (9 H, 2 anisyl CH_3, H3'e and H6', 6"), 4.61 (1H, H4'), 5.18 (1H, H5'), 5.35 (1H, H2'), 6.33 (1H, H1', $J_{1',2'}$ = 9.46), 6.8–8.2 (~25H, aromatic), 11.29 (1H, NHBz, dissociable). (Found: C, 65.71; H, 4.99; N, 4.79; S, 3.50. Calc. for $C_{46}H_{43}N_3O_{11}S$: C, 65.33; H, 5.09; N, 4.97; S, 3.79).

TLC examination (benzene–EtOH 9:1) showed that the mother liquor of recrystallization contained a considerable amount of detritylated product.

N^4-*Benzoyl*-1-(4-*azido*-2-O-*benzoyl*-3,4-*dideoxy*-6-O-*di*-p-*methoxytrityl*-β-D-ribo*hexopyranosyl*)*cytosine* (5). A mixture of 4 (8.5 g, 0.01 mol) and sodium azide (2.6 g, 0.04 mol) in HMPT (30 ml) was heated at 70° with stirring overnight. The mixture was poured into an ice-water mixture (400 ml) containing a few drops of pyridine. The ppt was collected by filtration, washed with water (400 ml containing a few drops pyridine), and then dissolved in chloroform (250 ml). The chloroform soln was washed with water (250 ml × 3), dried over Na_2SO_4, and evaporated to dryness to afford a colorless powder (5). The IR spectrum of this sample exhibited a strong absorption band at 2.160 cm^{-1} characteristic of the azido group. This product 5 was not purified further, but used directly in the next step.

N^4-*benzoyl*-1-(4-*azido*-2-O-*benzoyl*-3,4-*dideoxy*-β-D-ribo*hexopyranosyl*)*cytosine* (6). Compound 5 (5.0 g, 0.0063 mol) was dissolved in AcOH (16 ml). The soln was diluted with water (4 ml) and stirred at room temp. for 2 hr. After evaporation of the solvent, the residue was triturated with ether. The colorless solid was collected by filtration and crystallized from EtOH to afford 6 (2.6 g, 84%) as colorless needles, m.p. 163° (sintered), 200–217° (eff). PMR (DMSO-d_6), δ 2.13 (1H, q, H3'a, $J_{3'a,3'e} \simeq J_{3'a,4'} \simeq$ 11.0 Hz), 2.71 (1H, m, H3'e), 3.7–4.0 (4H, m, H6', 6", H5', H4'), 4.95 (1H, t, OH dissociable), 5.31 (1H, sextet, H2', $J_{2',1'} \simeq J_{2',3'a} \simeq$ 9.2 Hz, $J_{2',3'e} \simeq$ 5.2 Hz), 6.15 (1H, d, H1', $J_{1',2'} \simeq$ 9.2), 7.3–8.1 (~11 H, m, aromatic), 8.28 (1H, d, H6, $J_{5,6} \simeq$ 7.6 Hz), 11.2 (1H, s, NHBz, dissociable). (Found: C, 58.55; H, 4.61; N, 17.00. Calc. for $C_{24}H_{22}N_6O_6$: C, 58.78; H, 4.49; N, 17.14).

1-(4-*Azido*-3,4-*dideoxy*-β-D-ribo*hexopyranosyluronic acid*)*cytosine* (8). To a soln of 6 (2.4 g, 0.005 mol) in glacial AcOH (15 ml) was added 15 ml of oxidizing agent [3-fold excess, prepared by dissolving CrO_3 (6.7 g) in water (10 ml), and diluting to 100 ml with AcOH. The mixture was stirred overnight at room temp. after which it was partitioned between chloroform (250 ml) and water (200 ml). The chloroform was dried (Na_2SO_4) and concentrated to dryness to give crude 7.

The crude, blocked nucleoside 7 was dissolved in MeOH (80 ml) and treated with 20 ml of 1N NaOH overnight at room temp. The MeOH was evaporated and the residue was partitioned between water and ether (100 ml, each). The aqueous layer was separated and acidified to pH ~ 1 with conc HCl and then extracted with ether (100 ml × 2). The aqueous layer was passed through a column of Dowex 1 × 8 (OH^-) (100–200 mesh, 4.5 × 20 cm) and the column was washed successively with water (6 l) and 0.1 N formic acid (4 l). Compound 8 was eluted with 0.1 N formic acid. The eluent was evaporated to dryness. Compound 8 was obtained as colorless, fine needles (289 mg) which were recrystallized from water; m.p. 220–225° (dec), IR, ν_{max}^{KBr}: 3400, 2900, 2160 (N_3), 1710, 1600, 1560, 1520, 1400 1270 and 1090 cm^{-1}. (Found: C, 40.34; H, 4.24; N, 28.11. Calc. for $C_{10}H_{12}N_6O_6$: C, 40.54; H, 4.05; N, 28.38).

1-(4-*Amino*-3,4-*dideoxy*-β-D-ribo*hexopyranosyluronic acid*)*cytosine* (9). The azido derivative 8 (192 mg) was dissolved in DMF (75 ml) and water (25 ml), and hydrogenated over 10% Pd–C (75 mg) for 15 min with the initial pressure at 23 × 10^3 kg m^{-2}. After removal of the catalyst by filtration, the filtrate was evaporated to dryness to a crystalline solid that was recrystallized from DMF–water. Compound 9 was obtained as colorless needles (122 mg, 70%), m.p. 246–248° (dec), PMR (Fig. 1). (Found: C, 44.23; H, 5.38; N, 20.55. Calc. for $C_{10}H_{14}N_4O_6$: C, 44.44; H, 5.19; N, 20.74).

The *dihydrochloride of* 9 was prepared by dissolving 9 (5 mg) in 0.2 ml of 1N HCl. After 2 days at room temp. the separated crystals were collected (3.2 mg). The IR spectrum of this sample was idential with that of the natural product (Fig. 2).

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