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THE STRUCTURES OF PENTOPYRANINE A AND C, TWO CYTOSINE NUCLEOSIDES WITH α-L-CONFIGURATION

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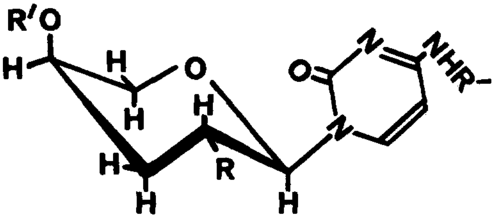
In continuing the search for intermediates lying on the pathway of blasticidin S biosynthesis1), hitherto unknown cytosine nucleoside complex comprising from six components has been isolated2) from the fermentation broth of *Streptomyces griseochromogenes*. This communication concerns with the structural elucidation of the two main components, designated pentopyranine A and C.

Pentopyranine C I, $C_9H_{13}O_4N_3$(M^+: m/e 227) mp. 143-145°C, $[\alpha]_D^{21}$+20° (c 1.2, H_2O) is a weak basic compound with pKa 4.2 and shows UV absorption spectrum [λ_{max} 278nm (ε=12,200) in 0.1N HCl, 270nm (ε=8,450) in 0.1N NaOH] almost identical with that of cytidine. These evidences together with the nmr spectral data [$\delta_{DSS}^{D_2O}$ 6.45 and 8.05 (each 1H, d, J=8Hz)] indicate that 1-substituted cytosine is the chromophore of I. The presence of a sugar moiety in I is shown by the IR spectrum (ν_{max}^{nujol} 3500-3200, 1140 cm^{-1}).

I was acylated to give triacetate II, $C_{15}H_{19}O_7N_3$(M^+: m/e 353), ν_{max}^{nujol} 1740, 1670, 1560, 1230 cm^{-1}, mp. 203°C, $\delta_{TMS}^{CDCl_3}$1.95(equatorial CH_3COO-), 2.15(axial CH_3COO-),3) 2.27(CH_3CON-), 7.50 and 7.80 (each 1H, d, J=7.5Hz) and tribenzoate III, $C_{30}H_{25}O_7N_3$(M^+: m/e 537), $\nu_{max}^{CHCl_3}$ 1720, 1675, 1560, 1270, 1120 cm^{-1}, $\delta_{TMS}^{CDCl_3}$~7.4 (2 x C_6H_5COO-) and ~7.9 (C_6H_5CON-), with acetic anhydride/pyridine and benzoyl chloride/pyridine, respectively. Since the amino group of cytosine nucleus in I was acylated under these conditions, there should exist two hydroxy groups in the sugar moiety of I.

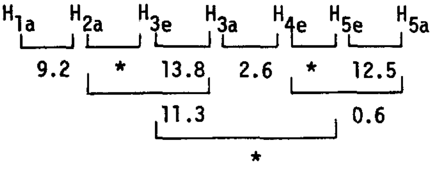
The nmr spectrum of I (see table) shows an anomeric proton, a methylene and four ill-resolved hydrogens attached to carbon bearing oxygen function. On benzoylation of I, two of the four hydrogens shifted to low field by 1.2-1.3 ppm and the rest remained without considerable changes in the chemical shift. (III, see table) This means that the latter two hydrogens bind to the carbon adjacent to the sugar ring oxygen and that the sugar takes pyranose form. The steric relation between H_1' and H_2' is determined to be *trans* diaxial based on the large coupling constants ($J_{1',2'}$=9.2Hz in I, 9.5 in III), and H_2' further coupled to the methylene protons. Small $J_{4',5a'}$ and $J_{3a',4'}$ clearly indicate that $H_{4'}$ is in equatorial position. It follows from these facts that I is 1-α-(3'-deoxy-L-arabinopyranosyl)-cytosine or its mirror image, 1-α-(3'-deoxy-D-arabinopyranosyl)-cytosine.

The absolute configuration of the sugar was determined to be L by comparing its 2,4-dinitrophenylosazone IV, $C_{17}H_{16}O_{10}N_8$ mp. 257-258°C (dec.) with an authentic sample of 3-deoxy-D-pentosone 2,4-dinitrophenylosazone[4)]. Whereas both compounds were completely identical in mps. and IR spectra, they showed opposite sign in optical rotation ($[\alpha]_D^{21}$authentic: +326°, c 0.218, lit 4, IV: -318° c 0.204, dioxane). Therefore, the structure of I is established as 1-α-(3'-deoxy-L-arabinopyranosyl)-cytosine as shown below (R=OH, R'=H).



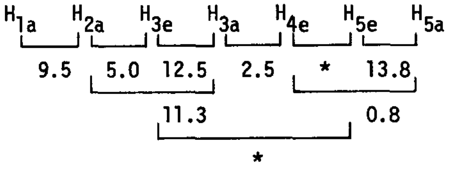
I (R=OH, R'=H) in D_2O/DCl

5.55 4.2 2.40 1.94 4.2 4.08 3.90



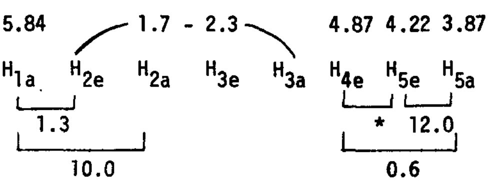
III (R=C_6H_5COO, R'=C_6H_5CO) in $CDCl_3$

6.25 5.52 2.52 2.26 5.40 4.37 4.06



VI (R=H, R'=CH_3CO) in $CDCl_3$

5.84 4.87 4.22 3.87

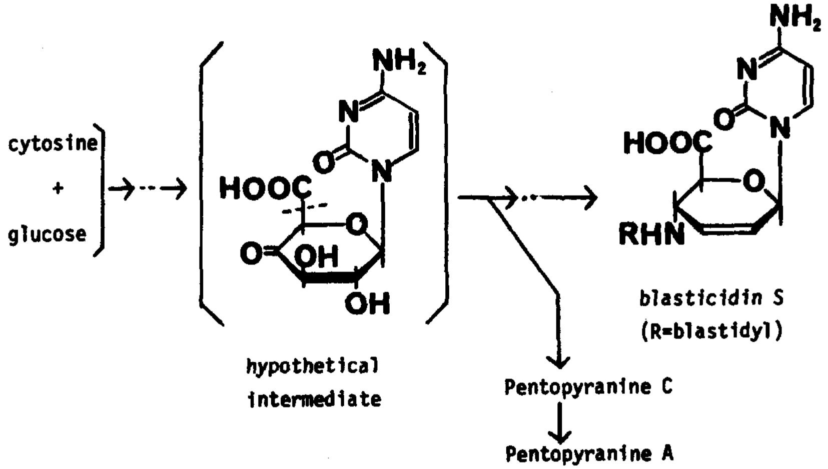


*Couplings between these protons (smaller than 2 Hz) were confirmed by spin decoupling experiments.

Pentopyranine A V, $C_9H_{13}O_3N_3$(M^+: m/e 211), mp. 258°C (dec.), $[\alpha]_D^{21}0°$ (c 0.75, H_2O) λ_{max} 278nm(ε=13,100) in 0.1N HCl 270nm(ε=8,850) in 0.1N NaOH, pKa 4.2, ν_{max}^{nujol} 3380, 3200, 1208, 1110 cm^{-1}, is very similar to I in physicochemical properties, but instead of triacetate, it gave diacetate VI, $C_{13}H_{17}O_5N_3$(M^+: m/e 295), mp. 201-202.5°C, ν_{max}^{nujol} 1710, 1670-1630, 1570, 1240 cm^{-1} $\delta_{TMS}^{CDCl_3}$ 2.13(axial CH_3COO-), 2.28(CH_3CON-) on acetylation with acetic anhydride/pyridine.

In the nmr spectrum of VI, an anomeric proton appeared as a doublet of doublets ($J_{1',2a'}$=10.0, $J_{1',2e'}$=1.3Hz) with negligible acyl shift. Through the comparison of the nmr spectra, the molecular formulae as well as the results of the acetylation between I and V, it becomes evident that one hydroxy group in I has been replaced by a hydrogen to give 2′,3′-dideoxy-pentopyranose taking Cl(L) conformation. The L configuration of the sugar was determined by comparing its 2,4-dinitrophenylhydrazone VII, $C_{11}H_{14}O_6N_4$(M^+: m/e 298), mp. 128-135°C, $[\alpha]_D^{21}$+2.7° (c 1.0, dioxane) with that of authentic 2,3-dideoxy-D-pentose, $[\alpha]_D^{20}$ -2.6° (c 1.0, dioxane) which was synthesized by the oxidation of 1,2-O-isopropylidene-(s)-pentane-1,2,5-triol$^{5)}$ followed by acid treatment. Thus, the structure of V is 1-α-(2′,3′-dideoxy-L-arabinopyranosyl)-cytosine.

Although various kinds of (deoxy)nucleosides and (deoxy)nucleotides have been isolated from nature as free forms$^{6)}$ and/or as components of DNA and RNA$^{7)}$, most of these possess β-D configuration. Therefore, to the best of our knowledge, pentopyranine A and C are the first naturally occurring nucleosides with α-L configuration and are presumed to be shunt pathway products of blasticidin S biosynthesis as shown below.



It may be of interest to note that an antibiotic cordycepin (3'-deoxyadenosine)[8] had been isolated from the fermentation broth of *Cordycepts militaris* as a sole 3'-deoxy nucleoside.

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*Author to whom inquiries should be adressed.

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