STUDIES ON THE BIOSYNTHESIS OF BLASTICIDIN S
V. ISOLATION AND STRUCTURE OF PENTOPYRANIC ACID

HARUO SETO, KAZUO FURIHATA and HIROSHI YONEHARA

Institute of Applied Microbiology, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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In the course of studies on the biosynthesis of blasticidin S,[1] a nucleoside antibiotic, we have isolated six novel cytosine nucleosides, named pentopyranines A to F, from the fermentation broth of *Streptomyces griseochromogenes*.[1,2,3] The structural relationship between these nucleosides and cytosinine, the nucleoside moiety of blasticidin S, implied the intermediacy of cytosine nucleosides with a hexuronic acid moiety (*e.g.* hypothetical intermediate **X**) in the biosynthesis of blasticidin S (see Fig. 1).

Accordingly, our attention was directed to the isolation of such nucleosides from the amphoteric fraction of the fermentation broth of *S. griseochromogenes*. As a result, we succeeded in the isolation of a cytosine nucleoside, designated pentopyranic acid, the structure of which has been determined to be 1-(β-D-glucopyranosyluronic acid)-cytosine, as described below.

Pentopyranic acid **I**, $C_{10}H_{13}O_7N_3$, is an amphoteric compound with pKa′ 2.3 (carboxylic acid) and 4.0 (amino group of cytosine nucleus) and showed UV absorption characteristic of 1-substituted cytosine [λ max 276 nm (ε 13400) in 0.01 N HCl, λ max 270 nm (ε 8000) in 0.01 N NaOH]. Although the presence of a sugar moiety in **I** was suggested by its ^{1}H-nmr spectrum at 100 MHz in conc.DCl, [δ^{DSS} 5.84: anomeric proton, intricately splitted multiplet (outer width ~8 Hz) due to high order coupling, 4.40: 1 H, intricately splitted multiplet (outer width ~9 Hz) due to high order coupling, 3.7~4.1: 3 H, envelope, 6.47 (d, $J_{5,6}$=8.0 Hz): H_5 of the cytosine nucleus and 8.08 (d, $J_{5,6}$=8.0 Hz): H_6 of the cytosine nucleus], the overlapping and high order coupling of these protons prevented to obtain detailed structural information.

On the other hand, the glucopyranosyluronic acid moiety in methyl pentopyrate tetra-acetate **II** ($C_{19}H_{23}O_{11}N_3$) was proved by the following ^{1}H-nmr spectral data and decoupling experiment: in D_6-DMSO, H-1: δ^{TMS} 6.31 (d, $J_{1,2}$=8.5 Hz), H-2: 5.45 (dd, $J_{1,2}$=8.5 Hz, $J_{2,3}$=9.0 Hz), H-3: 5.66 (t, $J_{2,3}$=9.0 Hz, $J_{3,4}$=9.0 Hz), H-4: 5.27 (dd, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), H-5: 4.78 (d, $J_{4,5}$=10.0 Hz), H-5 of the cytosine nucleus: 7.20 (d, $J_{5,6}$=7.5 Hz), H-6 of the cytosine nucleus: 8.36 (d, $J_{5,6}$=7.5 Hz), $\underline{CH_3}COO$: 1.86 (s, 3H), 2.00 (s, 6H), $\underline{CH_3}CON$: 2.10 (s, 3H), $COO\underline{CH_3}$: 3.75 (s, 3H), and $-NH\underline{C}OCH_3$: 10.93 (broad s, 1H). The coupling constants of these protons indicated that all the protons in the sugar moiety are situated in a *trans* diaxial relationship to each other, as shown in Fig. 2.

The following fragment ions observed in the EI mass spectrum (low resolution) of **II** confirmed the presence of a carboxylic acid in **I**: 410 ($M^+-CO_2CH_3$), 409 ($M^+-CH_3CO_2H$), 350 ($M^+-CO_2CH_3-CH_3CO_2H$), 349 ($M^+-2\times CH_3CO_2H$), 308 ($M^+-CO_2CH_3-CH_3CO_2H-CH_2CO$), 307 ($M^+-2\times CH_3CO_2H-CH_2CO$), 290 ($M^+-CO_2CH_3-2\times CH_3CO_2H$) and 248 ($M^+-CO_2CH_3-2\times CH_3CO_2H-CH_2CO$), relative intensity 11: 34: 100: 35: 33: 34: 93: 98, respectively.

Comparison of the CD spectrum of **I** with that of C-substance,[4] *i.e.* 1-(4-deoxy-4-amino-β-D-glucopyranosyluronic acid)-cytosine, showed that these two nucleosides possess the same absolute configuration. [in H_2O, **I**, 0.1 mg/ml, 217 nm ([θ]=−8300), 230 (0), 250 (+980), 260

Fig. 1

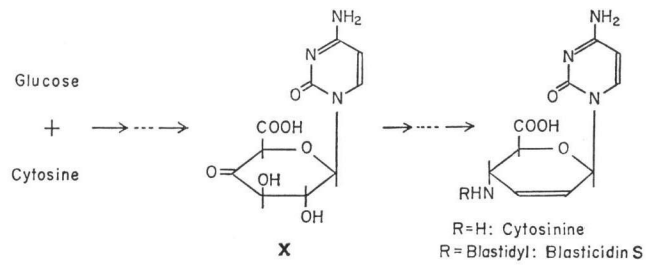
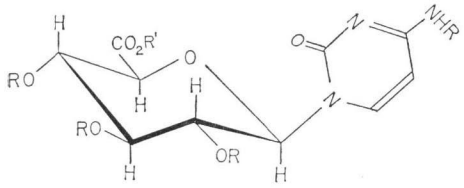


Fig. 2.



I, R=H, R'=H
II, R=Ac, R'=CH_3

(+370), 280 (+1700), 305 (0). C-substance, 0.1 mg/ml, 220 nm ([θ]=−6440), 235 (0), 250 (+1540), 257 (+770), 280 (+1820), 303 (0)].

Thus, the structure of **I** was determined as 1-(β-D-glucopyranosyluronic acid)-cytosine.

The complete identity of IR, UV, NMR spectra, optical rotation (in 0.01 N NaOH, $[\alpha]_D^{21}$ nat. +11°, synth. +12°, c 1.7) and m.p. of **I** and an authentic sample[5] corroborated the above conclusion.

The isolation of **I** from the fermentation broth of *S. griseochromogenes* is taken as an evidence that **I** is the direct precursor of a hypothetical intermediate **X** in Fig. 1 and that the oxidation of the hydroxymethyl group of the hexose moiety takes place prior to that at C-4.

An effort to isolate an intermediate situated between glucose and cytosine, and **I** is underway.

Experimental

Melting points, UV, mass and NMR spectra were taken as reported previously.[1] CD spectra were taken on a JASCO J-20 spectrometer.

Isolation of pentopyranic acid I

The fermentation broth of *S. griseochromogenes* was fractionated as described previously[2] and the amphoteric fraction was eluted with 0.5 N AcOH from a column of Amberlite IRA-410. The eluate was evaporated to dryness under reduced pressure and the residue was subjected to resin chromatography (Dowex-50W, X-2, 200~400 mesh, 2×90 cm, pyridine-formate buffer, pH 3.0, 0.2 M, each fraction 17 ml). Fractions 7~10 showing strong UV absorption were pooled and concentrated under reduced pressure. The dry residue was further purified by resin chromatography (Dowex-1, X-2, 2×90 cm, pyridine-acetate buffer, pH 5.0, 0.2 M each fraction 17 ml). Fractions 61~71 were combined and concentrated *in vacuo* to give crude crystals of **I** which were recrystallized from hot water. m.p. 215~228°C (dec.). Titration equivalent 340.

Anal. Calcd. for $C_{10}H_{13}O_7N_3 \cdot H_2O$: C, 39.35; H, 4.95; O, 41.93; N, 13.77.
Found: C, 39.57; H, 5.03; O, 41.91; N, 13.67.

Methyl pentopyranate tetra-acetate II

A solution of **I** (100 mg) in 50 ml of 5% methanolic hydrogen chloride was refluxed overnight and the reaction mixture was evaporated to dryness *in vacuo*. The dry residue was dissolved in distilled water and neutralized by the addition of Amberlite IR-4B. After removal of the resin by filtration, the filtrate was dried under reduced pressure to give a methyl ester of **I** (92 mg) which was, without purification, suspended in acetic anhydride (5 ml) added with five drops of pyridine. After 48-hour stirring, the reaction mixture was evaporated *in vacuo* to give **II** which was obtained as needles (55 mg) from methanol. Recrystallization from methanol gave an analytically pure sample (35 mg), m.p. 189~192°C.

Anal. Calcd. for $C_{19}H_{23}O_{11}N_3$: C, 48.41; H, 4.99; O, 37.53; N, 8.93.
Found: C, 48.61; H, 4.94; O, 37.49; N, 8.95.

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