NUCLEOSIDES. XCVI SYNTHESIS OF PENTOPYRANIC ACID*

KYOICHI A. WATANABE, DAVID H. HOLLENBERG and JACK J. FOX

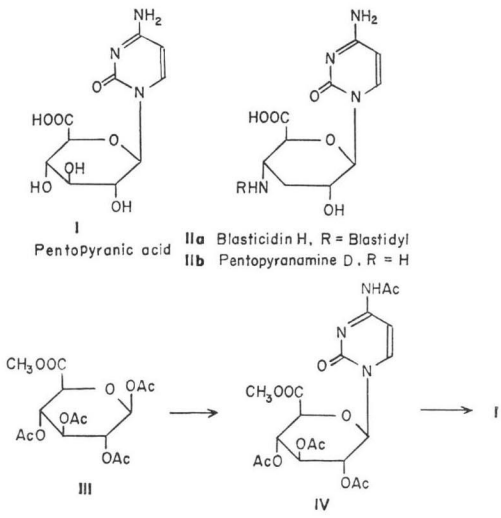
Laboratory of Organic Chemistry, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Institute, Sloan-Kettering Division of Graduate School of Medical Sciences, Cornell University, New York, N.Y. 10021, U.S.A.

(Received for publication January 9, 1976)

During the course of studies on the biosynthesis of blasticidin S, a nucleoside antibiotic, SETO[1,2] isolated a number of intermediate nucleosides, pentopyranines A~F, from the fermentation broth of *Streptomyces griseochromogenes*. The structures of pentopyranine A and C[3] were later confirmed by the total syntheses[4,5]. The structures of pentopyranine E and F were established[2] as 1-(α-L-arabinopyranosyl)cytosine and 1-(β-D-xylopyranosyl)cytosine, respectively, by direct comparison to known[6] nucleosides.

Very recently, two new nucleosidic products, pentopyranic acid (**I**)[7] and blasticidin H (**IIa**)[8] were isolated from the culture broth of *S. griseochromogenes*. The latter product is considered to be an immediate biogenetic precursor of blasticidin S. Pentopyranamine D, the nucleoside moiety of blasticidin H, was obtained by hydrolysis and assigned the structure **IIb**.[8] The isolation of these new nucleosides enabled SETO *et al.*[7] to propose a biosynthetic pathway of the important nucleoside antibiotic, blasticidin S.

We report herein the synthesis of 1-(β-D-glucopyranosyluronic acid)cytosine which has now been shown[7] to be identical with the natural product, pentopyranic acid (**I**). The synthesis



of **I** was achieved by condensation of methyl tetra-*O*-acetyl-β-D-glucopyranuronate (**III**) and bis(trimethylsilyl)-*N*-acetylcytosine by application of the procedure of NIEDBALLA and VORBRÜGGEN[9] to afford the protected nucleoside (**IV**). Acid hydrolysis of **IV** afforded **I**.

We had also synthesized 1-(4-amino-3,4-dideoxy-β-D-*ribo*hexopyranosyluronic acid)cytosine (**IIb**) in the course of synthetic studies on nucleoside antibiotics, gougerotin[10~13] and blasticidin S[14~16] and their analogs[17~20]. The synthesis of **IIb** and its identity with pentopyranamine D will be described elsewhere.

Experimental

N⁴-Acetyl-1-(methyl tri-*O*-acetyl-β-D-glucopyranosyluronate)cytosine (IV)

A mixture of N-acetylcytosine (3.06 g, 0.02 mol) and ammonium sulfate (3 mg) in hexamethyldisilazane (30 ml) was stirred and heated to reflux. When the reaction mixture became clear (2.5 hours), it was allowed to cool to room temperature. The excess hexamethyldisilazane was removed by evaporation using a mechanical vacuum pump. The residue was dissolved in 1,2-dichloroethane (100 ml) and added to a solution of methyl tetra-*O*-acetyl-β-D-glucopyranuronate[21] (**III**) (7.0 g, 0.019 mol) in 1,2-dichloroethane (50 ml). To the stirred solution was added $SnCl_4$ (5 ml) and the mixture was stirred overnight at room temperature and

* The authors acknowledge the support of the U.S. Public Health Service, Grant No. CA-08748 and CA-17085, and by a Fellowship from the Westchester Division of the American Cancer Society (to D.H.H.).

then heated to reflux for 1 hour. The mixture was allowed to cool to room temperature and then poured into saturated sodium hydrogen carbonate solution (250 ml). When the frothing ceased, the suspension was filtered through a Celite pad which was then thoroughly washed with dichloromethane. The organic layer was separated, washed with water (250 ml×2), dried (sodium sulfate), and evaporated to dryness. The residue was triturated with acetone (50 ml). The protected nucleoside (IV) (1.2 g) crystallized out as fine needles, mp 188~191°C.

Anal. Calcd. for $C_{19}H_{23}N_3O_{11}$: C, 48.61; H, 4.94; N, 8.95.
Found: C, 48.48; H, 5.17; N, 9.05.

From the mother liquor more nucleoside IV crystallized out, which was recrystallized from ethanol (420 mg, mp 188~191°C).

1-(β-D-Glucopyranosyluronic acid)cytosine [Pentopyranic Acid (I)]

The protected nucleoside (IV, 940 mg, 2 mmol) was dissolved in methanol saturated with HCl (10 ml). The mixture, after standing at room temperature for 1 hour was evaporated to dryness. The residue was dissolved in 6 N HCl (10 ml) and the solution was refluxed for 20 minutes, and then evaporated to dryness. The residue was dissolved in water (~10 ml) and the solution was passed through a column of Amberlite IR 45 (OH^-) (~35 ml, 10×3 cm, diam). The column was washed with water until the eluent exhibited no UV absorption. The UV absorbing fractions were combined and evaporated to dryness to a solid residue which was crystallized from water to give colorless crystals (362 mg, 63%), mp 215~230°C (with browning), 238~240°C (eff).

Anal. Calcd. for $C_{10}H_{13}N_3O_7 \cdot 2H_2O$: C, 37.16; H, 5.30; N, 13.00.
Found: C, 37.32; H, 5.23; N, 13.09.

For the identity of this sample with pentopyranic acid, see ref. 7.

References

1) Seto, H.: Isolation of pentopyranines A, B, C and D. Agr. Biol. Chem. 37: 2415~2419, 1973
2) Seto, H. & H. Yonehara: The structure of pentopyranines B, D, E, and F. Abstract 2G-3. Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, 1973
3) Seto, H.; N. Ōtake & H. Yonehara: The structures of pentopyranine A and C. Two cytosine nucleosides with α-L-configuration. Tetrahedron Lett. 1972: 3991~3994, 1972; Agr. Biol. Chem. 37: 2421~2426, 1973
4) Chiu, T. M. K.; H. Ohrui, K. A. Watanabe & J. J. Fox: Nucleosides. LXXXIV. Total synthesis of pentopyranine C, a nucleoside elaborated by *Streptomyces griseochromogenes*. J. Org. Chem. 38: 3622~3624, 1973
5) Watanabe, K. A.; T. M. K. Chiu, D. H. Hollenberg & J. J. Fox: nucleosides. LXXXVII. Total synthesis of pentopyranine A, an α-L-cytosine nucleoside elaborated by *Streptomyces griseochromogenes*. J. Org. Chem. 39: 2482~2486, 1974
6) Fox, J. J. & I. Goodman: The synthesis of nucleosides of cytosine and 5-methylcytosine. J. Am. Chem. Soc. 73: 3256~3258, 1951
7) Seto, H.; K. Furihata & H. Yonehara: Studies on the biosynthesis of blasticidin S. V. Isolation and structure of pentopyranic acid. J. Antibiotics 29: 595~596, 1976
8) Seto, H. & H. Yonehara: Studies on the isolation and structure of intermediates in the biosynthesis of blasticidin S. Abstract 1G-18, Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, 1975
9) Niedballa, U. & H. Vorbrüggen: A general synthesis of N-glycosides. I. Synthesis of pyrimidine nucleosides. J. Org. Chem. 39: 3654~3660, 1974
10) Watanabe, K. A.; M. P. Kotick & J. J. Fox: Nucleosides. LIX. Synthetic studies on nucleoside antibiotics. 1. Synthesis of 1-(4-amino-4-deoxy-β-D-glucopyranosyl) cytosines and their identities with products derived from gougerotin. Chem. Pharm. Bull. 17: 416~418, 1969
11) Watanabe, K. A.; M. P. Kotick & J J. Fox: Nucleosides. LXIII. Synthesis of 1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine, the nucleoside moiety of gougerotin. J. Org. Chem. 35: 231~236, 1970
12) Watanabe, K. A.; I. Wempen & J. J. Fox: Nucleosides. LXII. Synthetic studies on nucleoside antibiotics. 7. An improved synthesis of C-substance. Carbohyd. Res. 21: 148~153, 1972
13) Watanabe, K. A.; E. A. Falco & J. J. Fox: Nucleosides. LXXV. Synthetic studies on nucleoside antibiotics. 9. Total synthesis of gougerotin. J. Am. Chem. Soc. 94: 3272~3274, 1972
14) Goody, R. S.; K. A. Watanabe & J. J. Fox: Nucleosides. LXVI. Synthetic studies on nu-

cleoside antibiotics. 4. Synthesis of methyl 4-amino-2,3,4-trideoxy-β-D-*erythro*hex-2-enopyranosiduronic acid, the carbohydrate moiety of blasticidin S. Tetrahedron Lett. 1970: 293~296, 1970

15) WATANABE, K. A.; R. S. GOODY & J. J. FOX: Nucleosides. LXVIII. Synthetic studies on nucleoside antibiotics. 5. 4-Amino-2,3-unsaturated sugars related to the carbohydrate moiety of blasticidin S. Tetrahedron 26: 3883~3903, 1970

16) WATANABE, K. A.; I. WEMPEN & J. J. FOX: Nucleosides. LXIX. Synthetic studies on nucleoside antibiotics. 6. Synthesis of 1-(4-amino-2,3,4-trideoxy-β-D-*erythro*hex-2-enopyranosyl)-cytosine and derivatives related to the nucleoside moiety of blasticidin S. Chem. Pharm. Bull. 18: 2368~2371, 1970

17) WATANABE, K. A.; E. A. FALCO & J. J. FOX: Nucleosides. LXXIV. Synthetic studies on nucleoside antibiotics. 8. Synthesis of 1-[4-deoxy-4-(sarcosyl-D-seryl)amino-β-D-glucopyranosyl]-cytosine and related analogs of gougerotin. J. Org. Chem. 37: 1198~1201, 1972

18) CHIU, T. M. K ; D H. WARNOCK, K. A. WATANABE & J. J. FOX: Nucleosides. LXXXII. Synthetic studies on nucleoside antibiotics. 12 Synthesis of 1-[3,4-dideoxy-4-(sarcosyl-D-seryl)-amino-β-D-*ribo*-hexopyranosyl]cytosine, an analog of gougerotin. J. Heterocyclic Chem. 10: 607~610, 1973

19) WATANABE, K. A. & J. J. FOX: Nucleosides. LXXX. Synthetic studies on nucleoside antibiotics. 10. Synthesis of 1-[2,3,4-trideoxy-4-(sarcosyl-D-seryl)amino-β-D-*erythro*hex-2-enopyranosyluronic acid]cytosine, the nucleoside of blasticidin S bearing the dipeptide of gougerotin. Chem. Pharm. Bull. 21: 2213~2216, 1973

20) COUTSOGEORGOPOULOS, C.; A. BLOCH, K. A. WATANABE & J. J. FOX: Inhibitors of protein synthesis. 4. Studies on the structure-activity relationships of gougerotin and some of its analogs. J. Med. Chem. 18: 771~776, 1975

21) BOLLENBACK, G. N.; J. W. LONG, D. G. BENJAMIN & J. A. LINDQUIST: The synthesis of aryl-D-glucopyranosyluronic acids. J. Am. Chem. Soc. 77: 3310~3315, 1955