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SYNTHESIS OF CYTOSININE, THE NUCLEOSIDE COMPONENT OF ANTIBIOTIC BLASTICIDIN S

Tadao Kondo, Hisao Nakai and Toshio Goto

Department of Agricultural Chemistry, Nagoya University, Chikusa, Nagoya, Japan

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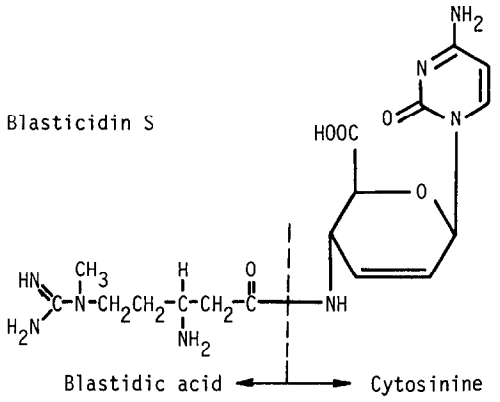
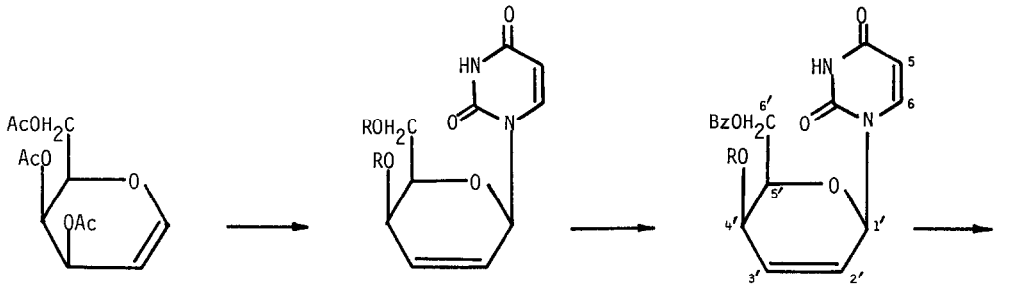
Blasticidin S is a nucleoside antibiotic produced by Streptomyces griseochromogenes and inhibits the virulent fungus, Piricularia oryzae, a serious cause of rice blast disease in Japan. The structure[1,2] and absolute configuration[3] were elucidated chemically by Ōtake et al. and confirmed by X-ray analysis.[4] Controlled acid hydrolysis of blasticidin S yielded cytosinine and blastidic acid.[2] Some synthetic approaches towards the nucleoside, cytosinine, have been attempted,[5,6] but unique arrangement of the functional groups has made the synthesis difficult and prevented from reaching the final goal. We are now successful in synthesizing cytosinine. Our route of synthesis is described in this communication.



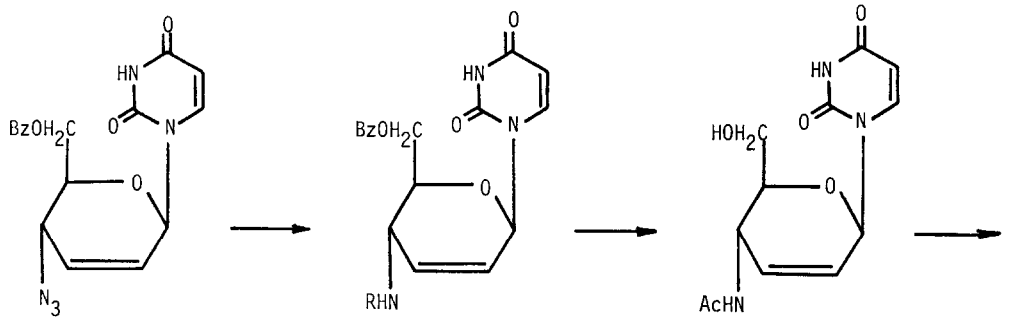
In the previous paper[7] we reported that 2'-enopyranosylpyrimidines could be synthesized directly from silylated pyrimidines and glycals in the presence of Lewis acid. Application of this method to the condensation of triacetylgalactal and bis(trimethylsilyl)uracil ($SbCl_5$ in EtOAc at 23° for 5 min.) resulted in formation of anomeric mixture of 1-(4',5'-di-O-acetyl-

2'3'-dideoxy-D-threo-hex-2'-enopyranosyl)uracils, from which crystalline α-anomer,[8] mp 148-149°, and amorphous β-anomer (I)[9] could be isolated in yields of 40 and 24%, respectively, by means of column chromatography on silica gel. The β-anomer (I) was hydrolyzed with sodium methoxide in methanol to give the diol (II), prisms,[10] mp 177-178° [$[\Phi]_{267\ nm}^{trough}$ -3700, $[\Phi]_{225\ nm}^{peak}$ +6500]. Controlled benzoylation of the diol (II) in pyridine by addition of a dilute solution of benzoyl chloride in CH_2Cl_2 at -20° afforded the 6'-benzoate (III), plates,[10] mp 175° [ν 1715 cm^{-1} (C=O)], which was then mesylated in pyridine with mesyl chloride giving amorphous 5'-mesylate (IV) [δ ppm($CDCl_3$) 3.12 (3H, s, CH_3SO_2)]. Displacement of the mesyl group in (IV) by an azide group was carried out by treatment of the mesylate (IV) with lithium azide in anhydrous DMF at 5° for 4 hr and the amorphous azide (V) [ν ($CHCl_3$) 2100 cm^{-1} (N_3)] was isolated by silica gel tlc. The azide group at C-4' is liable to rearrange to C-2' with double bond migration when the reaction temperature is elevated. Reduction of the azide (V) with $CrCl_2$ in aqueous acetone at room temp. according to Kirk and Wilson's method[11] gave crude amine (VI), which was acetylated with acetic anhydride in pyridine to give the amide (VII) [δ ppm($CDCl_3$) 2.10 (3H, Ac)]. Debenzoylation of the amide (VII) with methoxide in methanol gave the amidoalcohol (VIII), needles,[10] mp 202°. Oxidation of the amidoalcohol (VIII) to the amidocarboxylic acid was achieved successfully by addition of a large excess of chromic anhydride to a solution of (VIII) in acetone-DMF (1:1) at room temp. and by allowing the resulting mixture to stand for 30 min. The reaction mixture was subsequently treated with excess diazomethane in ether and, after evaporation of volatile solvents, the residue was chromatographed on silica gel column to remove chromium compounds giving N-acetyluracinine methyl ester (IX) as an amorphous solid [δ ppm($CDCl_3$-CD_3OD 1:1) 2.00 (3H, Ac), 3.80 (3H, CH_3O)]. Conversion of the ester (IX) into cytosinine (XII) was accomplished by the procedure of Fox et al.[12] Thus, the ester (IX) was refluxed in pyridine with P_2S_5 for 1 hr to give N-thioacetylthiouracinine methyl ester (X), yellow needles,[10] mp 204-205° [λ_{max}^{MeOH} 268 nm (ε 17,000), 328 nm (ε 21,200); δ ppm($CDCl_3$-CD_3OD 1:1) 2.48 (3H, CH_3CS), 3.71 (3H, CH_3O), 4.45 (1H, H-5'), 5.68 (1H, H-4'), $J_{4',5'}$=9 Hz]. To prevent amide formation in the next amination step, the methyl ester (X) was hydrolyzed with 1N NaOH in methanol at room temp. to the carboxylic acid (XI) [δ ppm(CD_3OD) 2.55 (3H, CH_3CS), no CH_3O signal], which was dissolved in methanol previously saturated with ammonia at -5° and heated in an autoclave at 100° for 24 hr. After evaporation of solvent, the residue was adsorbed on an Amberlite IRA-410 (OH type) column. Elution of the column with 0.5N HCl afforded cytosinine (XII) as crystalline hydrochloride, ir spectrum of which (KBr disc) is



(I) R=Ac (24%)
(II) R=H (77%)

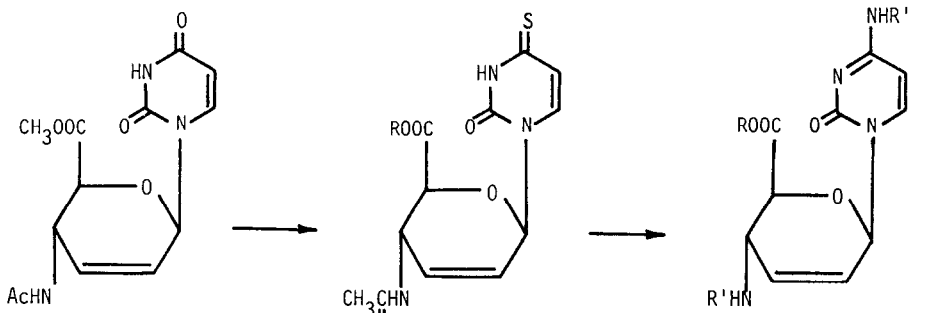
(III) R=H (49%)
(IV) R=Ms (93%)



(V) (78%)

(VI) R=H
(VII) R=Ac (60% from V)

(VIII) (quant.)



(IX) (42%)

(X) R=CH_3 (46%)
(XI) R=H (quant.)

(XII) R=H, R'=H
(XIII) R=CH_3, R'=Ac

Yields are given in parentheses.

superimposable to that of authentic specimen prepared from blasticidin S according to the method of Ōtake et al.[2] Further characterizations were made as follows: acetylation of cytosinine (XII) with acetic anhydride and pyridine followed by methylation with diazomethane in ether afforded N,N'-diacetylcytosinine methyl ester (XIII), colorless needles, mp 267-268° (dec.) [δppm($CDCl_3$-CD_3OD 1:1) 2.00 (3H, Ac), 2.28 (3H, Ac), 3.80 (3H, CH_3O), 6.75 (H-1'), 6.25 and 6.00 (H-2' and H-3'), 5.00 (H-4'), 4.45 (H-5'), 7.63 (H-5), 8.02 (H-6), $J_{1',2'}$=2.0, $J_{2',3'}$=10, $J_{4',5'}$=9, J_{56}=8 Hz; $[\Phi]^{peak}_{257\ nm}$ +3200 (dioxane-EtOH 1:1)]. Ir, nmr and ord spectra as well as mp of the synthetic XIII were identical with those of the authentic sample prepared from blasticidin S.[1] The yield of XIII from XI was ca. 25% which has not been optimized.

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REFERENCES AND FOOTNOTES

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8) α-Anomer: λ^{MeOH}_{max} 260 nm (ε 10,500), $\lambda^{MeOH-NaOH}_{max}$ 260 (7900); δppm($CDCl_3$) 6.45 (H-1'), 6.04 (2'), 6.55 (3'), 5.23 (4'), 4.4-3.9 (5' and 6'), 2.10 (Ac), 2.05 (Ac), 5.71 (H-5), 7.32 (6), 9.61 (NH), $J_{1',2'}$=3 Hz, $J_{2',3'}$=10, $J_{3',4'}$=5, $J_{4',5'}$=ca 0, $J_{1',3'}$=2.

9) β-Anomer: λ^{MeOH}_{max} 258 nm (ε 9800), $\lambda^{MeOH-NaOH}_{max}$ 258 (7500); δppm($CDCl_3$) 6.55 (H-1'), 5.92 (2'), 6.47 (3'), 5.17 (4'), 4.20 (3H, 5' and 6'), 2.16 (Ac), 2.10 (Ac), 5.85 (H-5), 7.35 (6), 9.47 (NH), $J_{1',2'}$=1.5 Hz, $J_{2',3'}$=10, $J_{3',4'}$=6.0, $J_{4',5'}$=ca 0, $J_{1',3'}$=1.5, $J_{1',4'}$=1.5. Structure assignments of these anomers have been carried out by carefull analyses of uv and nmr spectra and will be reported elsewhere.

10) Satisfactory analysis was obtained.

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