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Nucleosides. LXXX. Synthetic Studies of Nucleoside Antibiotics. 10.[1] 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

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A "hybrid" which contains the nucleoside moiety of blasticidin S and the dipeptide group of Gougerotin was synthesized by step-wise linkage of sarcosine and D-serine to cytosinine using the dicyclohexylcarbodeimide (DCC) procedure.

The nucleoside antibiotics, gougerotin and blasticidin S, share several structural features in common. Both are cytosine nucleosides containing a 4-amino-D-hexopyranuronic acid structure in which the amino group is acylated either by a peptide or an amino acid. The structure of gougerotin was firmly established by chemical and spectral studies,[4,5] and, more recently, we have achieved the total synthesis[1] of this antibiotic. The structure of blasticidin S was also established by chemical degradation,[6] X-ray crystallographic analysis[7] and by partial synthesis.[8]

In addition to their structural similarity, both gougerotin and blasticidin S exhibit several common biochemical activities. Both antibiotics are inhibitors of puromycin in the peptide release reaction.[9–13] Direct evidence that these antibiotics specifically inhibit peptide synthetase (peptidyl transferase) which is an integral part of the 50S ribosomal subunits has been obtained[14–18] and more recently it was demonstrated[19] that blasticidin S binds to 50S ribosomal subunits and this binding is inhibited by gougerotin but not by puromycin, lincomycin, erythromycin or chloramphenicol. Gougerotin and blasticidin S are reported to

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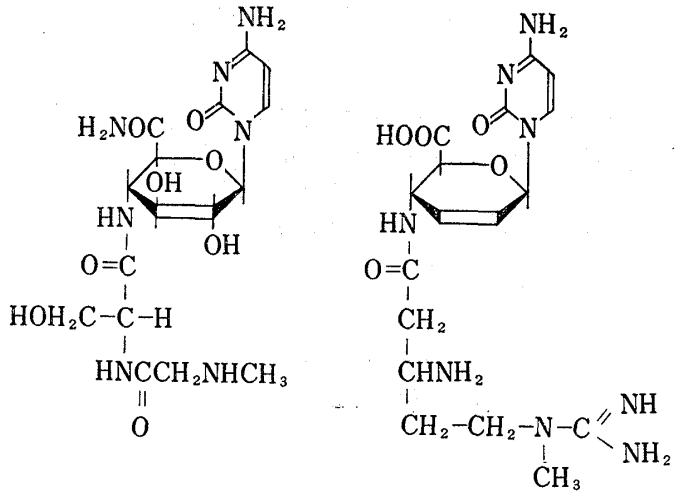

gougerotin blasticidin S

Chart 1

inhibit the multiplication of certain viruses.[20-23] Blasticidin S is a powerful agent[24] against the virulent fungus, *Piricularia oryzae*, a serious cause of rice blast disease while gougerotin has little effect against this pathogenic fungus.[25]

A number of analogs of blasticidin S have been tested as potential inhibitors of protein synthesis in cell-free systems. None of these analogs showed any significant inhibitory activity.[26] On the other hand, certain analogs of gougerotin bearing minor alterations in structure did exhibit significant biological activity.[27]

It was of interest, therefore, to synthesize a "hybrid" of gougerotin and blasticidin S, that is, a compound containing the nucleoside skeleton of blasticidin S (cytosinine) and the dipeptidyl moiety of gougerotin (sarcosyl-D-serine) for subsequent biochemical and biological examination.

The starting material, cytosinine[28] was obtained by alkaline hydrolysis of blasticidin S according to the procedure of Kawana, *et al.*[26b] and converted to the known ester dihydrochloride (**1**) with methanolic hydrogen chloride.[26b] Treatment of **1** with N-benzyloxycarbonyl-D-serine (N-Cbz-D-serine), dicyclohexylcarbodiimide (DCC) and triethylamine in a 1:1 mixture of methanol and acetonitrile gave the protected amino acyl nucleoside (**2**) in ~80% yield. Removal of the Cbz group of **2** was effected by ~5% hydrogen bromide in acetic acid to afford the crystalline dihydrobromide of **3** in 90% yield. Treatment of the dihydrobromide with Cbz-sarcosine, DCC and triethylamine, however, gave two products which were separated and purified by silica gel column chromatography. Nuclear magnetic resonance (NMR) examination of the major product showed that this compound is the N4'-acetyl derivative (**5**). Obviously, during hydrogen bromide-acetic acid treatment of **3**, acetylation of the seryl hydroxyl group occurred to a considerable extent to produce O-acetyl derivative (**3**) followed by O→N acetyl migration during the neutralization procedure with triethylamine. The desired protected dipeptidyl nucleoside (**4**) was obtained as a minor product as colorless microcrystals. The NMR spectrum (in DMSO-d_6) of this compound showed CH_3 (acetyl) δ=1.99 (3H), NCH_3 δ=2.98 (3H), CH_3 (ester) δ=3.55 (3H), CH_2 (sarcosine) δ=3.98 (2H), CH_2 (serine) δ=4.25 (2H), CH (serine) δ=4.58 (1H), H1', H2'(3') and H5 δ=5.87 (3H), H3'(2') δ=6.48 (1H), H6 and benzyl aromatic δ=7.35 (6H). These spectral data support structure **4**. Compound

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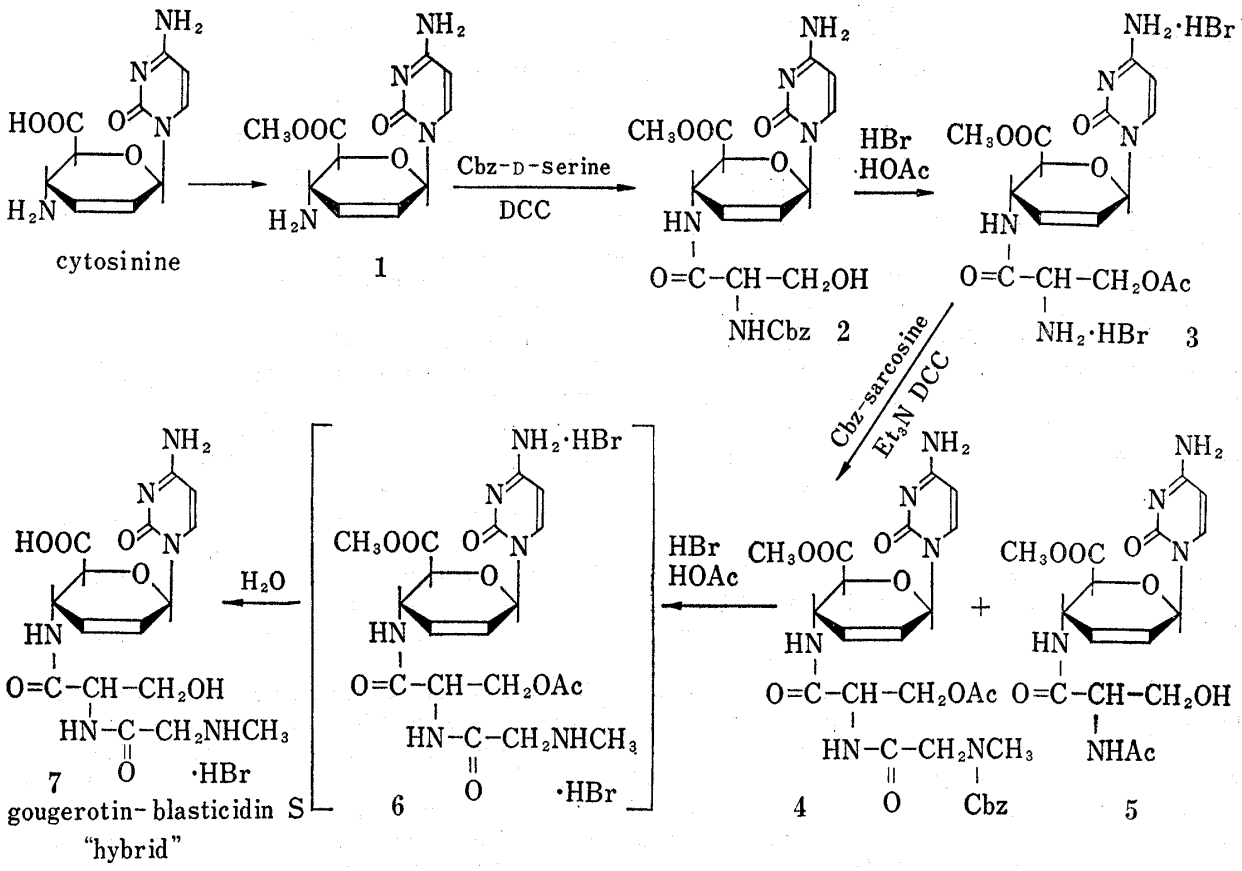

Chart 2

(**4**) was treated with hydrogen bromide–acetic acid mixture to form **6**, which, without isolation, was de-esterified in acid. The product **7** was purified by preparative paper chromatography and was obtained as pale yellow microcrystals. The NMR spectrum (D_2O, DSS external standard) of this product was completely consistent with structure **7**; $-NCH_3$ signal at $\delta=2.77$, serine CH_2 doublet at $\delta=3.84$, sarcosine CH_2 singlet at $\delta=3.97$, 3H signal for H5, H1′ and H2′ (or H3′) centered at 6.0, H3′ (or H2′) at $\delta=6.46$ and H6 doublet at $\delta=7.60$.

The possibility that some racemization may have occurred during the synthesis of **7** is highly unlikely. We have shown previously that during the synthesis of other gougerotin analogs[29] by the DCC procedure, no evidence of racemization was obtained. The structure of **7** is therefore 1-[2,3,4-trideoxy-4-(sarcosyl-D-seryl)amino-*β*-D-*erythro*-hex-2-enopyranosyl)-uronic acid]cytosine, a "hybrid" of gougerotin and blasticidin S.

Biochemical and biological studies on **7** are in progress and will be reported elsewhere.

Experimental

1-[Methyl 2,3,4-trideoxy-4-(N-benzyloxycarbonyl-D-seryl)amino-*β*-D-*erythro*-hex-2-enopyranosyluronate]-cytosine (2)——To a solution of **1**[26b] (678 mg, 2 mmoles), N-Cbz-D-serine (750 mg, 3 mmoles) and triethylamine (405 mg, 4 mmoles) in a mixture of absolute methanol (3 ml) and acetonitrile (3 ml) was added DCC (620 mg) and the mixture was shaken for 20 hr. Dicyclohexylurea was filtered and washed with methanol (10 ml). The combined filtrate and washings were evaporated to dryness and the residue was triturated with ether (20 ml), whereupon crystallization occurred. After filtration, the crystalline material was again triturated several times with ether and finally dissolved in methanol (25 ml). The solution was treated with a methanol-washed mixture of Dowex 1 (OH^-, 1.5 ml) and Dowex 50 (H^+, 1.5 ml) in an ice bath for 4 min and the mixed resins were filtered off. The filtrate was evaporated to afford a crystalline residue which was recrystallized from ethanol to give 745 mg of **2** as fine needles, mp 162—173° effervesced at 175—186°; $[\alpha]_D^{27}$

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+71° (c=1.1, MeOH). UV λ_{max}^{MeOH} nm: 270 and 238; λ_{min}^{MeOH} nm 261 and 232; $\lambda_{max}^{1\% HCl in MeOH}$ nm: 280; $\lambda_{min}^{1\% HCl in MeOH}$ nm: 245. NMR (DMSO-d_6) CH_3 (ester) δ=3.51 (3H); CH_2 (serine) centered at 3.55 CH (serine) δ=4.41; CH_2 (benzyl) δ=5.03 (2H); H1′, H2′ (or 3′) and H5 $\delta\sim$5.9 (3H); H3′ (or 2′) δ=6.50 (1H); benzyl aromatic δ=7.37 (5H) and H6 δ=7.55 (1H). This product was not further purified but used directly in the next step.

1-[Methyl 2,3,4-trideoxy-4-(O-acetyl-D-seryl)amino-β-D-*erythro*-hex-2-enopyranosyluronate]cytosine Dihydrobromide (3)——The Cbz derivative (2) (700 mg) obtained above was finely pulverized and suspended in ~5% HBr in acetic acid (15 ml) and the mixture was stirred for 4.5 hr, then diluted with ether (15 ml). Crystals (674 mg) separated which were filtered quickly and dried *in vacuo* in a desiccator over P_2O_5 and KOH overnight. NMR (D_2O, DDS external standard). CH_3 (acetyl) δ=2.18 (3H); CH_3 (ester) δ=3.35 (3H); CH_2 (serine) δ=3.77 (2H); H1′, H2′, H3′ at δ=6.07, 6.23, 6.59; H5, δ=6.26; H6, δ=7.83. This product was not characterized further but used directly in the next step.

1-[Methyl 2,3,4-trideoxy-4-(N-benzyloxycarbonylsarcosyl-O-acetyl-D-seryl)amino-β-D-*erythro*-hex-2-enopyranosyluronate]cytosine (4)——To the solution of the above hydrobromide salt (516 mg, 1 mmole), Cbz-sarcosine (330 mg, 1.5 mmoles) and Et_3N (203 mg, 2 mmoles) in MeOH (3 ml) and MeCN (3 ml) was added DCC (330 mg) and the mixture was shaken for 24 hr. Dicyclohexylurea was filtered and washed with methanol. The combined filtrate and washings were evaporated and the residue was triturated with ether (20 ml×3). The colorless powder was dissolved in MeOH (20 ml) and the solution was stirred with a mixture of Dowex-1 (OH^-) (1 ml) and Dowex-50 (H^+) (1 ml) for 4 min at 0°. The resin was filtered and the filtrate was evaporated to dryness and the residue (which contained 2 components as judged by thin-layer chromatography (TLC)) was chromatographed over a Silica gel G column (60 mg) using 6:1 $CHCl_3$–MeOH solvent. The first fraction contained the Cbz-dipeptidyl compound (4) (71 mg); mp 110° (sintered), 184—197° (decomp., eff), $[\alpha]_D^{27}$ +76° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm: 270 and 238; λ_{min}^{MeOH} nm: 261 and 230; $\lambda_{max}^{0.1N HCl in MeOH}$ nm: 280, $\lambda_{min}^{0.1N HCl in MeOH}$ nm: 244.

1-[2,3,4-Trideoxy-4-(sarcosyl-D-seryl)amino-β-D-*erythro*-hex-2-enopyranosyluronic acid]cytosine Hydrobromide (7)——The protected dipeptidyl nucleoside (6, 57 mg) was stirred in 4.1 ml of ~5% HBr in AcOH for 5 hr. Water (5 ml) was added to the reaction and the mixture was allowed to stand for 2 hr. The solution was applied directly on Whatman No. 1 paper (46×57 cm) and the paper was developed with *n*-BuOH–H_2O–AcOH (4:2:1) for 40 hr in a descending fashion. The main band was excised, extracted with water, and evaporated to dryness. The residue was co-evaporated several times with ethanol until colorless microcrystals were obtained, 21 mg, mp 184° (sintered) 205—207° (decomp., eff), $[\alpha]_D^{26}$ +101° (c=0.1, H_2O). *Anal.* Calcd. for $C_{16}H_{22}O_7N_6 \cdot HBr \cdot H_2O$: C, 37.73; H, 4.94; N, 16.50. Found: C, 38.04; H, 5.18; N, 16.64.

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