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Nucleosides. LXIX.[1] Synthetic Studies on Nucleoside Antibiotics. (6). Syntheses 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

The chemistry of the structurally related nucleoside antibiotics gougerotin and blasticidin S has been intensively investigated[2] in our laboratory. We have reported (Chart 1) the syntheses of methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid[3] and 1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid) cytosine (C-substance),[4] the carbohydrate and the nucleoside moieties of gougerotin respectively.[5] Recently we have achieved the synthesis of methyl 4-amino-2,3,4-tri-deoxy-α-D-*erythro*-hex-2-enopyranosiduronic acid,[6] a member of a new class of carbohydrates related to the sugar moiety of blasticidin S.

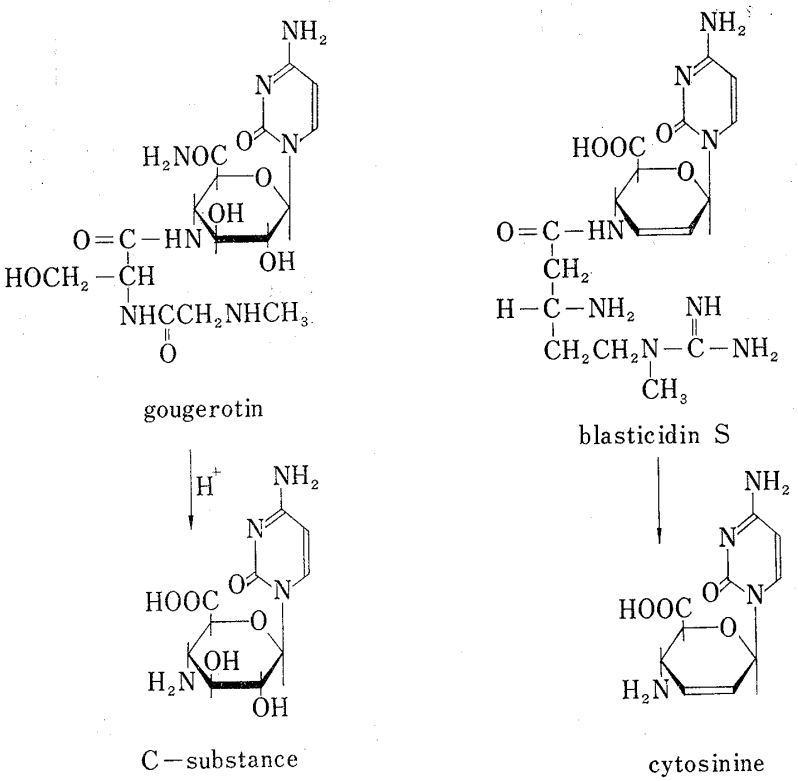

Chart 1

This communication deals with the synthesis of 1-(4-amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranosyl)cytosine (**10**) [isolated as its crystalline triacetyl derivative (**11**)] and

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2) J.J. Fox, K.A. Watanabe, and A. Bloch, *Progr. Nucleic Acid Res. Mol. Biol.*, **5**, 251 (1966) and references therein.
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with the identity of **11** with a product derived from blasticidin S by Yonehara and Ōtake.[7] The structure of blasticidin S had been firmly established by degradation[8] and X-ray[9] studies. The preparation of **10** and **11** constitutes the first synthetic proof of the nucleoside moiety of this antibiotic (Chart 2).

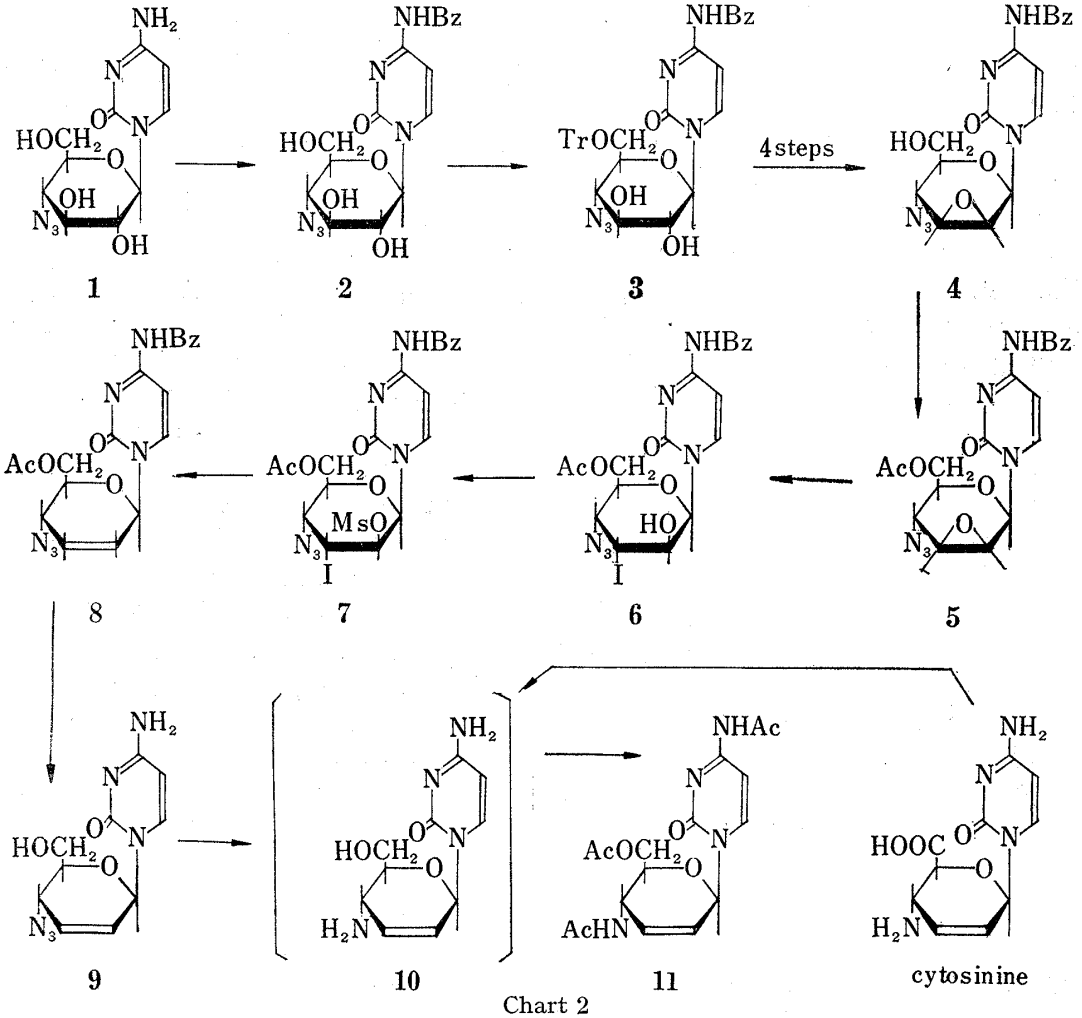

Chart 2

Compound **1**[4] was selectively benzoylated to **2** with benzoic anhydride in methanol,[10] 90% yield, mp 190—192,[11] $[\alpha]_D^{27}$ +56° (DMF). Tritylation of compound **2** gave compound **3** in 94% yield, mp 190—198° (eff.), $[\alpha]_D^{27}$ +38° (DMF). Compound **3** was tosylated, the product was refluxed with sodium methoxide in methanol, then rebenzoylated with benzoic anhydride in pyridine. Detritylation was carried out in a 1:1 mixture of dichloromethane and methanol containing a catalytic amount of concentrated HCl and was followed by chromatography on Silica gel G using 10% ethanol in benzene as solvent. Crystalline epoxide (**4**) mp 186—189°, $[\alpha]_D^{27}$ +134° (pyridine) was obtained in 28% overall yield from compound **3**.

7) H. Yonehara and N, Ōtake, *Tetrahedron Letters*, **1966**, 3785.
8) H. Yonehara, S. Takeuchi, N. Ōtake, T. Endo, Y. Sakagami and Y. Sumiki, *J. Antibiot.*, Ser. A, **16**, 195 (1963); N. Ōtake, S. Takeuchi, T. Endo, and H. Yonehara, *Tetrahedron Letters*, **1965**, 1404, 1411; *idem*, *Agr. Biol. Chem.* (Tokyo), **30**, 126, 132 (1966); J.J. Fox and K.A. Watanabe, *Tetrahedron Letters*, **1966**, 897.
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11) All compounds with melting points reported herein gave satisfactory elemental analyses.

Acetylation of **4** gave the crystalline acetate (**5**) in 92% yield, mp 145—146° (eff.), $[\alpha]_D^{27}$ 117° (pyridine), which upon treatment with sodium iodide in acetone in the presence of a small amount of sodium acetate and acetic acid,[12] afforded the crystalline iodohydrin (**6**) in 89% yield, mp 222—224° (decomp.), $[\alpha]_D^{27}$ +94° (DMF).

The NMR data (in pyridine-d_5 with TMS as internal standard) for **6** was consistent with a 3′-iodo derivative of the altro configuration: H1′ (δ=7.00, singlet); H3′ (δ=5.25, triplet; $J_{2',3'} \cong J_{3',4'} \cong 3.2$ Hz); H2′ (δ=4.99, deformed doublet); H6′,6″ (δ=4.70), H5′ (δ=4.33, deformed quartet), H4′ (δ=4.21, quartet, $J_{4',5'} \cong 11.0$). On addition of D_2O, the H_2' signal at δ=4.99 changed to a sharp doublet with a spacing of ~3.2 Hz. These data also establish the manno configuration for epoxides **4** and **5** and, consequently, showed that monotosylation of **3** had occurred mainly on position 2′.

Mesylation of **6** at ~0° gave the crystalline iodo-mesylate (**7**), mp 166—167° (decomp.), $[\alpha]_D^{27}$ +94° (DMF). IR λ_{max}^{KBr} 4.8 μ (N_3); 5.8 (ester); 5.9 (amide); 6.0, 6.2, 6.8 (pyrimidine); 7.4, 8.6 (sulfonate). When mesylation of **6** was performed at room temperature, the crystalline 2′,3′-unsaturated nucleoside (**8**) was obtained directly in 79% yield, mp 159—161° (decomp.), $[\alpha]_D^{27}$ +179° (pyridine). De-acylation of **8**[13] with sodium methoxide afforded the crystalline nucleoside (**9**), mp 202—206° (eff.), $[\alpha]_D^{27}$ +216° (DMF). Compound **9** was treated with sodium borohydride in a 3:1 mixture of isopropanol and DMF at reflux temperature for 24 hr.[14] After removal of the isopropanol *in vacuo*, the mixture was diluted with water and placed on a column of Amberlite IRC-50 (H^+), washed with water, and the reduced nucleoside (**10**) was eluted from the column with 1N ammonium hydroixde. Evaporation of the eluate gave a residue, compound **10**, which, without purification, was treated with acetic anhydride in pyridine. Compound **11**, 1-(4-acetamido-6-O-acetyl-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranosyl)-N^4-acetylcytosine, was isolated as fine colorless needles after column chromatography on Silica Gel Gusing 10% methanol in chloroform (v/v) followed by recrystallization from ethanol, mp 228—231° (to a brown liquid). When this compound was admixed with a sample of **11** derived from blasticidin S,[7] no depression of melting point was observed. The IR spectrum of synthetic compound **11** was identical with that exhibited by the Blasticidin S — derived material (see Figure).

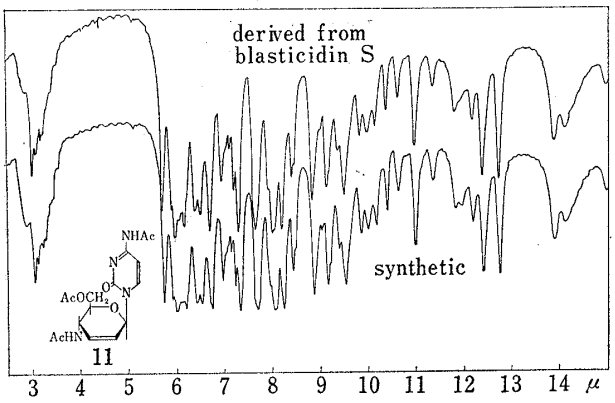

Fig. 1

Studies are in progress in our laboratory on the total synthesis of cytosinine from derivatives such as **4** as part of our general program directed toward the synthesis of nucleoside antibiotics and their analogs.

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13) This de-acylation step is essential because all attemps to reduce the azide of **8** led to loss of UV absorption of the aglycon. We have previously shown that N^4-acylated cytosine nucleosides undergo ring reduction easily [See H.A. Friedman, K.A. Watanabe, and J.J. Fox, *J. Org. Chem.*, **32**, 3775 (1967)].
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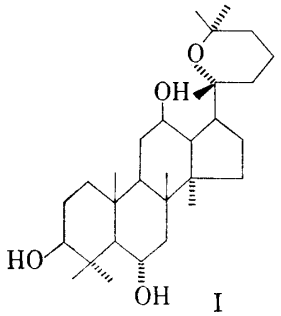
Isolation of Panaxatriol from *Panax ginseng* Callus

Panax ginseng C.A. MEYER (Araliaceae, ginseng) is a perennial herb indigenous to the forests of the eastern Asia and cultivated in Northern China, Korea and Japan. Ginseng root is widely used as a tonics in the Orient from ancient.

Saponins and sapogenins of ginseng were elucidated by Shibata, *et al.*[1] and Elyakov, *et al.*,[2] but that of gineng callus not investigated yet. Now we wish to report the isolation of panaxatriol (I),[1b,2] a genin of ginsenoside Rg_1,[1c,d] in high yield from ginseng callus.

The callus derived from petiole of cultivated ginseng was grown on Murashige and Skoog's agar medium (minus glycine) containing 2,4-dichlorophenoxyacetic acid 1 ppm. The callus has been subcultured at about 25° in the dark and at four to five weeks intervals for about three years.

The callus (500 g fresh weight, 25 g dry weight) harvested was homogenized with cold methanol 650 ml in a Waring blender, refluxed for three hours and filtered. The filtrate was concentrated to a small volume under reduced pressure. To the extract was added cold methanol and the methanol soluble portion was again evaporated to dryness *in vacuo*. Then, the residue was washed with ether. The ether insoluble portion after dissolving water was extracted with *n*-butanol. The butanol layer was evaporated to dryness. The crude saponin (2.8 g) obtained was submitted to thin-layer chromatography on silica gel G (upper layer of *n*-BuOH–AcOH–H_2O=5:1:4) to show almost the same pattern as ginseng saponins. Especially, a large amount of ginsenoside Rg and a small amount of Rb in ginseng callus were detected. The crude saponin was hydrolyzed by refluxing with 5% sulfuric acid in 50% aqueous ethanol. After working up in the usual way, the hydrolysate was put on a silica gel column and gradually eluted with benzene, benzene–ethyl acetate (2:1), and benzene–ethyl acetate (1:1) (one fraction 20 ml). Fraction No. 90—170 gave a crystalline compound, which was recrystallized from



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