Isolation of 1-β-D-Arabinofuranosylcytosine from the Mushroom *Xerocomus nigromaculatus* HONGO

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1-β-D-Arabinofuranosylcytosine (ara-C), an anticancer drug, has been isolated for the first time from a natural source, the mushroom *Xerocomus nigromaculatus* HONGO.

Keywords basidiomycetes; ara-C; 1-β-D-arabinofuranosylcytosine; cytotoxic activity; *Xerocomus nigromaculatus*

Mushrooms are well known to produce unusual compounds with a wide variety of biological activities, and a number of metabolites showing antimicrobial and cytotoxic activities have been isolated from them, such as pleurotellol, alliacol A, strobilurin, saponaceolide and so on.[1] During the course of our screening for biologically active metabolites from mushrooms (Basidiomycetes), a potent cytotoxin (IC_{50} value for P388: 0.004 μg/ml), tentatively named Xn-I, was isolated from *Xerocomus* (*X.*) *nigromaculatus* HONGO (Japanese name: Kuroazaawatake).[2] After structural studies, this compound was identified as 1-β-D-arabinofuranosylcytosine (ara-C) on the basis of physico-chemical and spectral evidence (Fig. 1). We wish to report herein the first isolation of this synthetic anticancer drug, ara-C, from a natural source.

The MeOH extract of *X. nigromaculatus* HONGO, collected in Miyagi prefecture in Aug. 1989, was partitioned between ethyl acetate and H_2O. The water-soluble fraction was chromatographed on an active carbon column using H_2O, MeOH and $CHCl_3$–MeOH as eluants, then an active fraction ($CHCl_3$: MeOH = 1 : 2, 1 : 3 eluates) was further purified by high performance liquid chromatography (HPLC) (RP-18, solvent system: MeOH : H_2O = 1 : 50) followed by crystallization from EtOH–H_2O to afford Xn-I (**1**), mp 223—224 °C, $[\alpha]_D^{27}$ +152° (c = 0.15, H_2O), as colorless needles. The isolation procedure described above is summarized in Chart 1.

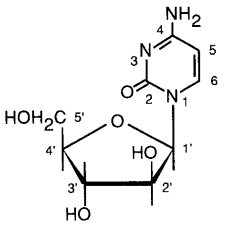

Fig. 1. 1-β-D-Arabinofuranosylcytosine (ara-C)

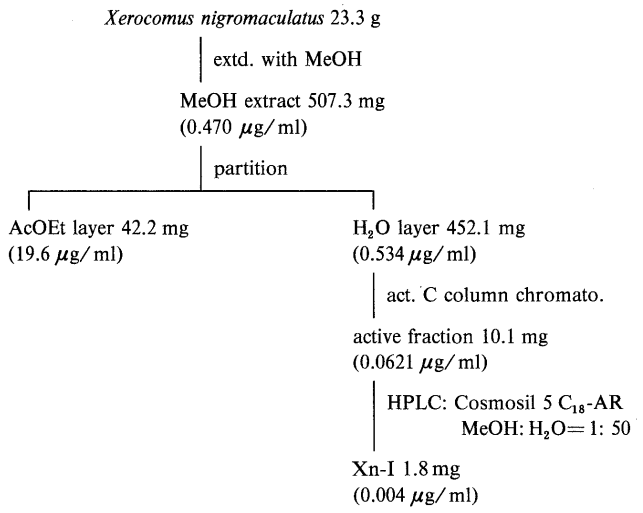

Chart 1. Isolation Procedure for Xn-I

Values in parenthesis indicate the 50% inhibitory concentration for P388 cell growth *in vitro*.

Xn-I (**1**), $C_9H_{13}N_3O_5$, exhibited a protonated molecular ion peak [$(M+H)^+$] at m/z 244 in the field desorption mass spectrum (FD-MS). The ultraviolet (UV) spectrum showed absorption maxima at 272 and 197 nm, which are similar to those of cytidine. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** (Table I) presented a pair of doublet signals at δ 6.03 and 7.80 due to heterocyclic protons of a cytosine moiety. Further, it showed a doublet signal at δ 6.20 assignable to an anomeric proton and a series of signals at δ 3.83—4.40 due to carbinyl protons, implying the presence of a sugar moiety.

The cross peaks observed in the ^{1}H–^{1}H correlation spectroscopy spectrum of **1** clarified the relationship of the sugar protons (Fig. 2) and the coupling constants of these protons were therefore determined (Table I) through the detailed analysis of the ^{1}H-NMR spectrum of **1**.[3] These results led us to conclude that the sugar moiety is an arabinofuranose, and hence Xn-I was supposed to be ara-C. Finally, this was confirmed by comparing **1** with an authentic specimen in respect to physico-chemical and spectral data, together with the HPLC behavior. Consequently, Xn-I was unequivocally identified as ara-C.

ara-C is pyrimidine antimetabolite and one of the most effective anticancer drugs available for acute myelogenous leukemia. ara-C was first synthesized by Walwick *et al.* in 1959,[4] but has not previously been found to occur naturally. Therefore, this is the first report of ara-C as a natural product.

TABLE I. ^{1}H-NMR Spectral Data for Xn-I[a]

Position	δ_{ppm}
1′	6.20 (1H, d, J = 4.9)
2′	4.40 (1H, dd, J = 4.9, 3.9)
3′	4.11 (1H, dd, J = 5.4, 3.9)
4′	4.00 (1H, ddd, J = 5.9, 5.4, 3.4)
5′	3.91 (1H, dd, J = 12.7, 3.4)
	3.83 (1H, dd, J = 12.7, 5.9)
5	6.03 (1H, d, J = 7.3)
6	7.80 (1H, d, J = 7.3)

a) The data was taken in D_2O at 500 MHz.

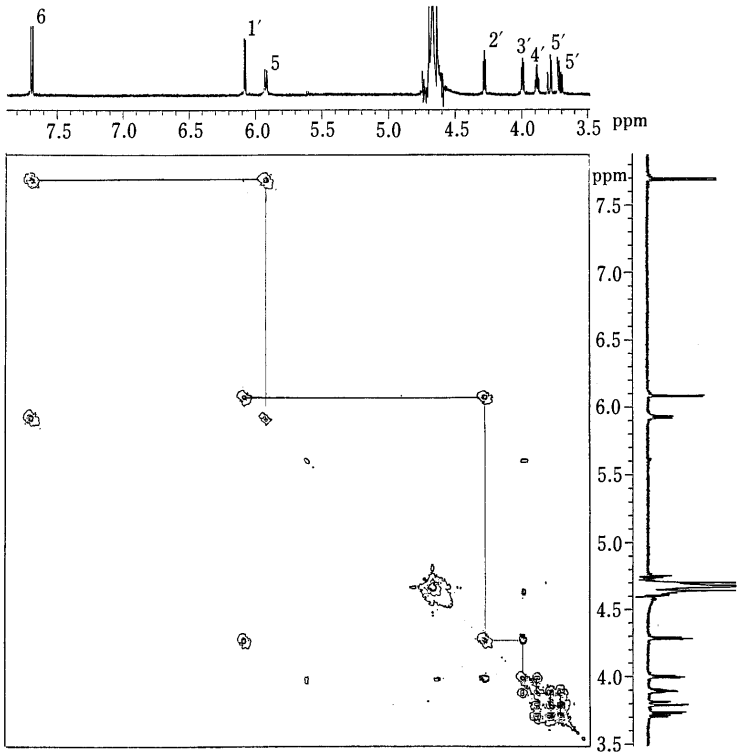

Fig. 2. ^1H–^1H COSY Spectrum of Xn-I in D_2O

The data was measured relative to the HOD peak at δ 4.67.

Experimental

The melting point was measured on a Yanagimoto micro hot plate and is uncorrected. The UV spectrum was recorded on a Hitachi 323 spectrometer. Optical rotation value was measured on a JASCO DIP-370 polarimeter. ^1H-NMR spectra were recorded on a JEOL GX-500 (500 MHz) using tetramethylsilane (TMS) as an external standard. Chemical shifts are shown in δ (ppm) and multiplicities are given as follows: singlet = s, doublet = d, triplet = t, multiplet = m, and broad = br. Coupling constants (J) are shown in hertz (Hz). The FD-MS was taken on a JEOL DX-303 spectrometer. Thin layer chromatography (TLC) analyses were performed on Kieselgel $60F_{254}$ (Merck) and spots were detected under UV irradiation (254 nm) and by heating on a hot plate after spraying anisaldehyde–sulfuric acid reagent.

Isolation Procedure The fruiting bodies of *X. nigromaculatus* HONGO (23.3 g), collected in Miyagi prefecture in Aug. 1989, were extracted with MeOH (50 ml, three times) at room temperature for 2 d. After removal of the solvent, the residue (507.3 mg) was partitioned between EtOAc (10 ml, twice) and H_2O (10 ml). The water-soluble fraction (452.1 mg) was chromatographed on an active carbon column (2 g: 2cm i.d. × 4 cm) using H_2O, MeOH, and MeOH–$CHCl_3$ as eluants. Fractions (10.1 mg) eluted with MeOH–$CHCl_3$ (3 : 1, 2 : 1) were further purified by HPLC [column: Cosmosil $5C_{18}$-AR (10 mm i.d. × 250 mm), solvent system: MeOH–H_2O (1 : 50), flow rate: 2 ml/min] followed by crystallization from EtOH–H_2O afford Xn-I (1.8 mg) as colorless needles.

Xn-I (1) TLC: *Rf* 0.37 (*n*-BuOH : AcOH : H_2O = 4 : 1 : 1), mp 223—224 °C. $[\alpha]_D^{27}$: +152° (c = 0.15, H_2O). FD-MS *m/z*: 244 [$(M+H)^+$]. UV $\lambda_{max}^{H_2O}$ nm (log ε): 272 (4.08), 197 (4.39). ^1H-NMR (D_2O) δ: Table I.

Assay for Cytotoxic Activity RPMI 1640 medium supplemented with 10% fetal bovine serum, 20 μM 2-mercaptoethanol and 100 μg/ml of kanamycin was used as the cell culture medium. The sample material was dissolved in dimethylsulfoxide (DMSO) at a concentration of 100, 10 or 1 μg/ml. A suspension of P388 lymphocytic cells (10^4 cells/ml) in the medium was prepared. The cell suspension (2 ml) was incubated in a culture tube (Falcon No. 2054), then a sample solution was added. After incubation at 37 °C for 72 h, the cell number in the culture suspension was determined with a Coulter counter (model ZB). The control cell suspension was prepared without the sample material according to the procedure described above. All assays were performed in triplicate and 50% inhibitory activities (IC_{50}) were calculated from the difference of the cell number in the culture suspensions in the presence and absence of sample material.

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References and Notes

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