ISOLATION AND CHARACTERIZATION OF A NEW ARISTEROMYCIN ANALOG

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The chemical structure of aristeromycin M, a new carbocyclic nucleoside, was elucidated by spectroscopic analysis and chemical transformation from aristeromycin.

Aristeromycin[1~3], a carbocyclic analog of adenosine, was isolated from *Streptomyces citricolor* IFO 13005 B-16575, and has become of interest in regards to its biological activities[4~7].

While isolating aristeromycin on a large scale, we found a minor substance less polar than aristeromycin in the cultured broth.

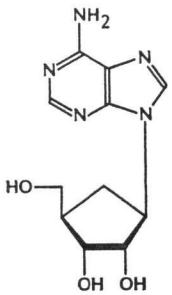
The present paper describes the isolation and the structure determination of a new carbocyclic nucleoside aristeromycin M (Fig. 1).

A cultured broth (100 liters) of *S. citricolor* was treated in a manner similar to that described in the literature[1]. The crude solid thus obtained was dissolved in water, and 31 g of aristeromycin was fractionally crystallized. The mother liquor was passed through a column of Diaion HP-20 resin (700 ml). The column was washed with water and eluted with 6% aqueous ethanol, then with 10% aqueous ethanol. The latter effluents gave 0.55 g of crude aristeromycin M.

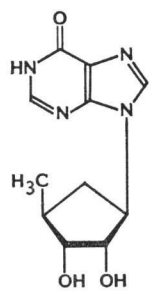
Structural Elucidation

The UV spectra of aristeromycin M were closely related to those of inosine. The ^{1}H NMR (Joel JNM-GX400 FT NMR spectrometer, solvent; D_2O) (Fig. 2) presented two sharp singlets at δ 8.168 (1H) and δ 8.236 (1H) due to the heterocyclic protons of the hypoxanthine moiety ($C_5H_3N_4O$).

Fig. 1. Structures of carbocyclic nucleosides.



Aristeromycin



Aristeromycin M

A sharp doublet (3H) at δ 1.217 (J=7.1 Hz) indicated the presence of a secondary methyl group. The remaining protons ($C_5H_8O_2$) observed as a series of peaks at δ 1.692~4.815 were analyzed by spin decoupling experiments summarized in Table 1. Based on these results, the multiplet at δ 2.160 (1H) was assigned to the methyne proton surrounded by methyne, methylene and methyl groups. As the compound consumed one equivalent of potassium periodate, the presence of a *cis* glycol system was suggested. These data showed that the compound has the

Fig. 2. ^{1}H NMR spectrum of aristeromycin M.

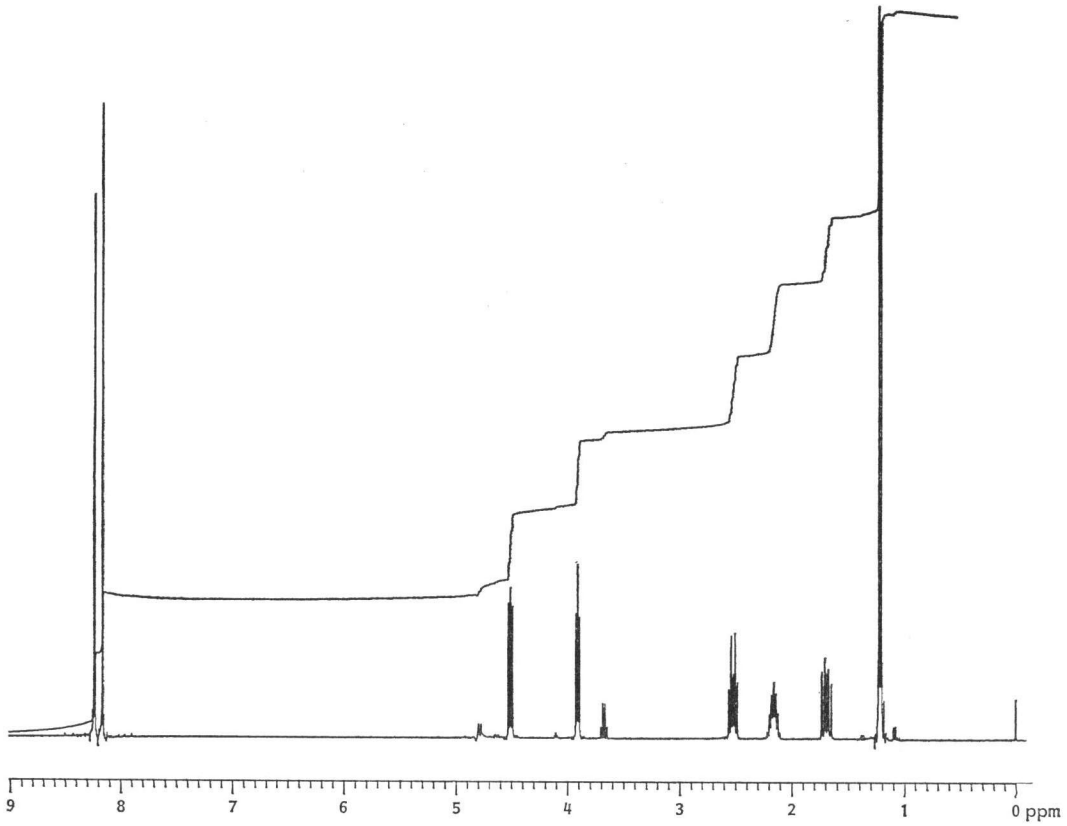
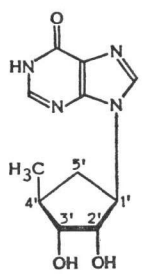
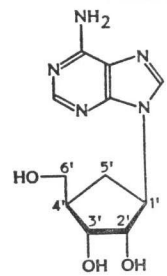


Table 1. Chemical shifts and coupling constants of aristeromycin analogues (D_2O).

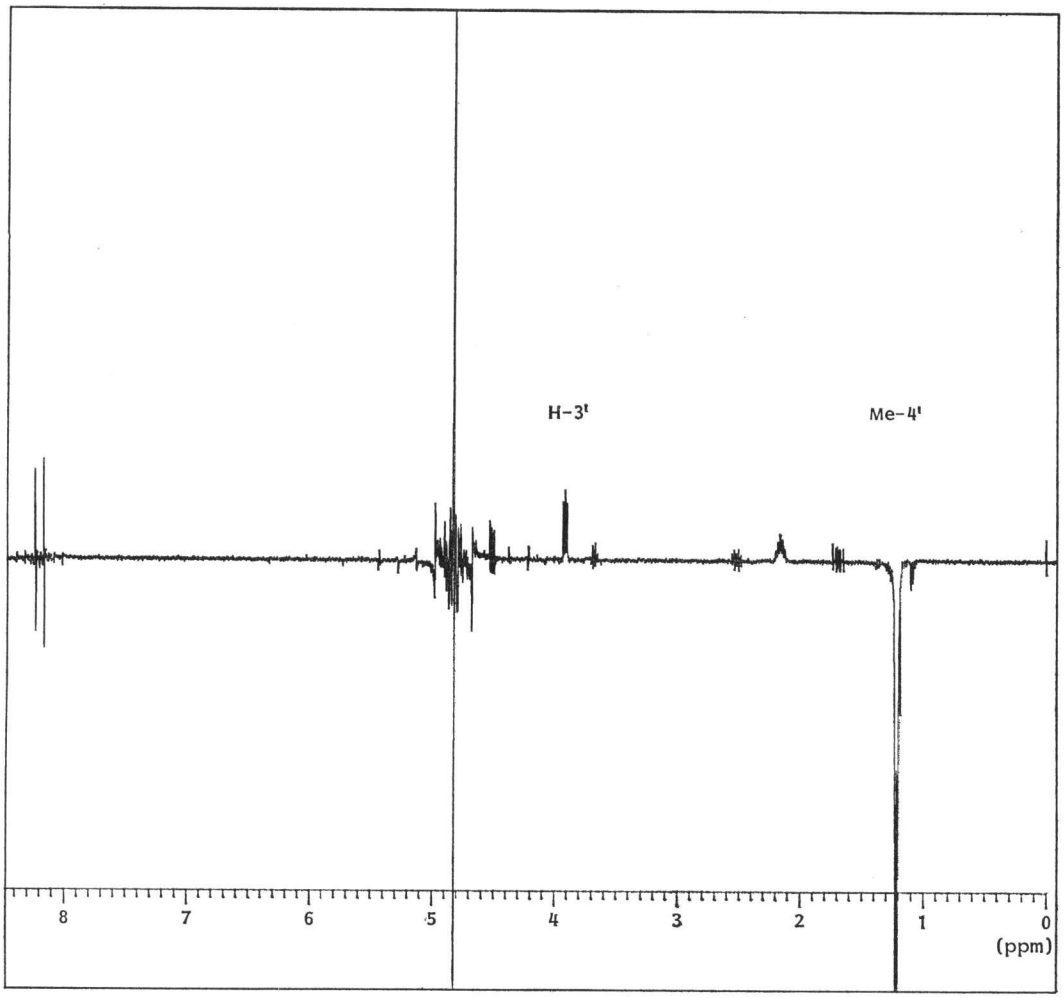


Proton	δ (ppm)	J (Hz)
H1′	4.815 dt	10.7, 8.1, 8.1
H2′	4.510 dd	8.1, 5.9
H3′	3.911 t	5.9
H4′	2.160 m	5.9, 9.5, 8.1, 7.1
H5′	2.521 dt	13.1, 8.1, 8.1
	1.692 ddd	13.1, 9.5, 10.7
4′-Me	1.217 d	7.1



Proton	δ (ppm)	J (Hz)
H1′	4.817 ddd	8.9, 8.4, 9.3
H2′	4.498 dd	9.3, 5.9
H3′	4.119 dd	5.9, 3.4
H4′	2.332 m	3.4, 10.9, 8.4, 6.4
H5′	2.518 dt	13.2, 8.4, 8.4
	1.782 ddd	13.2, 10.8, 8.9
H6′	3.769 dABq	11.2, 6.4
	3.749	

Fig. 3. FT NOE difference spectrum of aristeromycin M.



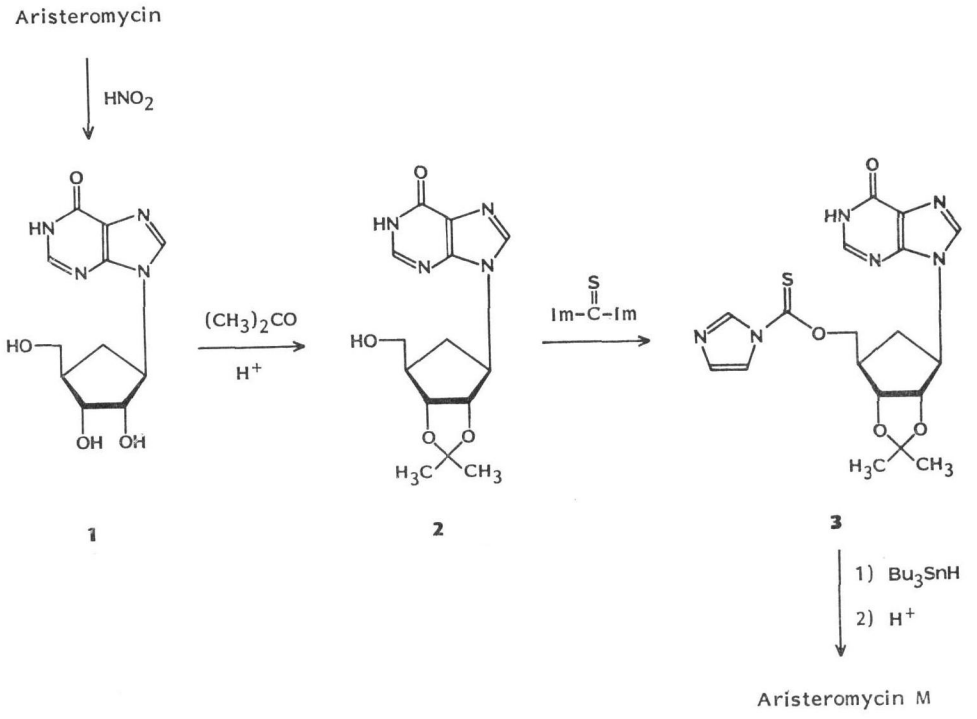
cyclopentane ring system similar to that of aristeromycin. To determine the relative configuration of the substituents attached to the cyclopentane ring, we examined FT NOE difference spectrum of aristeromycin M, in which a strong NOE between C4′-methyl and C3′-methyne groups was observed (Fig. 3). The above data showed that the methyl group is oriented to the opposite side of the hydroxyl groups.

Chemical Synthesis of Aristeromycin M

The absolute configuration of aristeromycin was established by X-ray analysis[2]. If aristeromycin and aristeromycin M are congeners, the former should be converted chemically into the latter, and then the absolute configuration of the latter would be confirmed. Based on this consideration, chemical modification of aristeromycin was carried out (Fig. 4).

A carbocyclic analog of inosine (**1**)[8], obtained from aristeromycin by deamination, was converted into the 2′,3′-*O*-isopropylidene derivative (**2**). It was allowed to react with thiocarbonyldiimidazole in dichloromethane to give the 6′-*O*-thiocarbonylimidazolyl derivative (**3**) in high yield. The free

Fig. 4. Synthesis of aristeromycin M from aristeromycin.



radical deoxygenation of **3** with tri-*n*-butyltin hydride[9~11] was applied giving a poor yield of the desired product accompanied by an appreciable amount of by-products. The reaction mixture was hydrolyzed under acidic conditions, followed by silica gel column chromatography to afford aristeromycin M. Mass, UV, and CD spectra of the synthetic specimen were identical to those of natural aristeromycin M in all respects. The absolute configuration of aristeromycin M was determined as 9-[(1*R*, 2*S*,3*R*,4*S*)-2,3-dihydroxy-4-methylcyclopentan-1-yl]hypoxanthine.

It is difficult to rule out the possibility that aristeromycin M may be an artifact arising from the predicted adenine analog, but it can be assumed to be a possible precursor of aristeromycin biosynthesis.

The antimicrobial activity (*in vitro*) against 19 strains of bacteria and 24 strains of fungi including phytopathogenic ones was not observed. The LD_{50} in mice was over 400 mg/kg (ip administration). The antiviral test for aristeromycin M analogs is in progress, and the result will be published elsewhere.

Experimental

General Methodology

UV spectra were recorded on Hitachi EPS-2T and Jasco Uvidec 320 spectrometers. ^1H NMR spectra were obtained with a Joel-GX400 spectrometer in D_2O using 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. Chemical shifts were given in parts per million downfield from the standard. CD spectra were recorded on a Jasco 500A spectrometer. A Nichiden JMS-01SC mass spectrometer was used for mass spectrometry. Melting points were determined on a Yanagimoto hot plate apparatus and were uncorrected. A pre-coated silica gel plate and Silica Gel 60 (70~230 mesh, E. Merck) were used for thin-layer chromatography and column chromatography, respectively. The solvent was removed *in vacuo* at a temperature below 40°C with a rotary evaporator.

9-[(1*R*,2*S*,3*R*,4*R*)-4-Hydroxymethyl-2,3-*O*-isopropylidenecyclopentane-1-yl]hypoxanthine (**2**)

A mixture of **1** (0.5 g), *p*-toluenesulfonic acid (1.0 g) and acetone (30 ml) was stirred at room temperature for 20 hours and poured into a 10% ammonium hydroxide solution. The resulting solution was concentrated giving a solid that was purified by chromatography on a silica gel column (30 g, $CHCl_3$ - MeOH=9: 1). The product was crystallized from EtOH to give **2** (340 mg). MP 196~197°C.

9-[(1*R*,2*S*,3*R*,4*R*)-4-Thiocarbonylimidazolyloxymethyl-2,3-*O*-isopropylidenecyclopentane-1-yl]-hypoxanthine (**3**)

To a suspension of **2** (50 mg) in dry CH_2Cl_2 (20 ml) was added thiocarbonyldiimidazole (100 mg), and the reaction mixture was stirred for 2 days at room temperature. The insoluble products (41 mg) were collected by filtration and recrystallized from MeOH giving colorless prisms (30 mg). MP >300°C. NMR (Varian EM390 spectrometer, DMSO-d_6 - D_2O) δ 1.42 and 1.67 (6H, s×2, *iso*-propyl), 7.35 (2H, m, CH_2O), 7.80 and 8.00 (2H, d×2, imidazole protons), 8.23 and 8.45 (2H, s×2, aromatic protons), 8.73 (1H, s, imidazole proton).

Anal Calcd for $C_{18}H_{20}N_6O_4S$: C 51.91, H 4.84, N 20.18.
Found: C 51.19, H 4.77, N 19.73.

Aristeromycin M

Natural Sample: MP 260~261°C (recrystallized from EtOH). UV λ_{max} nm (ε) 251 (11,400, pH 1.0), 250 (11,800, pH 7.0), 255.5 (12,900, pH 12.0). CD $\Delta\varepsilon$=+11.8.

Anal Calcd for $C_{11}H_{14}N_4O_3$: C 52.79, H 5.64, N 22.39.
Found: C 52.79, H 5.69, N 22.05.

Synthetic Sample: A mixture of α,α'-azobisisobutyronitrile (100 mg) and *n*-Bu_3SnH (0.5 ml) in dry dioxane (0.5 ml) was added to a refluxing solution of **3** (150 mg) in dry dioxane (1 ml) over 1 hour. The solvent was removed, and the residue was dissolved in H_2O (pH 1.5). The solution was heated at 70°C for 2 minutes, neutralized and concentrated. The residue was applied to a silica gel column chromatography (10 g). The column was eluted with $CHCl_3$ - MeOH (5: 1) to give a mixture of aristeromycin M and organotin compounds. After rechromatography on silica gel and recrystallization from MeOH, pure aristeromycin M was obtained as colorless prisms (25 mg). CD $\Delta\varepsilon$=+11.8. MS *m/z* 250 (M, $C_{11}H_{14}N_4O_3$), 232 (M−H_2O).

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