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The Structure of Aristeromycin[1,2]

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Aristeromycin, a new antibiotic isolated from the culture broth of *Streptomyces citricolor*, exhibits inhibition of the growth of *Pyricularia oryzae* and *Xanthomonas oryzae*. The structure of aristeromycin, apart from stereochemistry, was assumed as (A) on the basis of physicochemical and chemical properties of aristeromycin and its pentaacetate. By X-ray analysis of aristeromycin hydrobromide the assumed structure was ascertained and the absolute configuration was established as (1′R, 2′S, 3′R, 4′R)-9-[β-2′α, 3′α-dihydroxy-4′β-(hydroxymethyl)cyclopentyl]adenine.

Aristeromycin, a new antibiotic, was isolated in 1967 by Kusaka, *et al.*[4] from the culture broth of *Streptomyces citricolor*. The antibiotic shows inhibitory activity against *Xanthomonas oryzae* and *Pyricularia oryzae in vitro* as well as *in vivo*. Aristeromycin (**I**) is colorless prism, mp 213—215° (decomp.), $[\alpha]_D^{25}$ −52.5°. From its elementary and X-ray analysis, and mass spectrometric method the molecular formula was presumed to be $C_{11}H_{15}O_3N_5$ (M.W. 265). From the measurement of a peak of *m/e*: 265 by high resolution method the accurate molecular weight was found to be 265.116. As difference between the calculated and measured values of the molecular weight is 1 m.m.u., $C_{11}H_{15}O_3N_5$ was decided as the molecular formula.

Since the antibiotic is negative to Barton's, Fehling, ninhydrin, Molisch and Sakaguchi's reagents, it was assumed not to contain phenolic hydroxyl, reducing sugar and α-amino acid moiety. The maximum values in the ultraviolet (UV) spectra of aristeromycin were as follows: $\lambda_{max}^{pH2.3}$ mμ (log ε): 260 (4.154), $\lambda_{max}^{pH6.7}$ mμ (log ε): 262 (4.167), $\lambda_{max}^{pH12.0}$ mμ (log ε): 262 (4.161). The UV spectra resemble those of adenine derivatives, especially adenosine λ_{max}^{pH2} mμ (log ε): 257 (4.164), λ_{max}^{pH11} mμ (log ε): 260 (4.173).

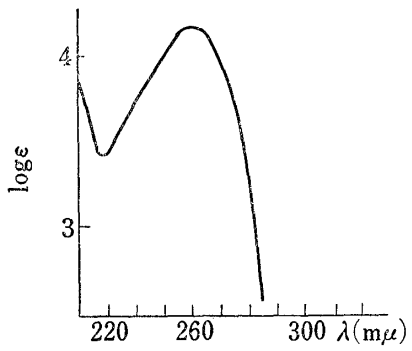


Fig. 1. UV Spectrum of Aristeromycin

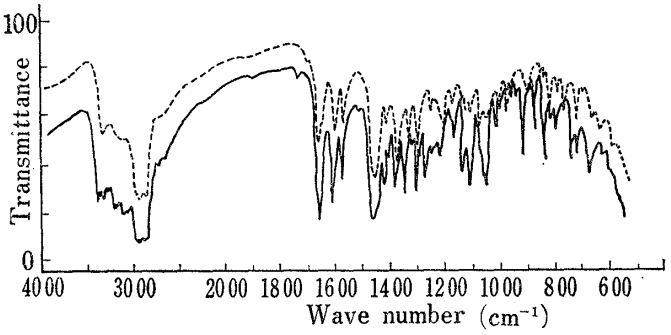


Fig. 2. IR Spectra of Aristeromycin and Adenosine (Nujol mull)

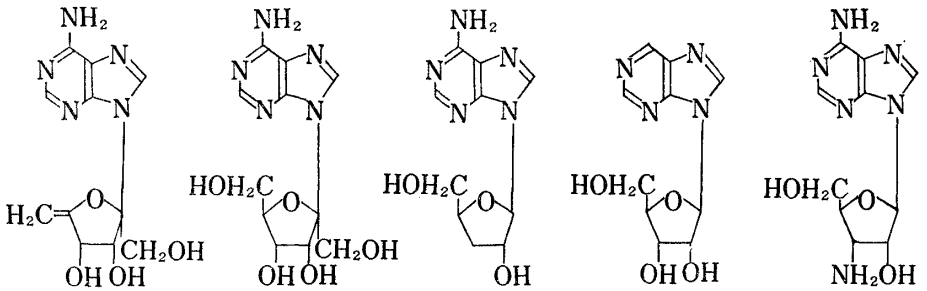
——: aristeromycin ------: adenosine

1) T. Kishi, M. Muroi, T. Kusaka, M. Nishikawa, K. Kamiya and K. Kizuno, *Chemical Communications*, No. 17, 852 (1967).
2) This report was presented at the 156th meeting of the Japan Antibiotics Research Association, July, 21, 1967.
3) Location: *Juso-nishino-cho, Higashiyodogawa-ku, Osaka.*
4) T. Kusaka, H. Yamamoto, M. Shibata, M. Muroi, T. Kishi and K. Mizuno, *J. Antibiotics* (Tokyo), **21**, 255 (1968).

As shown in Fig. 2 the infrared (IR) spectrum of aristeromycin closely resembles those of adenosine and angustmycin C (III), especially the absorption of 1660, 1600 cm^{-1} probably due to $\nu_{C=C}$, $\nu_{C=N}$ of purine nucleus, and 3100—3400 cm^{-1} of $\nu_{OH,NH}$, 1030—1070 cm^{-1} of δ_{OH}.

In the nuclear magnetic resonance (NMR) spectrum of aristeromycin (Fig. 3), two aromatic protons are observed at δ-values 8.17 ppm (1H, singlet) and 8.10 ppm (1H, singlet) probably due to hydrogen at C_2 and C_8 of adenine nucleus. Two protons at *ca.* 7.2 ppm (2H, singlet like) which disappears on addition of D_2O are probably due to aminogroup. These data of the UV, IR, NMR spectra and elementary analysis suggest that aristeromycin is an adenine derivative.

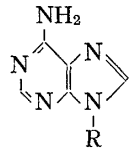
Among known antibiotics, those having a UV spectrum like adenine derivatives are angustmycin A (II), angustmycin C (III), cordycepin (IV), nebularine (V), and 3'-amino-3'-deoxyadenosine (VI).



II III IV V VI

Chart 1

A strong peak (*m/e*: 135) observed in the mass spectrum of I also supported the presence of adenine nucleus ($C_5H_5N_5$=135).



$R=C_6H_{11}O_3$

As the unsaturation number of $R=C_6H_{11}O_3$ is one, it may form a ring or contain an unsaturated double bond. As I was not reduced by catalytic reduction with Pd-carbon and as no olefinic proton was observed in the NMR spectrum, the presence of unsaturated double bond was not supposed in the R-group. As I gradually decolorizes the aqueous solution of potassium permanganate and is positive to the benzidine reagent after treatment with periodic acid, it was assumed to contain a vicinal hydroxyl group. On paper ionophoresis, I and adenosine does not migrate in the Theorell buffer at pH 9.0 but in the same buffer containing boric acid, they migrate 2.6 and 2.3 cm, respectively toward the anode. From these facts the R-group should contain at least two hydroxyl group and it is supposed to be *cis*-diol.

Aristeromycin acetate (VII) obtained by acetylation in pyridine is obviously negative to the periodic acid-benzidine reagent. In the NMR spectrum of VII (Fig. 3), there were found three singlets at 1.92, 2.07, and 2.09 ppm corresponding to each three protons probably due to O-acetyl and one singlet at 2.31 ppm corresponding to six protons due to N-acetyl. The highest mass number in the mass spectrum of VII was *m/e*: 475 which was in good accord with its molecular weight. From these facts it was assumed that the three oxygens in the R-group $C_6H_{11}O_3$ form hydroxyl groups.

When solutions of I in 2N HCl or 2N H_2SO_4 were boiled for 10 hr, the corresponding purine base and reducing sugar were not detected and the original antibiotic was recovered. Thus it was thought that usual N-glycosidic linkage is not present in I. A doublet signal (in $CDCl_3$, 2H, d, J=5 Hz) at 3.54 ppm in I is assumed to be methylene proton of hydroxymethyl from its chemical shift, from the fact that such methylene is observed at 3.86 in adeno-

sine (2H, d, J=5 Hz) and from the fact that these protons were shifted to 4.23 in the pentaacetate. Consequently it might be reasonable to assume a cyclopentane ring having one *cis*-diol and one hydroxymethyl groups as the presumed structure of the R-group. Aside from signals due to the hydroxymethyl and hydroxyl groups, there were multiple signals at 1.9—2.5 (3H), 3.91 (1H) 4.32 (1H) and 4.84 ppm (1H). Of them, those at 3.91 and 4.32 ppm would be attributed to hydroxyl methine protons since they shift to the lower field in the spectrum of VII (5.34 and 5.68 ppm, respectively). The proton at 4.84 ppm might be due to a methine group attached to a nitrogen atom in the adenine nucleus. The relatively lower chemical shift might be due to the paramagnetic shielding of the adenine ring current.

In the NMR spectrum of VII there were observed one methylene proton of hydroxymethyl group at 4.23 as doublet, one methine proton of $C_{1'}$ at 4.96 ppm which does not shift in VII.

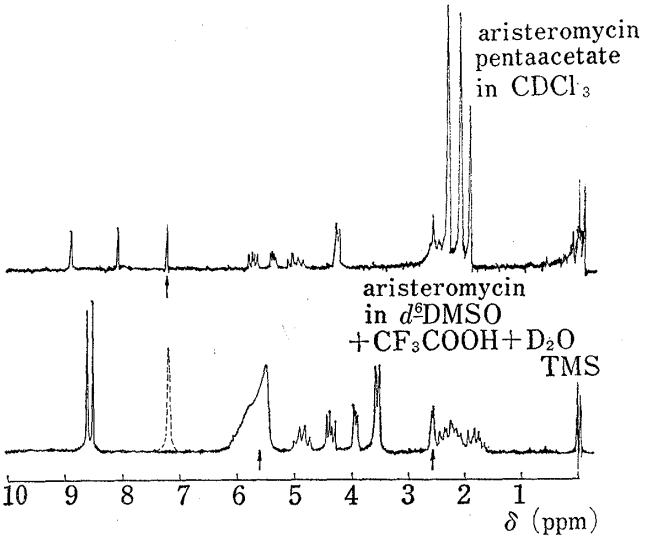


Fig. 3. Comparison of the NMR Spectra of Aristeromycin Pentaacetate and Aristeromycin

The peak, indicated with an arrow, is one by solvent.

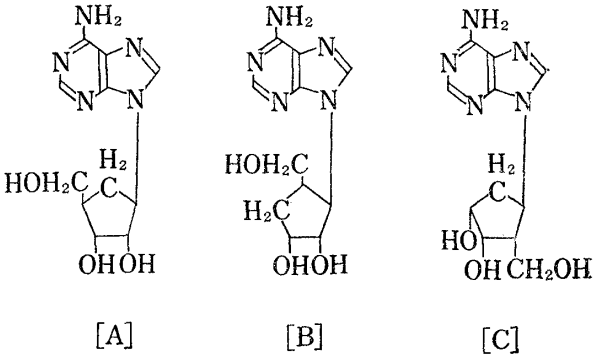


Chart 2. Proposed Structure of Aristeromycin

From these facts, the R-group is assumed to be a five membered ring formed by combining $-\overset{H}{\underset{OH}{C}}-\overset{H}{\underset{OH}{C}}-$, $-\overset{H}{\underset{CH_2OH}{C}}-$, CH_2 and CH. The following three illustrations are proposed for the plane structure of I (Chart 2).

To make clear the mutual relation of the substituents attached to the cyclopentane ring, I and VII were investigated by NMR spin decoupling method. When the methine proton of $C_{1'}$ was irradiated, methine proton at 5.7 ppm was decoupled from quartet to doublet in VII. Conversely by the irradiation of the proton at 5.76 ppm ($C_{2'}$), the proton at 5.34 ppm was decoupled together with the proton at $C_{1'}$. When the proton at $C_{3'}$ was irradiated, the proton at $C_{2'}$ was decoupled from quartet to doublet. From the fact, the possibility of formula (C) was excluded. The difference between the formula (A) and (B) is that the CH_2 and $CH\text{-}CH_2OH$ group exist at $C_{4'}$ and $C_{5'}$ or $C_{5'}$ and $C_{4'}$.

When careful irradiation was carried out with VII in the high field, only methine at $C_{1'}$ was decoupled in the irradiation at 2.3—2.05 ppm. It was found that two protons of methylene appeared as a multiplet at 2.2—2.6 ppm and the methine proton of $CH(CH_2OH)$ exists at *ca.* 2.6 ppm and overlaps with the methylene. It resulted that $C_{5'}$ is methylene and consequently $C_{4'}$ is methine having hydroxymethyl group. It suggests that the formula

(A) is I. Moreover, the signals of methine protons at $C_{2'}$ and $C_{3'}$ were observed as a quartet similarly since they may be influenced by neighboring hydrogens, thus the formula (A) is most reasonable.

The proposed structure was supported by the result of the NMR spin decoupling of I. It is certain that methine proton at $C_{4'}$ and methylene proton at $C_{5'}$ lie one above the other at *ca.* 2.3 ppm and the latter also lies in the high field (Fig. 5).

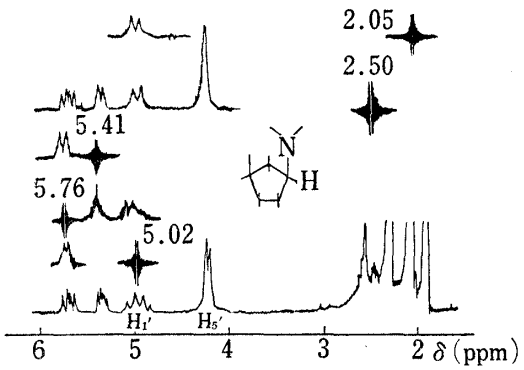


Fig. 4. NMR Spin Decoupling of Aristeromycin Pentaacetate ($CDCl_3$)

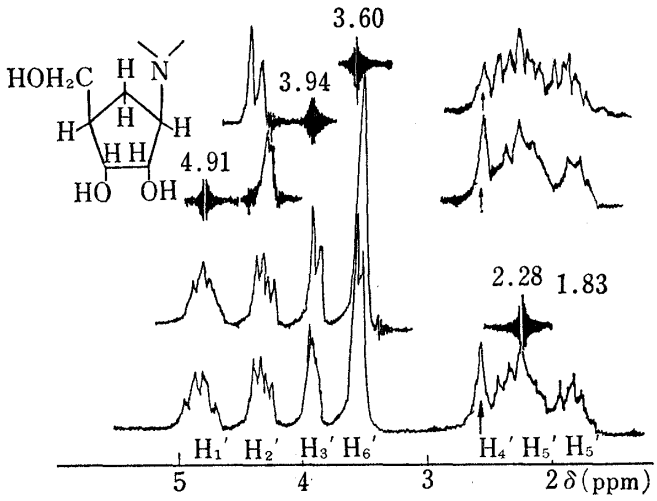


Fig. 5. NMR Spin Decoupling of Aristeromycin (DMSO·D_2O·F_3CCOOH)

The peak, indicated with an arrow, is one by solvent.

The coupling constants of each neighboring proton in I and its pentaacetate are: $J_{1'2'}$=9 Hz, $J_{2'1'}$=9 Hz, $J_{2'3'}$=5 Hz, $J_{3'2'}$=5 Hz, $J_{3'4'}$=2.5 Hz, $J_{1'5'}$=9 Hz and $J_{6'4'}$=5 Hz.

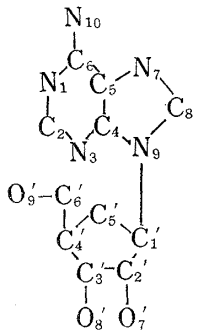
For the confirmation of the suggested structure and for the determination of the absolute configuration of the molecule, X-ray analysis of aristeromycin hydrobromide was carried out. A preliminary study indicated that the crystal belonged to the monoclinic system, the unit cell parameters being a=10.73, b=7.25, c=9.15 Å and β=110°24. Assuming two molecules per asymmetric unit, Dc was calculated to be 1.723, which agreed well with Dm, 1.727 (by the flotation method). Systematic absence of OkO reflections when k is odd showed that the possible space group was either C_2^2 ($P2_1$) or C_{2h}^2 ($P2_1/m$). Since the crystal contains optically active molecules, only $P2_1$ can be accepted.

As the first step of the structure analysis, a three-dimensional sharpened Patterson function was computed. The bromine coordinates (x=0.31, y=0.00, z=0.09) were obtained from the highest peak in Harker section, H(U½W). The minimum function diagram calculated on the basis of these bromine coordinates, clearly visualized the adenine skeleton of the molecule, which lies in the (0 1 0) plane.

The structure of the sugar moiety was not clarified from this diagram because of the mirror image ambiguity inevitable in this case. By careful analysis of the behavior of temperature factors in least-squares treatment, however, the true structure was differentiated from its mirror image and the whole stereochemical structure was elucidated as shown in Fig. 6. The atomic coordinates and isotropic temperature factors obtained by further refinement using least-squares method are shown in Table I. The average value of the standard deviation for the coordinates shown in the table is 0.018 Å.

The temperature factor of $C_{5'}$ seemed to be a little too small for a carbon atom. But bond lengths, $C_{1'}$-$C_{5'}$ and $C_{4'}$-$C_{5'}$, and bond angle, $C_{1'}$-$C_{5'}$-$C_{4'}$, indicated that the atom was

TABLE I. The Atomic Coordinates and Isotropic Temperature Factors



Atom	x/a	y/b	z/c	B
Br	0.6863	0.9925	0.9122	2.819
C_2	0.0787	0.0349	0.2216	2.473
C_4	0.2634	0.0151	0.1534	2.295
C_5	0.1774	0.9959	0.9897	1.755
C_6	0.0393	0.0223	0.9533	1.596
C_8	0.3844	0.9764	0.0033	2.015
$C_{1'}$	0.5017	0.0025	0.2947	1.758
$C_{2'}$	0.5809	0.1756	0.2930	2.709
$C_{3'}$	0.7424	0.1118	0.3765	1.396
$C_{4'}$	0.7334	0.9112	0.4162	2.420
$C_{5'}$	0.5979	0.8252	0.3028	1.204
$C_{6'}$	0.8513	0.8055	0.4020	1.928
N_1	0.9972	0.0308	0.0726	2.353
N_3	0.2149	0.0215	0.2677	1.912
N_7	0.2594	0.9806	0.8981	2.142
N_9	0.3802	0.9970	0.1560	1.177
N_{10}	0.9511	0.0197	0.8074	2.328
$O_{7'}$	0.5515	0.3174	0.3801	3.170
$O_{8'}$	0.8009	0.2289	0.5028	2.195
$O_{9'}$	0.0290	0.3772	0.4871	2.452

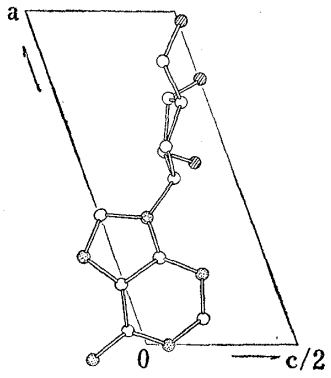


Fig. 6. The Stereo-model of Aristeromycin

oxygen: ◍ nitrogen: ◍
carbon: ○

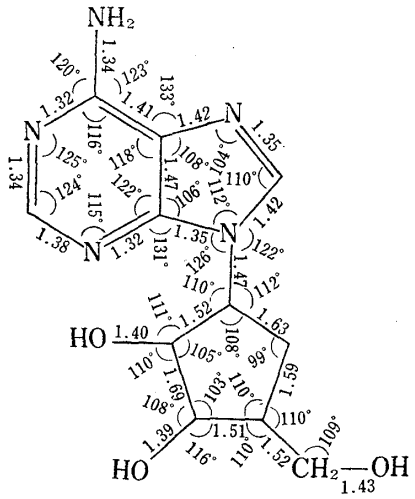


Fig. 7. The Bond Lengths and Angles of Aristeromycin

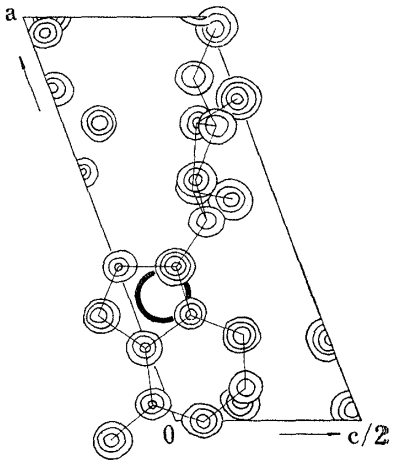


Fig. 8. The Final Three-dimensional Electron Density Distribution Shown by Means of Superimposed Contour Sections Drawn parallel to (0 1 0)

carbon. The results of elementary analysis and chemical evidences described above eliminated any possibility of assigning this atom for other species, nitrogen or oxygen.

The bond lengths and angles evaluated on this work are shown in Fig. 7. The final electron density distribution is shown by means of superimposed contour sections in Fig. 8. The R-value was 0.125.

The absolute configuration of the molecule was determined to be (1'R, 2'S, 3'R, 4'R)-9-{β-2'α,3'α-dihydroxy-4'β-(hydroxymethyl)cyclopentyl}adenine by the use of anomalous dispersion of the bromine atom. Relative intensities of the pairs of reflections (h k l) and ($\bar{h}$ $\bar{k}$ $\bar{l}$) used for this analysis are listed in Table II.

Table II. Comparison of the Observed and Calculated Intensity Differences Used for the Establishment of Absolute Configuration

hkl	Fc(hkl)/ Fc($\bar{h}\bar{k}\bar{l}$)	Io(hkl)/ Io($\bar{h}\bar{k}\bar{l}$)	hkl	Fc(hkl)/ Fc($\bar{h}\bar{k}\bar{l}$)	Io(hkl)/ Io($\bar{h}\bar{k}\bar{l}$)
511	1.04	>1	614	1.11	>1
1123	1.04	>1	1025	0.88	<1
1114	0.83	<1	1035	1.28	>1
824	1.23	>1	1045	0.88	<1

A difference Fourier synthesis was computed to locate hydrogen atoms. Of a total of 16 hydrogen atoms in the asymmetric unit, three attached to carbon atoms $C_{1'}$, C_2, and C_8, respectively and two attached to nitrogen atom N_{10} were located unambiguously. A planar trivalent structure was found for N_{10}.

Since N_9 was also in a trivalent state, the proton originated from hydrogen bromide should be located at N_1 or N_3 or N_7. Peaks attributable to hydrogen were found at geometrically reasonable positions near N_1 and N_7.

It was difficult to determine, at the present stage, which of them was true one.

The final R-value, taking these hydrogen into account, was 0.123 and the coordinates of hydrogen atoms are shown in Table III.

Table III. Atomic Coordinates of Hydrogen

Atom	x/a	y/b	z/c	Atom	x/a	y/b	z/c
H_2	0.049	0.015	0.304	H_{10b}	0.849	0.993	0.778
H_8	0.476	0.001	0.979	H_1	0.285	0.900	0.074
$H_{1'}$	0.470	0.972	0.386	H_7	0.223	0.054	0.782
H_{10a}	0.981	0.035	0.725				

Shealy and Clayton[5] have synthesized 9-{β-DL-2'α,3'α-dihydroxy-4'β-(hydroxymethyl)-cyclopentyl}adenine, mp 238—242° (decomp.), UV λ_{max} mμ (ε): 261 (14800) in phosphate buffer. The compound is optical inactive, but I is optical active ($[\alpha]_D^{25}$ −52.5°) and its stereochemical structure is (1'R, 2'S, 3'R, and 4'R).

Experimental

Aristeromycin——Aristeromycin was obtained as colorless prisms, melted at 213—215° (decomp., uncorr.). *Anal.* Calcd. for $C_{11}H_{15}O_3N_5$: C, 49.80; H, 5.70; N, 26.40; O, 18.09. Found: C, 49.88; H, 5.65; N, 26.46; O, 18.59. High resolution analysis of mass spectrometry (*m/e*): Calcd. for $C_{11}H_{15}O_3N_5$: 265.117481. Found: 265.116494. Specific rotation $[\alpha]_D^{25}$ −52.5° (c=1.0, DMF).

5) Y.F. Shealy and J.D. Clayton, *J. Am. Chem. Soc.*, **88**, 3885 (1966).

Rf values in paper chromatography (Whatman No. 1)

n-BuOH (H$_2$O saturated)	*Rf* 0.25
n-BuOH:AcOH:H$_2$O (4:1:5)	0.38
n-BuOH:Pyridine:H$_2$O (5:3:1)	0.61

Aristeromycin Hydrobromide——To a solution of 265 mg of aristeromycin in 50 ml of water was added 0.2 ml of HBr (47%). After evaporating to dryness *in vacuo*, the residue was dissolved in 20 ml of MeOH and left standing at room temperature. Aristeromycin hydrobromide separated out as colorless prisms was recrystallized from MeOH, mp 221.5° (browning) 229° (decomp.). *Anal.* Calcd. for $C_{11}H_{15}O_3N_5 \cdot$ HBr: C, 38.16; H, 4.66; N, 20.23; Br, 23.08. Found: C, 37.93; H, 4.56; N, 20.18; Br, 23.83. Unit cell parameters were measured on Weissenberg films obtained with CuKα radiation, using a Nonius Weissenberg camera calibrated against NaCl.

Intensity measurements for the structure analysis were carried out with a Hilger Watts' linear diffractometer, using MoKα radiation. A total of 1442 diffractions were taken of 7 layers about the b-axis. By the use of statistical methods, the absolute scale was established and the overall temperature factor was estimated to be 2.75A^2.

For the measurement of anomalous dispersion, CuKα radiation was used.

Aristeromycin Pentaacetate——To a suspension of 500 mg of aristeromycin in 8 ml of pyridine was added dropwise 4 ml of Ac_2O with stirring and cooling with ice water. The mixture was stirred for a while at room temperature and left standing at 37° for 40 hr. The reaction minture was poured in ice water, extracted with 300 ml of AcOEt, washed with water and the extract was concentrated *in vacuo* to give a sirupy substance. The product was chromatographed on thin layer plates (Merck silica gel HF_{254}, Solvent AcOEt) and the portion showing the largest *Rf* value was collected, extracted with AcOEt and the extract was washed with water, dried and concentrated *in vacuo* to yield a colorless sirupy substance. The highest mass number *m/e*: 475 ($C_{11}H_{15}O_3N_5$ (265)+$C_2H_2O \times 5$ (210)), UV spectrum: UV λ_{max}^{EtOH}=272 mμ, NMR spectrum (Fig. 3).

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