STUDIES ON NEPLANOCIN A, NEW ANTITUMOR ANTIBIOTIC. II

STRUCTURE DETERMINATION

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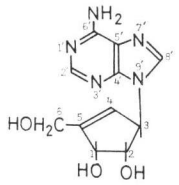
The structure and stereochemistry of neplanocin A was determined on the basis of its spectral and chemical evidences as [1*R*-(1α,2α,3β)]-3-(6-amino-9*H*-purin-9-yl)-5-(hydroxymethyl)-4-cyclopentene-1,2-diol. For the final proof of the structure, X-ray crystallographic analysis of neplanocin A was carried out.

In a previous paper[1], the producing organism, isolation and characterization of neplanocin A, a new antitumor antibiotic from the culture filtrate of *Ampullariella regularis* A11079, were described. This antibiotic exhibits significant antitumor activity against L1210 leukemia in mice and represents a new member of the nucleoside family from natural sources. This paper deals with the structural elucidation of neplanocin A.

Structural Elucidation of Neplanocin A

Neplanocin A (**1**), $C_{11}H_{13}N_5O_3$, displayed UV maxima at 262 (log ε 4.20, water, pH 7), 262 (log ε 4.19, pH 11), and 260 nm (log ε 4.18, pH 2). The UV spectra, therefore, resembled those of adenine derivatives, especially adenosine. The IR spectrum of **1**[1] also similar to that of adenosine, showed absorptions at 1650, 1600 and 1570 cm^{-1} ($\nu_{C=C}$, $\nu_{C=N}$), typical of a purine moiety and at 3100~3400 cm^{-1} ($\nu_{OH,NH}$). The only difference between the spectrum of **1** and that of adenosine was the presence of the additional absorption of 1640 cm^{-1} ($\nu_{C=C}$) in **1**. In the mass spectrum (EI, 70 eV) of **1**, the ion peak at *m/z* 136 supported the presence of an adenine moiety. This ion occurred by the cleavage of the bond between the base and the sugar moiety accompanied with the transfer of two hydrogens to the base[2]. The ^{1}H-NMR spectrum of compound **1**[1] revealed two very sharp singlets at δ 8.16 and 8.09 due to the aromatic protons at C(2) and C(8) of the adenine moiety and a broad peak (2H) at δ 7.21 due to an amino group. The remaining protons of the spectrum consisted of a series of peaks from δ 4.1 to 5.8 (9H). The addition of a small amount of acetic acid-d_4 to **1** in dimethylsulfoxide-d_6 (Fig. 1) caused deuterium exchange of the protons on nitrogen at δ 7.21 and of 3 protons at δ 5.17 (d, J=6.5 Hz), 4.98 (d, J=5.5 Hz) and 4.93 (t, J=5.5 Hz). These data suggested the presence of two secondary and one primary hydroxyl group. A multiplet (2H) at δ 4.16, a doublet of doublet (1H) at δ 4.49 and a doublet of doublet of doublet (1H) at δ 4.34 in **1** were assumed to be due to a hydroxymethyl proton and two hydroxymethine protons since the splitting of the respective protons changed by the addition of acetic acid-d_4. To assign each of the signals, decoupling experiments were carried out as summarized in Table 1. In addition, the spec-



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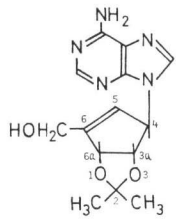

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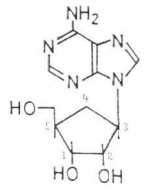

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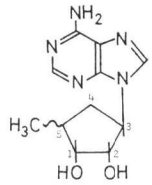

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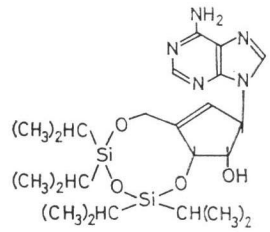

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trum exhibited the homoallylic coupling[3] between the proton at C(3) and the geminal ones at C(6), because the dihedral angle between the plane of the C(4)-C(5) double bond and the C(3) proton is *ca.* 90°. From these results (Table 1), the signal at δ 5.74 can be assigned to a vinylic proton and that at δ 5.37 to a methine group to which a nitrogen atom in the adenine moiety is attached. The above data suggest that neplanocin A is 3-(6-amino-9*H*-purin-9-yl)-5-(hydroxymethyl)-4-cyclopentene-1,2-diol.

The proposed structure is supported by the following chemical evidence. Neplanocin A rapidly consumed one equivalent of KIO_4 and gave an *O*-isopropylidene derivative (**2**), indicative of a *cis*-vicinal hydroxyl group. Catalytic hydrogenation of **1** gave a dihydro derivative

Fig. 1. ^{1}H-NMR spectrum of neplanocin A (Me_2SO-d_6+CD_3COOD).

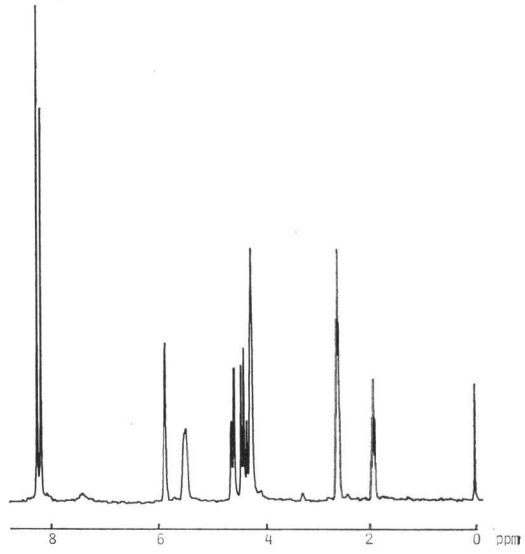


Table 1. ^{1}H-NMR decoupling data of neplanocin A.

Decoupled proton	Irradiated at (ppm)				
	4.16 6-H	4.34 2-H	4.49 1-H	5.37 3-H	5.74 4-H
1-H (d, J=5.5)		singlet			
2-H (dd, J=5.5, 5.5)			doublet (J=5.5)	doublet (J=5.5)	
3-H (ddd, J=5.5, 1.5, 2.0)	deformed	deformed			deformed
4-H (dd, J=1.5, 1.0)	doublet (J=1.5)			broad singlet	
6-H (dd, J=2.0, 1.0)				broad singlet	broad singlet

which was identical with aristeromycin[4] (**3**). A main product (**4**, $C_{11}H_{15}N_5O_2$), was obtained by the elimination of the allylic primary hydroxyl group prior to the hydrogenation of the C(4)-C(5) double bond of **1**.

Therefore, the structure of neplanocin A is established as [1*R*-(1α, 2α, 3β)]-3-(6-amino-9*H*-purin-9-yl)-5-(hydroxymethyl)-4-cyclopentene-1, 2-diol (**1**). The ^{13}C-NMR spectrum of neplanocin A also supports the structure of **1**. The signals of all 11 carbon atoms in neplanocin A were assigned (Table 2) on the basis of SFOR techniques and a comparison of the spectrum of adenosine[5].

Table 2. ^{13}C-NMR chemical shifts for neplanocin A.

Carbon	Chemical shift	Multiplicity
C (1)	72.3	d
C (2)	76.7	d
C (3)	64.4	d
C (4)	123.5	d
C (5)	150.1	s
C (6)	58.6	t
C (2′)	152.3	d
C (4′)	149.6	s
C (5′)	119.2	s
C (6′)	156.0	s
C (8′)	139.6	d

X-Ray Analysis of Neplanocin A

X-Ray Intensity Measurement

Recrystallization of **1** from a methanol solution afforded colorless, transparent, single crystals. Preliminary oscillation and Weissenberg photographs indicated unambiguously the space group of $P2_12_12_1$. Accurate cell parameters were determined by a least-squares fit of 2θ angles of 50 reflections in the range $23° < 2\theta < 35°$ measured on an automated four-circle diffractometer with Mo $K\alpha$ radiation (λ=0.71069 Å).

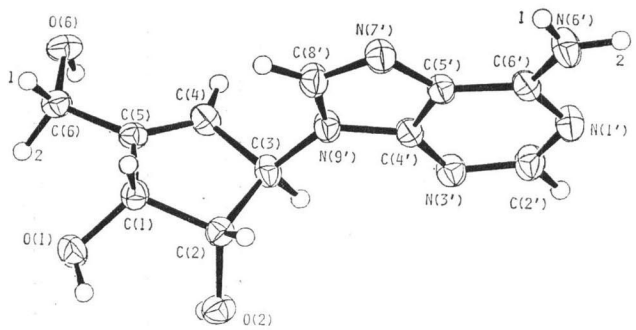
A specimen, approximately 0.18×0.19×0.38 mm, was used for data collection. Integrated intensities of the independent reflections for 2θ less than 60° were measured with the $\omega-2\theta$ scan mode at the ω scan rate of 2° min^{-1} by use of graphite-monochromated Mo $K\alpha$ radiation. The scan width in ω was $(1.0+0.34\tan\theta)°$ with stationary background counts of 10 s duration on either side of the peak. No remarkable intensity variation was observed for the five monitoring reflections during the data collection. The intensities were corrected for the Lorentz and polarization effects. No corrections were made for absorption or extinction. Of the 1878 reflections measured, the 1705 reflections with $|F_o| < 3\sigma(F)$ were considered observed and used for the structure determination.

Determination and Description of the Structure

The structure of **1** was solved by the direct method using the Multan 78 program package[6]. An E map calculated with a phase set having the highest figure-of-merit revealed all the non-hydrogen atoms. Their positional and anisotropic thermal parameters were refined by the block-diagonal least-squares calculations. A difference Fourier map revealed all the hydrogen atoms; they were refined isotropically. In the final least-squares cycle the weighing scheme, $1/\omega=\sigma^2(F)+0.0004|F_o|^2$, was used. The final residual indices were: R=0.038 and $R_w=[\Sigma w||F_o|-|F_c||^2/\Sigma w|F_o|^2]^{1/2}$=0.043. All computations were carried out on ACOS S700 computer at Crystallographic Research Center, Institute of Protein Research, Osaka University, and FACOM 230/38 computer at Information Processing Research Center, Kwansei Gakuin University.

The crystal data are summarized as follows: Orthorhombic, space group $P2_12_12_1$; a=13.746(1), b=12.045(1), c=6.899(1) Å, V=1142.3(2) Å^3; Z=4, D_x=1.5308(3) g cm^{-3}. Fig. 2 shows the mole-

Fig. 2. A drawing of molecular structure of neplanocin A.



cular structure.*

The X-ray structure thus confirms unequivocally the chemical structure described above. The adenine moiety is planar within ±0.01 Å. N(6′) deviates from the adenine plane by 0.045(3) Å. The bond lengths and angles in the adenine moiety agree satisfactorily with those found in other structures containing an adenine moiety[7~9]. In the sugar moiety the bond lengths and angles are in an expected range. The cyclopentenyl ring twists in such a way that C(1) deviates by 0.16 Å toward N(9′), and C(2) by 0.15 Å opposite to N(9′), from the plane of C(5), C(4), and C(3). No definite convention that describes the conformation of the cyclopentenyl ring has been proposed. Nevertheless, if the endo-exo notation is applied to the present case, and if N(9′) is taken as the reference atom, the present cyclopentenyl ring is designated as C(2)-exo-C(1)-endo. This contrasts with the C(2)-endo conformation** found in 1,6-*O*-(tetraisopropyldisiloxane-1,3-diyl)neplanocin A (**5**), a close relative of **1**.

The interplanar angle between the adenine plane and the mean cyclopentenyl plane is 81.5°. The torsion angle about the N(9′)-C(3) bond, Φ[C(8′)-N(9′)-C(3)-C(4)], is 61.3(3)°, which lies in the anti range. The corresponding torsion angles in **5** are 34.4(7)° and 33.0(7)° for the two independent molecules involved in its crystals**; in aristeromycin that is 99°[4].

In the crystal the molecules are linked to each other by hydrogen bonds to form a tight three-dimensional network. N (1′) does not participate in any hydrogen bond, and no close interaction is found involving N(1′), the closest distance being N(1′) . . . H(C2) of 2.68 Å.

Experimental

Melting points were observed in a Yanaco micro-melting point apparatus and were uncorrected. IR spectra (KBr) were recorded with a Hitachi 260-50 spectrophotometer and UV spectra with a Hitachi 323 instrument. The optical rotation at 589 nm was measured with a JASCO automatic polarimeter DIP-180. The NMR spectra were taken with a JEOL JNM-FX100 spectrometer at 99.55 (^{1}H) and 25.00 (^{13}C) MHz with TMS as an internal reference. The mass spectra were taken with a JEOL JMS-D300 spectrometer. Glass plates coated with Kieselgel-GF_{254} served for TLC. Column chromatography was carried out using silica gel (Merck, Kieselgel 60).

[1*R*-(1α,2α,3β)]-3-(6-Amino-9*H*-purin-9-yl)-5-(hydroxymethyl)-4-cyclopentene-1,2-diol (**1**)

1 was obtained as colorless prisms, mp 220~222°C, $[\alpha]_D^{23}$ −157° (*c* 0.5, H_2O). *Anal.* Calcd. for

* The list of the X-ray data can be obtained from the authors on request.

** Private communication from M. YAMASAKI, Y. YAMAGATA, T. FUJIWARA, K. TOMITA, K. FUKUKAWA, T. UEDA and T. HIRANO. The authors are deeply indebted to them.

$C_{11}H_{13}N_5O_3$: C, 50.19; H, 4.98; N, 26.60. Found: C, 49.96; H, 5.00; N, 26.43. MS: calcd. for $C_{11}H_{13}N_5O_3$: 263.1019. Found: 263.1026.

[3a*S*-(3aα, 4β, 6aα)]-4-(6-Amino-9*H*-purin-9-yl)-3a,6a-dihydro-2,2-dimethyl-4*H*-cyclopenta-1,3-dioxole-6-methanol (**2**)

To a suspension of **1** (500 mg) in acetone (30 ml) was added 70% $HClO_4$ (1.43 g) with stirring at 0°C and kept overnight at room temperature. The solution was neutralized by the addition of finely powdered K_2CO_3 and the insoluble inorganic salts were removed by filtration. The filtrate was evaporated and the residue was crystallized from methanol to give 403 mg (70%) of **2**, mp 261~262°C, $[\alpha]_D^{23}$ −105.4° (*c* 1.0, MeOH). *Anal.* Calcd. for $C_{14}H_{17}N_5O_3$: C, 55.44; H, 5.65; N, 23.09. Found: C, 55.39; H, 5.54; N, 22.93. IR ν_{max}^{KBr}: 3270, 3150, 1685, 1615, 1565 cm^{-1}. UV λ_{max}^{MeOH}: 262 nm (log ε 4.18). NMR: $\delta^{Me_2SO\text{-}d_6}$: 1.27 (3H, s), 1.38 (3H, s), 4.15 (2H, m, 6-H), 4.69 (1H, d, *J*=5.5 Hz, 1-H), 5.07 (1H, t, *J*=5.5 Hz, OH), 5.34 (1H, d, *J*=5.5 Hz, 2-H), 5.46 (1H, broad s, 3-H), 5.72 (1H, broad s, 4-H), 7.26 (2H, broad s, NH_2), 7.96 (1H, s), 8.16 (1H, s). MS (*m/z*): 303 (M^+, 4%), 288 (M^+-CH_3, 3), 245 ($M^+-CH_3COCH_3$, 42), 216 (25), 135 (53), 111 (100).

Hydrogenation of **1**

1 (500 mg) in 25% aqueous methanol (120 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of platinum oxide (93 mg). After the absorption of hydrogen was completed, the catalyst was filtered and the filtrate was evaporated *in vacuo*. The residue was chromatographed over silicagel (25 g) and eluted with acetone - H_2O (60: 1) to afford [1*R*-(1α,2α,3β)]-3-(6-amino-9*H*-purin-9-yl)-5-methylcyclopentane-1,2-diol (**4**, 270 mg), which was then crystallized from ethanol as colorless prisms; mp 234~236°C. *Anal.* Calcd. for $C_{11}H_{15}N_5O_2$: C, 53.00; H, 6.07; N, 28.10. Found: C, 58.08; H, 6.27; N, 28.04. IR ν_{max}^{KBr}: 3440, 3380, 3330, 3170, 3070, 1665, 1610, 1570 cm^{-1}. UV $\lambda_{max}^{H_2O}$: 262 nm (log ε 4.16). In the ^{1}H-NMR spectrum (Me_2SO-d_6) of this compound the signal of the C(5) methyl linked to the cyclopentane ring was shown at δ 1.10 as a major signal (3H×2/3, d, *J*=6.5 Hz) and at δ 1.21 as a minor signal (3H×1/3, d, *J*=6.5 Hz). MS (*m/z*); 249 (M^+, 7%), 232 (3), 214 (2), 190 (4), 178 (2), 162 (31), 136 (100), 135 (50).

Further elution with the same solvent gave [1*R*-(1α,2α,3β,5β)]-3-(6-amino-9*H*-purin-9-yl)-5-hydroxymethylcyclopentane-1,2-diol (**3**, 140 mg) which was crystallized from aqueous methanol as colorless prisms; mp 216~218°C, $[\alpha]_D^{27}$ −54.4° (*c* 0.5, DMF). *Anal.* Calcd. for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.70; N, 26.40. Found: C, 49.84; H, 5.70; N, 26.40. IR ν_{max}^{KBr}: 3390, 3320, 3200, 3100, 1655, 1610, 1575 cm^{-1}. UV $\lambda_{max}^{H_2O}$: 262 nm (log ε 4.18). MS (*m/z*): 265 (M^+, 4%), 248 (2), 247 (2), 234 (3), 218 (2), 190 (4), 178 (2), 162 (25), 136 (100), 135 (48), This was identical with the authentic aristeromycin in all respects.

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References

1) YAGINUMA, S.; N. MUTO, M. TSUJINO, Y. SUDATE, M. HAYASHI & M. OTANI: Studies on neplanocin A. New antitumor antibiotic. I. Producing organism, isolation and characterization. J. Antibiotics 34: 359~366, 1981
2) PORTER, Q. N. & J. BALDAS: Mass Spectrometry of Heterocyclic Compounds. p. 487, John Wiley & Sons, New York, 1971
3) BHACCA, N. S. & D. H. WILLIAMS: Applications of NMR Spectroscopy in Organic Chemistry. pp. 110~114, Holden-Day, San Francisco, 1964
4) KISHI, T.; M. MUROI, T. KUSAKA, M. NISHIKAWA, K. KAMIYA & K. MIZUNO: The structure of aristeromycin. Chem. Pharm. Bull. 20: 940~946, 1972

5) JOHNSON, L. F. & W. C. JANKOWSKI: Carbon-13 NMR Spectra. p. 376, John Wiley & Sons, New York, 1972
6) MAIN, P.; S. E. HULL, L. LESSINGER, G. GERMAIN, J-P. DECLERCQ & M. M. WOOLFSON: A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data. MULTAN 78, University of York, 1978
7) VOET, D. & A. RICH: The crystal structures of purines, pyrimidines and their intermolecular complexes. Progr. Nucl. Acid Res. Mol. Biol. 10: 183~265, 1970
8) LAI, T. F. & R. E. MARSH: The crystal structure of adenosine. Acta Crystallogr., Sect. B 28: 1982~1989, 1972
9) NEIDLE, S.; W. KÜHLBRANDT & A. ACHARI: The crystal structure of an orthorhombic form of adenosine-5'-monophosphate. Acta Crystallogr., Sect. B 32: 1850~1855, 1976