ALDECALMYCIN, A NEW ANTIMICROBIAL ANTIBIOTIC FROM *Streptomyces*

III. DETERMINATION OF ABSOLUTE CONFIGURATION

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The planar structure of aldecalmycin (**1**) had been determined in the previous paper, together with the conformations of the substituted *trans* decalin ring having one double bond and the sugar; β-glucopyranoside type. The absolute configuration of **1** was a following problem to be solved. X-Ray crystallographic analysis of crystalline 4′,6′-*O*-benzylidenedihydroaldecalmycin (**4**) revealed the relative configuration. On the other hand, the stereoisomerism of the sugar was determined to be D by the optical rotation value of tetra-*O*-acetyl-α-methylglucopyranoside derived from **1**. These results established the absolute configuration of **1**.

A new antimicrobial antibiotic, aldecalmycin (**1**) was isolated from the culture broth of *Streptomyces* sp. MJ147-72F6. **1** had antibacterial activities against methicillin-resistant *Staphylococcus aureus* (MRSA)[1]. The structure elucidation by NMR spectroscopy was described in previous paper[2]. In this paper, we describe the absolute structure of **1**.

Results and Discussion

Stereoisomerism of the Sugar

Methanolysis of aldecalmycin was performed with Amberlist 15 in MeOH. The residue contained a mixture of methylglucopyranoside and many decomposition products. Many trials to get the aglycon of aldecalmycin was unsuccessful. The residue was chromatographed on a Sephadex LH-20 column to isolate the mixture of α,β-methylglucopyranosides. Treatment of this mixture with acetic anhydride in pyridine at room temperature gave tetra-*O*-acetates. The main product of the acetates was purified by centrifugal partition chromatography (CPC). The structure was confirmed to be 2,3,4,6-tetra-*O*-acetyl-α-methylglucopyranoside by the ^{1}H and ^{13}C NMR spectra. The stereoisomerism of the α-methylglucopyranoside was determined to be D by comparing the optical rotation ($[\alpha]_D^{22}$ +117.0°) with that of published data[3].

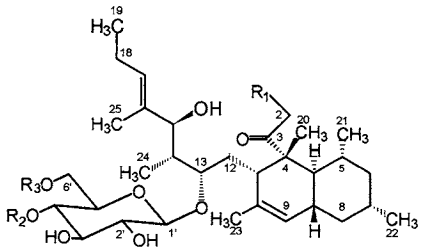
Preparation of 4′,6′-*O*-Benzylidenedihydroaldecalmycin (**4**)

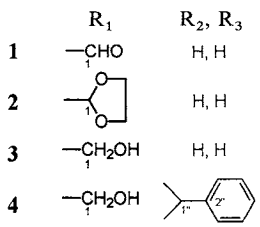
Aldecalmycin (**1**) and derivatives (**2**), (**3**)[2] shown in Fig. 1, were amorphous powder. We synthesized another derivative suitable for the X-ray analysis.

Treatment of **3** with benzaldehyde and D-camphorsulfonic acid in dioxane gave benzylidene derivative (**4**). The molecular formula of **4** was determined as $C_{40}H_{60}O_9$ by HRFAB-MS. The NMR data of **4** are summarized in Table 1. All of the connectivities between protons and carbons were assigned by the ^{13}C-^{1}H

COSY spectrum. The chemical shifts, 4′-H and 6′-H of the sugar moiety of **4** were shifted lower field as compared with those of **3**. Furthermore, the ^1H detected heteronuclear multiple-bond correlation spectroscopy (HMBC) spectrum of **4** revealed that 1″-H was connected to C-4′ and C-6′ with ether linkage (Fig. 2). These results showed that **4** was 4′,6′-*O*-benzylidenedihydroaldecalmycin.

Fig. 1. Structures of aldecalmycin (**1**) and its derivatives (**2**, **3** and **4**).





X-Ray Crystallographic Study of the Compound **4**

The compound **4** was recrystallized from an EtOH - H_2O solution to give a colorless prismatic crystal. The crystal belongs to the monoclinic system. The crystal data are summarized in Table 2. The absolute configuration of **4** was totally elucidated by the X-ray analysis and stereoisomerism of

Fig. 2. HMBC data of **4**.

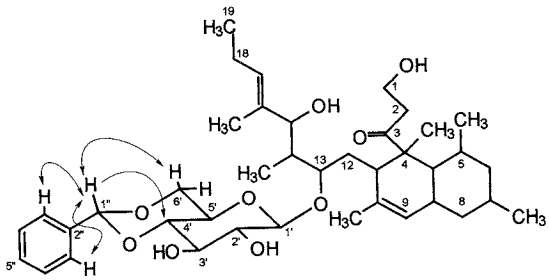


Table 1. NMR data of **4** in $CDCl_3$.

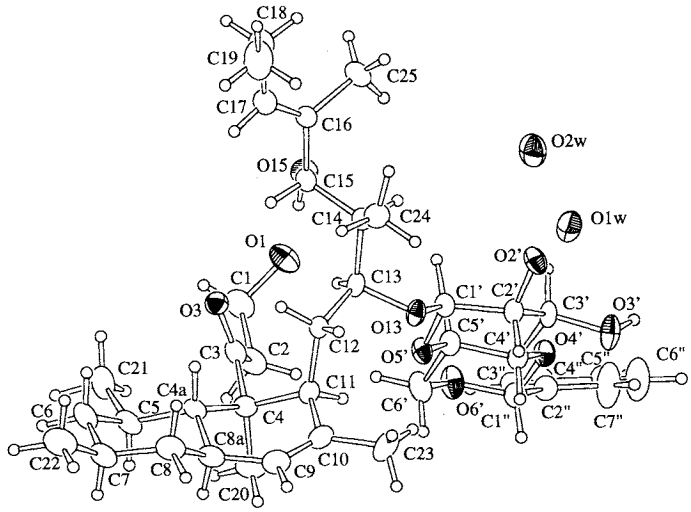
Position	^{13}C	^1H (multiplicity)	*J* value (Hz)	Position	^{13}C	^1H (multiplicity)	*J* value (Hz)
1	56.5	3.62 (m)		18	20.8	2.05 (quintet)	$J=7.6$
		3.95 (ddd)	$J=3.2, 8.4, 11.4$	19	14.0	0.97 (t)	$J=7.6$
2	42.3	2.81 (ddd)	$J=3.2, 5.0, 18.5$	20	17.1	1.18 (s)	
		2.95 (ddd)	$J=3.2, 8.4, 18.5$	21	23.3	0.53 (d)	$J=6.9$
3	216.2			22	22.2	0.91 (d)	$J=5.9$
4	51.6			23	22.3	1.68 (s)	
4a	44.7	1.63 (m)		24	9.8	0.61 (d)	$J=7.3$
5	36.9	1.35 (m)		25	10.2	1.59 (s)	
6	46.2	1.00 (m)		1′	100.2	4.40 (d)	$J=7.6$
		1.63 (m)		2′	74.8	3.42 (ddd)	$J=2.6, 7.6, 8.8$
7	33.4	1.59 (m)		2′-OH		2.47 (d)	$J=2.6$
8	42.4	0.93 (m)		3′	73.2	3.82 (m)	
		1.77 (m)		3′-OH		2.96 (d)	$J=2.4$
8a	41.1	1.73 (m)		4′	80.8	3.58 (m)	
9	123.6	5.02 (br s)		5′	66.1	3.55 (m)	
10	135.3			6′	68.5	3.82 (m)	
11	43.8	2.06 (br d)	$J=7.8$			4.40 (dd)	$J=4.2, 10.0$
12	30.1	1.09 (dd)	$J=12.4, 15.0$	1″	102.0	5.55 (s)	
		1.75 (m)		2″	137.0		
13	77.2	3.51 (m)		3″	126.3	7.50 (m)	
14	38.9	1.93 (m)		4″	128.3	7.37 (m)	
15	79.6	3.78 (d)	$J=12.8$	5″	129.3	7.37 (m)	
16	134.5			6″	128.3	7.37 (m)	
17	131.0	5.43 (t)	$J=7.6$	7″	126.3	7.50 (m)	

Chemical shifts in ppm from TMS as an internal standard.

Table 2. Crystal data of **4**.

Empirical formula	$C_{40}H_{60}O_9 \cdot 2H_2O$		$\beta = 107.586(7)°$
Formula weight	720.94		$V = 2003.0(4)Å^3$
Crystal color, habit	Colorless, prismatic	Space group	$P2_1$
Crystal dimensions	$0.10 \times 0.20 \times 0.30$ mm	Z value	2
Crystal system	Monolinic	D_x	$1.195\,g/cm^3$
Lattice parameters	$a = 13.904(2)Å$	F_{000}	784.00
	$b = 9.677(1)Å$	μ(CuKα)	$6.99\,cm^{-1}$
	$c = 15.617(1)Å$		

Fig. 3. ORTEP drawing of **4** showing the atom labeling scheme.



the sugar moiety (D-glucose) described above. The ORTEP drawing of **4** is shown in Fig. 3. There is an intramolecular hydrogen bond between O (1) and O (15) (2.850(5) Å). The absolute configuration of **4** is the same as that of diplodiatoxin[4,5] on its bicyclic ring.

Experimental

General

MP was determined on a Yanagimoto micro melting point apparatus. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX400 and a JNM-EX270 spectrometer. Mass spectra were obtained with a JEOL JMS-SX102 spectrometer.

Preparation of 2,3,4,6-Tetra-*O*-acetyl-α-D-methylglucopyranoside

Aldecalmycin (**1**, 125.2 mg) was dissolved in MeOH (12.5 ml) which was mixed with Amberlist 15 (6 ml, dry vol.) and the mixture was refluxed at 85°C for 6 days. The mixture was filtered to remove the resin and the filtrate was evaporated to dryness. The residue was dissolved in a small amount of MeOH and applied on a Sephadex LH-20 column (110 ml) which was eluted with MeOH to give a mixture of methylglucopyranoside (17.0 mg). The material was dissolved in pyridine (1.7 ml), to which was added acetic anhydride (110 μl) and was placed at room temperature for over night. The mixture was added H_2O (100 μl) to stop the reaction and was extracted with EtOAc (20 ml), which was washed with saturated sodium bicarbonate aqueous solution, 10% potassium bisulfate aqueous solution and H_2O. The extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The residue was purified by CPC-L.L.N (model NMF, Sanki Engineering Limited) that the solvent system with *n*-hexane-acetonitrile (1:1) to give colorless oil of 2,3,4,6-tetra-*O*-acetyl-α-D-methylglucopyranoside

(8.4 mg, yield; 11.0%): $[\alpha]_D^{22}$ +117.0° (*c*, 0.76 EtOH); FAB-MS *m/z* 385 $(M+Na)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.98 (3H, s), 2.00 (3H, s), 2.05 (3H, s), 2.08 (3H, s), 3.39 (3H, s), 3.96 (1H, ddd, J=2.4, 4.9, 10.3 Hz), 4.09 (1H, dd, J=2.4, 12.5 Hz), 4.24 (1H, dd, J=4.9, 12.5 Hz), 4.88 (1H, dd, J=3.9, 10.3 Hz), 4.93 (1H, d, J=3.9 Hz), 5.04 (1H, dd, J=9.3, 10.3 Hz), 5.45 (1H, dd, J=9.3, 10.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6 (q), 20.7 (q×3), 55.4 (q), 61.9 (t), 67.1 (d), 68.5 (d), 70.1 (d), 70.8 (d), 96.8 (d), 169.6 (s), 170.0 (s), 170.1 (s), 170.6 (s).

Preparation of Benzylidene Derivative (**4**)

Dihydroaldecalmycin (**3**, 62.4 mg) was dissolved in dioxane (2 ml) containing D-camphorsulfonic acid (8 mg), to which was added benzaldehyde (400 μl) and the reaction mixture was placed at 35°C for 30 hours. The mixture was added triethylamine (24 μl) for neutralization and was concentrated under reduced pressure to dryness. The residue was chromatographed on a silica gel column (40 ml) with toluene - EtOAc (3:2) to give white solid of **4** (40.6 mg, yield; 56.3%). The solid was crystallized from a *n*-hexane - dichloromethane solution to give colorless needle. **4**: mp. 204~207°C, $[\alpha]_D^{24}$ −82.9° (*c*, 0.39, $CHCl_3$), FAB-MS *m/z* 707 $(M+Na)^+$, HRFAB-MS Calcd for $C_{40}H_{60}O_9Na$: 707.4135, Found: *m/z* 707.4139 $(M+Na)^+$, IR ν_{max} (KBr) cm^{-1} 3480, 2950, 2910, 1700, 1460, 1390, 1080, 1020, 1000, 750, 700.

X-Ray Crystallography

The crystal of **4** was recrystallized from an EtOH - H_2O solution. A colorless prism of $C_{40}H_{60}O_9 \cdot 2H_2O$ having dimensions of 0.10×0.20×0.30 mm was mounted on a Rigaku AFC-7R diffractometer with graphite monochromated Cu-Kα radiation. Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 22 reflections in the range $50<2\theta<56°$. Crystal data are shown in Table 2. The reflection data were collected at a temperature of 22°C using the ω-2θ scan technique to a maximum 2θ value of 120.1°, and scans of $(1.52+0.30\tan\theta)°$ were made at a speed of 16.0°/minute (in omega). Of the 3333 reflections which were collected, 3189 reflections were unique. Three standard reflections were monitored for every 150 reflections with no significant intensity decay. An absorption correction using the program DIFABS[6] was applied which resulted in transmission factors ranging from 0.88 to 1.03. The data were corrected for Lorenz and polarization effects. The structure was solved by the direct method (SIR88[7]) and expanded using Fourier techniques (DIRDIF92[8]). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms except for those of O(1), O(2'), O(1w) and O(2w) were included but not refined. The final cycle of full-matrix least-squares refinement was based on 2415 observed reflections ($I>2\sigma(I)$) and 460 variable parameters and converged with unweighted and weighted agreement factors of R=0.043 and Rw=0.053. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.12 and −0.11 e/Å^3, respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

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