ALDECALMYCIN, A NEW ANTIMICROBIAL ANTIBIOTIC FROM *Streptomyces*

II. STRUCTURE ELUCIDATION BY NMR STUDIES

RYUICHI SAWA, YOSHIKAZU TAKAHASHI, TSUTOMU SAWA,
HIROSHI NAGANAWA and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

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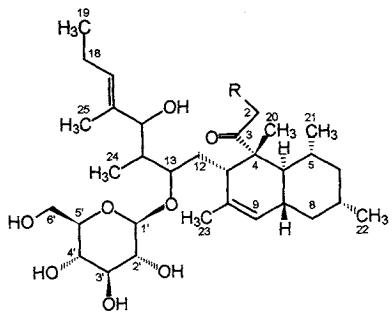
A new antibiotic, aldecalmycin (**1**) was isolated from the culture broth of *Streptomyces* sp. MJ147-72F6. The ^{1}H and ^{13}C NMR spectra of **1** were complicated due to the presence of a β-ketoaldehyde moiety. Therefore, **1** was converted into an ethylene ketal derivative (**2**) and into a dihydroaldecalmycin (**3**). These derivatives gave assignable NMR spectra. The planar structure was elucidated by using ^{1}H-^{1}H COSY, 2D-HOHAHA, ^{1}H-^{13}C COSY and HMBC spectra of **2**. The conformation of the decalin ring was elucidated by using 1D-HOHAHA, NOE difference and NOESY spectra of **3**. The geometry of double bond in the side chain was determined by NOE difference and NOESY spectra of **3**.

In the course of screening soil microorganisms for their ability to produce new antibiotics, we have found aldecalmycin (**1**), from a culture broth of *Streptomyces* sp. MJ147-72F6. In the preceding paper[1], taxonomy, production, fermentation, isolation, physico-chemical and biological properties of aldecalmycin were reported. In this paper, we describe the structure elucidation of aldecalmycin (Fig. 1).

Results and Discussion

The ^{1}H NMR spectrum of aldecalmycin was complicated in the various solvents due to a keto-enol tautomerism[1]. Therefore, we synthesized some aldehyde-masked derivatives to obtain assignable NMR spectra. The ethylene ketal derivative (**2**) and the cyanoborohydride reduction product, dihydroaldecalmycin (**3**), were readily obtained and used to elucidate the planar structure and the relative stereochemistry, respectively.

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



Planar Structure of Compound 2

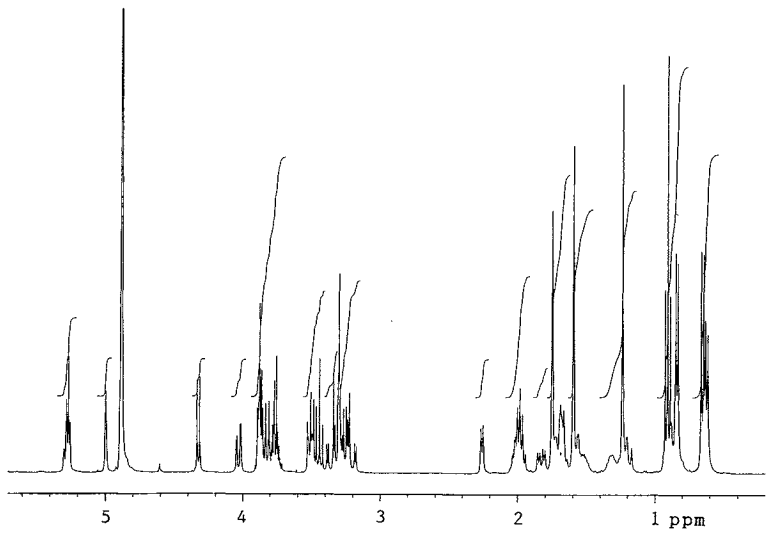
Treatment of **1** with ethylene glycol and D-camphorsulfonic acid in dioxane at 22°C for 23 hours gave ethylene ketal derivative (**2**). Physico-chemical properties of **2** are shown in Table 1. The molecular formula of **2** was determined as $C_{35}H_{58}O_{10}$ by HRFAB-MS. The ^{1}H NMR spectrum of **2** is shown in Fig. 2 and the ^{13}C and ^{1}H NMR data of **2** are summarized in Table 2. All one bond connections

Table 1. Physico-chemical properties of aldecalmycin (**1**), ethylene ketal derivative (**2**) and dihydroaldecalmycin (**3**).

	1	**2**	**3**
Appearance	White powder	White powder	White powder
MP (°C)	125~128	113~117	124~127
Optical rotation $[\alpha]_D$	−78.7° (26°C, *c* 1.0, MeOH)	−73.6° (26°C, *c* 1.0, MeOH)	−65.3° (23°C, *c* 0.27, MeOH)
Molecular formula	$C_{33}H_{54}O_9$	$C_{35}H_{58}O_{10}$	$C_{33}H_{56}O_9$
Elemental analysis			
Calcd:	C 65.64, H 9.18, O 25.18 (as $C_{33}H_{54}O_9 \cdot \frac{1}{2}H_2O$)		C 65.43, H 9.48 (as $C_{33}H_{56}O_9 \cdot \frac{1}{2}H_2O$)
Found:	C 65.48, H 9.29, O 25.47		C 65.51, H 9.47
FAB-MS (*m/z*)	593 $(M-H)^-$	637 $(M-H)^-$	619 $(M+Na)^+$
HRFAB-MS (*m/z*)			
Calcd:	593.3689 (as $C_{33}H_{53}O_9$)	637.3952 (as $C_{33}H_{57}O_{10}$)	619.3822 (as $C_{33}H_{56}O_9Na$)
Found:	593.3687 $(M-H)^-$	637.3935 $(M-H)^-$	619.3825 $(M+Na)^+$
UV λ_{max} nm ($E^{1\%}_{1cm}$)			
in MeOH	272 (30), 299 (31)	End absorption	End absorption
in HCl-MeOH	271 (24), 303 (sh, 13)		
in NaOH-MeOH	304 (395)		
IR ν max (cm^{-1}) KBr	3430, 2960, 2910, 1694, 1626, 1456, 1379, 1074, 1038, 995	3440, 2960, 2910, 1698, 1636, 1456, 1379, 1134, 1076, 1019	3420, 2960, 2910, 1700, 1460, 1380, 1080, 1040, 1020, 1000
TLC (Rf value)[a]	0.39	0.46	0.38

[a] Silica gel TLC (Merck Art. 5715): $CHCl_3$-MeOH (20:3).

Fig. 2. ^1H NMR spectrum of **2** in CD_3OD-C_6D_6 (10:1) (400 MHz).



between ^1H and ^{13}C were elucidated by the ^1H-^{13}C COSY experiment. The NMR data and the molecular formula indicated the presence of seven methyls, eight methylenes, fourteen sp^3 methines and two sp^2 methines, four quaternary carbons and five exchangeable hydroxyl groups in compound **2**.

The ^1H-^1H COSY spectrum of **2** revealed four partial structures shown in Fig. 3. The partial structure from C-1′ to C-6′ suggested an existence of a sugar. This sugar should be β-glucopyranoside type because four vicinal coupling constants were all large (A). The characteristic signals at δ 3.78 and 3.89, which were

Table 2. ^{13}C and ^{1}H NMR data of **2** and **3**.

Position	(2)			(3)		
	^{13}C	^{1}H (multiplicity)	J value (Hz)	^{13}C	^{1}H (multiplicity)	J value (Hz)
1	102.0	5.28 (t)	J=4.8	57.5	3.65 (dt)	J=4.8, 11.3
					3.92 (ddd)	J=4.5, 7.8, 11.3
2	45.6	3.22 (dd)	J=4.8, 19.2	43.8	2.89 (ddd)	J=4.5, 4.8, 19.2
		3.37 (dd)	J=4.8, 19.2		3.13 (ddd)	J=4.8, 7.8, 19.2
3	214.7			216.3		
4	52.8			52.6		
4a	46.1	1.68 (m)		46.3	1.61 (m)	
5	38.0	1.33 (m)		38.2	1.39 (m)	
6	47.5	0.91 (m)		47.8	0.95 (m)	
		1.59 (m)			1.65 (m)	
7	34.5	1.54 (m)		34.8	1.60 (m)	
8	43.6	0.84 (m)		43.9	0.86 (m)	
		1.74 (m)			1.75 (m)	
8a	42.4	1.68 (m)		42.6	1.73 (m)	
9	124.1	5.01 (br s)		124.3	4.99 (br s)	
10	137.1			137.5		
11	45.1	2.27 (br d)	J=6.4	45.5	2.11 (br d)	J=6.2
12	32.3	1.21 (dd)	J=12.6, 14.8	32.3	1.07 (dd)	J=11.8, 15.2
		1.84 (dd)	J=6.4, 14.8		1.79 (ddd)	J=1.5, 6.2, 15.2
13	78.1	3.52 (m)		76.7	3.55 (ddd)	J=1.5, 2.7, 11.8
14	40.9	2.03 (ddq)	J=3.0, 6.6, 10.4	39.7	1.95 (ddq)	J=2.7, 7.1, 10.0
15	80.2	3.83 (d)	J=10.4	80.4	3.77 (d)	J=10.0
16	136.0			136.5		
17	131.1	5.29 (br t)	J=7.4	131.2	5.40 (br t)	J=7.6
18	21.6	1.99 (quintet)	J=7.4	21.7	2.06 (quintet)	J=7.6
19	14.3	0.92 (t)	J=7.4	14.3	0.99 (t)	J=7.6
20	17.5	1.25 (s)		17.4	1.24 (s)	
21	24.1	0.63 (d)	J=7.0	23.8	0.59 (d)	J=6.8
22	22.7	0.85 (d)	J=6.0	22.7	0.92 (d)	J=6.3
23	22.6	1.76 (br s)		22.8	1.71 (br s)	
24	10.6	0.66 (d)	J=6.6	10.4	0.60 (d)	J=7.1
25	10.5	1.61 (br s)		10.4	1.58 (br s)	
1′	101.7	4.33 (d)	J=8.2	100.5	4.28 (d)	J=7.6
2′	75.1	3.25 (dd)	J=8.2, 9.0	75.4	3.15 (dd)	J=7.6, 8.9
3′	78.1	3.45 (t)	J=9.0	78.3	3.35 (dd)	J=8.5, 8.9
4′	71.6	3.51 (t)	J=9.0	72.0	3.24 (dd)	J=8.5, 9.6
5′	77.3	3.3*[1]		78.2	3.3*[1]	
6′	62.4	3.88 (dd)	J=4.6, 12.0	63.2	3.69 (dd)	J=5.6, 11.5
		4.04 (dd)	J=3.2, 12.0		3.96 (dd)	J=1.8, 11.5
Ketal 1	65.5	3.78 (m)*[2]				
Ketal 2	65.7	3.89 (m)*[2]				

(**2**): 400 MHz (^{1}H) and 100 MHz (^{13}C) in CD_3OD-C_6D_6 (10:1).
(**3**): 270 MHz (^{1}H) and 67.5 MHz (^{13}C) in CD_3OD.
Chemical shifts in ppm from TMS as an internal standard.
*[1] Partially obscured by the solvent signal. *[2] These values may be interchanged.

coupled with each other, were assigned to an oxygen bearing methylene. The signal at δ 5.28 was assigned to the methine proton, 1-H attached to a carbon bearing two oxygens. This proton was coupled to the methylene protons, 2-H. These signals were assignable to the newly constrained ketal moiety (B). The partial structures of the unsaturated alkyl chain (C) and the saturated alkyl chain (D) were also deduced from the ^{1}H-^{1}H COSY spectrum.

Fig. 3. Partial structures (A, B, C and D) of **2** by ^{1}H-^{1}H COSY.

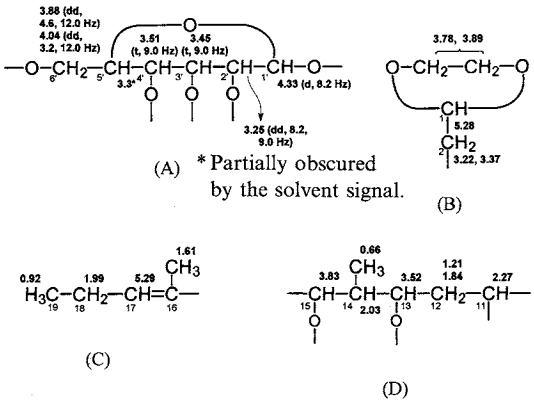


Fig. 4. Partial structure (D ~ E) of **2** by 2D-HOHAHA and HMBC.

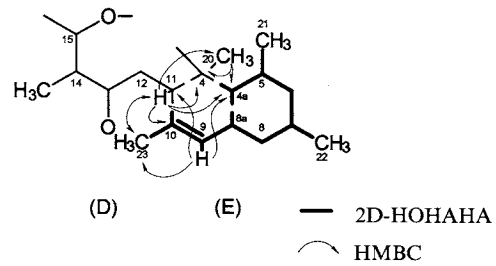
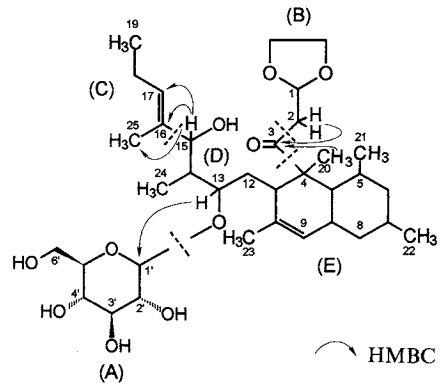


Fig. 5. Connectivities among partial structures (A, B, C, D and E) by HMBC.



The partial structure (E), which was composed by many overlapping signals (δ 0.8 ~ 1.8) was determined by two dimensional homonuclear Hartmann-Hahn (2D-HOHAHA)[2] and heteronuclear multiple-bond correlation (HMBC)[3] experiments. The 2D-HOHAHA spectrum revealed the connectivities from 4a-H to 23-H as shown in Fig. 4. The HMBC spectrum revealed the following two- and three-bonds connectivities between ^{1}H and ^{13}C:

i) from 9-H (δ 5.01) to C-4a (δ 46.1), C-23 (δ 22.6) and C-11 (δ 45.1),

ii) from 11-H (δ 2.27) to C-10 (δ 137.1), C-23, C-4 (δ 52.8), C-20 (δ 17.5) and C-4a,

iii) from 23-H (δ 1.76) to C-11,

iv) from 20-H (δ 1.25) to C-4 and C-4a.

Both spectral analyses indicated a substituted decalin ring possessing one double bond as shown in (E). Furthermore, the connectivity between (D) and (E) was established because C-11 was common to both.

The connectivities of the five partial structures (A ~ E) were also elucidated by the HMBC spectrum. The following cross peaks between ^{1}H and ^{13}C showed the connectivities between each partial structure:

i) from 13-H (δ 3.52) to C-1′ (δ 101.7); (A)-(D),

ii) from 15-H (δ 3.83) to C-16 (δ 136.0), C-17 (δ 131.1), and C-25 (δ 10.5); (C)-(D),

iii) from both 2-H (δ 3.22, 3.37) and 20-H to the carbonyl C-3 (δ 214.7); (B)-(E).

From these results, all connectivities of the partial structures were clarified as shown in Fig. 5. Five exchangeable hydroxyl groups should be connected to C-2′, C-3′, C-4′, C-6′ (sugar moiety) and C-15 by considering the molecular formula. Thus, the planar structure of **2** was determined and the structure of aldecalmycin (**1**) followed with C-1 of the ethylene ketal moiety of **2** being replaced with an aldehyde group.

Relative Stereochemistry of the Decalin Ring

The relative stereochemistry of the decalin ring was studied by spectral analyses of dihydroaldecalmycin (**3**) which was synthesized by reduction of **1** with sodium cyanoborohydride. Physico-chemical properties of **3** are shown in Table 1. The molecular formula of **3** was determined as $C_{33}H_{56}O_9$ by HRFAB-MS and

Fig. 6. 1D-HOHAHA spectrum of **3** irradiating at δ 0.59 (21-H) (400 MHz).

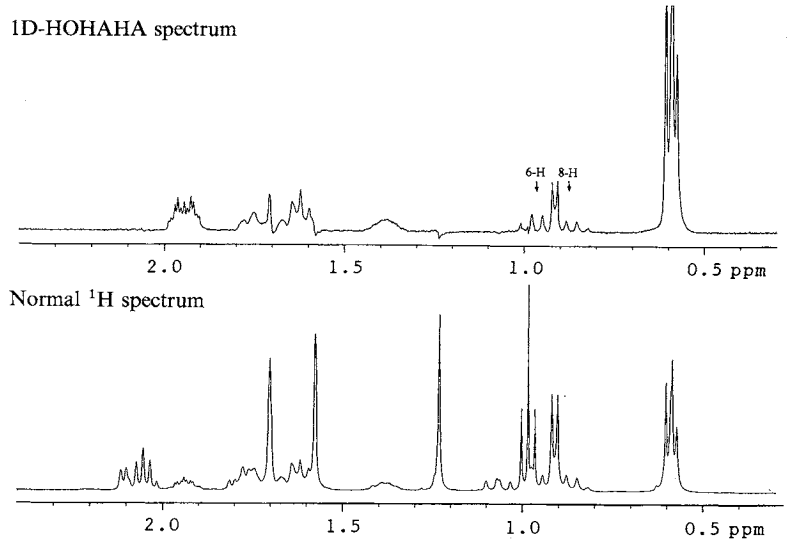
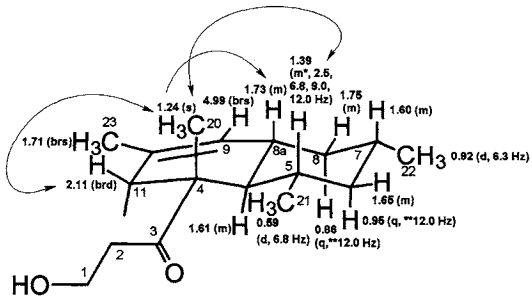


Fig. 7. Relative stereochemistry of the decalin ring by 1D-HOHAHA and NOEs.



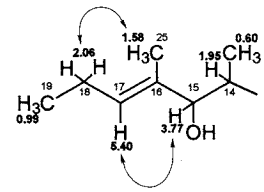
* These J values were measured by spin decoupling; {0.59} (21-H).
** These J values were observed by 1D-HOHAHA; {0.59} (21-H).

elemental analysis. The ^{1}H and ^{13}C NMR data of **3** are shown in Table 2. These assignments were determined mainly by ^{1}H-^{13}C COSY and 2D-HOHAHA spectra. By comparing with the NMR data of **2**, methylene signals at δ 3.65 and 3.92 were assigned to the hydroxymethyl group which was introduced by reduction of the aldehyde group. The fact that these protons (1-H) coupled with 2-H also confirmed the conversion of the aldehyde to the alcohol. The structure of **3** is shown in Fig. 1.

To determine the conformation of saturated part of the decalin ring (cyclohexane ring), the coupling constants of the cyclohexane ring should be determined. But the ^{1}H-signals of the decalin moiety were overlapping as shown in Fig. 6. Therefore, a 1D-HOHAHA experiment and a spin decoupling experiment were performed to determine these coupling constants. Two quartets (J=12.0 Hz) of 8-H_{ax} (δ 0.86) and 6-H_{ax} (δ 0.95) were revealed by the 1D-HOHAHA experiment irradiating at δ 0.59. In the spin decoupling experiment, irradiation at δ 0.59 (21-H) caused the methine signal, 5-H at δ 1.39 (multiplet)

to collapse to a readable signal of ddd (J=12.0, 9.0 and 2.5 Hz). Thus, the coupling constants between vicinal protons on the cyclohexane ring protons with the exception of those between two *equatorial* protons of methylene were all large. The large coupling constants suggested that the cyclohexane ring is in the chair conformation and the four methine protons on the cyclohexane ring are all *axial*; the junction of the decalin ring is *trans* and the two methyl groups (C-21, C-22) are *equatorial*.

Fig. 8. Geometry of double bond (C-16, C-17) by NOEs.



The conformation of the cyclohexene ring was determined by NOESY and NOE difference experiments. NOEs were observed between 5-H and 20-H, 8a-H and 20-H, 11-H and 20-H. These NOEs, especially that observed between 8a-H at the ring junction and 20-H of the cyclohexene ring established the half-chair conformation for the cyclohexene ring. All of these results are summarized in Fig. 7.

Geometry of the Double Bond

The geometry of the double bond (C-16, C-17) was determined by NOE difference and NOESY spectra of the compound **3**. NOEs observed between 15-H and 17-H, 25-H and 18-H established the geometry as 16*E* as shown in Fig. 8.

Aldecalmycin was related to lydicamycin[4] on its planar structure described in the previous paper[5] but the ring system of aldecalmycin is not a type of lydicamycin but of diplodiatoxin[6]. The absolute configuration and biosynthesis of aldecalmycin will be described in accompanying papers[7,8].

Experimental

General

MPs were determined on a Yanagimoto micro melting point apparatus. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were recorded with a Hitachi I-5020 spectrometer. UV spectra were taken on a Hitachi U-3210 spectrometer. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM-GX400 and JNM-EX270 spectrometers. Mass spectra were obtained with a JEOL JMS-SX102 spectrometer.

Preparation of Aldecalmycin Ethylene Ketal (2)

Aldecalmycin (**1**, 58.0 mg) and D-camphorsulfonic acid (5.8 mg) were dissolved in dioxane (5.8 ml), to which was added ethylene glycol (145 μl) and the reaction mixture was placed at 22°C for 23 hours. The mixture was concentrated *in vacuo* and the residue was dissolved with ethyl acetate (40 ml). The solution was washed with saturated sodium bicarbonate aqueous solution (20 ml). The solution was dried over anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The residue was chromatographed on a silica gel column developed with EtOAc-MeOH (15:1) to give a white powder (24.6 mg). This material was purified by a Sephadex LH-20 column chromatography with MeOH. Final purification was carried out on a centrifugal partition chromatography (CPC-L.L.N model NMF, Sanki Engineering Limited) with the solvent system, *n*-hexane-MeOH, to give a white amorphous powder of **2** (15.9 mg, yield; 25.6%).

Preparation of Dihydroaldecalmycin (3)

Aldecalmycin (**1**, 239 mg) was dissolved in MeOH (24 ml) in the presence of AcOH (120 μl), to which was added sodium cyanoborohydride (120 mg) and the reaction mixture was stirred at room temperature for 14 hours. The reaction mixture was concentrated under reduced pressure to dryness. The residue was

dissolved in MeOH (1 ml) and applied to a Sephadex LH-20 column (200 ml) and was eluted with MeOH to give a white amorphous powder of **3** (205.4 mg, yield; 85.7%).

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