BIOSYNTHESIS OF ALDECALMYCIN

Sir:

A new antibiotic, aldecalmycin (**1**) was found in the culture broth of *Streptomyces* sp. MJ147-72F6. The antibiotic is active against methicillin-resistant *Staphylococcus aureus* (MRSA)[1]. The ^{1}H and ^{13}C NMR spectra of **1** were complicated due to a presence of β-ketoaldehyde moiety. Therefore, the structure of **1** was established by various NMR experiments of some aldehyde-masked derivatives as shown in Fig. 1[2]. The stereochemistry of **1** was determined by X-ray crystallographic study of the 4′,6′-*O*-benzylidenedihydroaldecalmycin[3]. As a series of studies in aldecalmycin, we now describe the biosynthesis of the antibiotic by the feeding experiments using ^{13}C-labeled compounds.

A slant culture of the strain MJ147-72F6 was inoculated into 500-ml Erlenmeyer flask containing 110 ml of seed medium consisting of galactose 2.0%, dextrin 2.0%, Bacto-soytone (Difco) 1.0%, corn steep liquor (Iwaki) 0.5%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2% and a drop of silicone (Shin-etsu Chemical Industry) (pH 7.4 before sterilization) and cultured at 27°C for 3 days on a rotary shaker (180 rpm). Two ml of the seed culture was transferred into five 500-ml Erlenmeyer flasks containing 110 ml of production medium composed of yeast extract 0.5%, glucose 1.0%, potato starch 2.0%, Casamino acids (Difco) 0.5% and $CaCO_3$ 0.4% (pH was not adjusted).

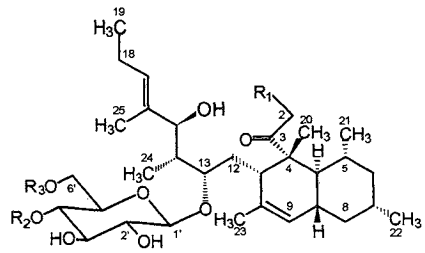
At 24 hours after inoculation, ^{13}C-labeled compound (30 mg per flask) was added. Another same amount of ^{13}C-labeled compound was added at 36 hours after inoculation and then the culture was incubated for further 12 hours. In case of [1-^{13}C]D-glucose, the amount was 25 mg per flask. The feeding intervals were referred to the production of aldecalmycin[1]. The sodium [1-^{13}C]acetate (99% atom % ^{13}C), sodium [2-^{13}C]acetate (99% atom % ^{13}C), sodium [1-^{13}C]propionate (99% atom % ^{13}C), and [1-^{13}C]D-glucose (99% atom % ^{13}C) were obtained from Aldrich Chemical Co., U.S.A. as ^{13}C-labeled compounds.

The culture broth was centrifuged and the supernatant was extracted with BuOAc at pH 3.0. The extract was washed with H_2O and was concentrated *in vacuo* to dryness. The residue was dissolved in MeOH and applied on Sephadex LH-20 column (160 ml). The column was eluted with MeOH and the active fractions against *Bacillus stearothermophilus* were collected and dried under reduced pressure. The crude powder was purified by CPC using the solvent system with $CHCl_3$ - MeOH - H_2O (5 : 6 : 4). Without further purifications, crude **1** (*ca.* 70% purity) was used for the next conversion.

The crude compound was dissolved in MeOH (1 ml) in the presence of acetic acid (8 μl), to which was added sodium cyanoborohydride (8 mg) and the reaction mixture was stirred at a room temperature for 3 hours. The reaction mixture was concentrated under reduced pressure to dryness. The residue was dissolved in MeOH and applied on a Sephadex LH-20 column (160 ml). Elution with MeOH gave white amorphous powder of ^{13}C-labeled dihydroaldecalmycin (**3**, 2.8 ~ 3.4 mg).

Enriched carbons of the ^{13}C-labeled **3** obtained by the feeding experiments were measured by ^{13}C NMR spectra. The enrichment ratios were calculated from the relative signal intensity of C-4′ as 1.0 (Table 1). In case of [1-^{13}C]acetate, C-1, C-8, C-11 were enriched. In case of [2-^{13}C]acetate C-2, C-8a, C-12, all methyl carbons (C-19, C-20, C-21, C-22, C-23, C-24 and C-25) and their adjacent carbons (C-4, C-5, C-7, C-10, C-14, C-16 and C-18) were enriched. This result suggested that randomization had been occurred in the latter case. They might be considered as an indirect incorporation of the precursor through propionate. Incorporation experiment of [1-^{13}C]propionate was carried out to make sure the consideration. The addition of [1-^{13}C]propionate resulted in seven enriched

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



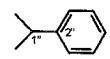
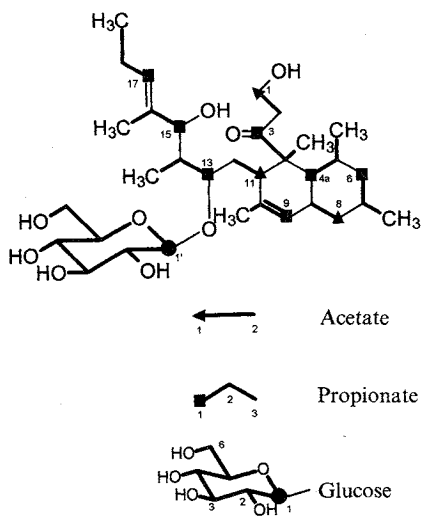
	R_1	R_2,R_3
1	—CHO	H, H
2		H, H
3	—CH_2OH	H, H
4	—CH_2OH	

Table 1. Incorporation of isotopic precursors by ^{13}C NMR experiments for **3**.

Position	Enrichment ratio				
	δ_C (ppm)	[1-^{13}C]Acetate	[2-^{13}C]Acetate	[1-^{13}C]Propionate	[1-^{13}C]Glucose
1	57.5	5.4*	1.5	0.8	1.1
2	43.8	0.7	7.1*	0.5	1.4
3	216.3	0.9	2.0	15.4*	0.6
4	52.6	0.9	5.6*	0.3	1.3
4a	46.3	1.3	2.7	24.4*	1.0
5	38.2	0.8	6.2*	0.3	1.3
6	47.8	1.5	3.4	29.6*	1.1
7	34.8	0.9	6.8*	0.4	1.4
8	43.9	4.3*	1.2	0.8	0.9
8a	42.6	0.9	6.9*	0.3	1.4
9	124.3	1.1	2.1	21.7*	0.8
10	137.5	0.7	4.4*	0.2	1.2
11	45.5	4.6*	1.0	0.8	0.9
12	32.2	0.7	5.8*	0.4	1.1
13	76.7	1.4	2.7	29.5*	1.0
14	39.7	0.8	6.1*	0.3	1.4
15	80.4	1.5	3.3	21.4*	1.1
16	136.5	0.9	5.2*	0.3	1.2
17	131.2	1.2	2.7	20.2*	1.2
18	21.7	1.3	8.1*	0.5	1.5
19	14.3	1.5	5.5*	0.9	2.0
20	17.4	1.0	5.0*	1.0	1.2
21	23.8	0.7	3.6*	0.7	1.0
22	22.7	1.3	6.3*	1.3	1.4
23	22.8	1.4	6.3*	1.0	1.6
24	10.4	1.2	5.6*	0.8	1.4
25	10.4	1.2	5.4*	0.8	1.6
1′	100.5	0.8	1.0	0.7	2.2*
2′	75.4	1.0	1.2	0.7	0.8
3′	78.3	1.1	1.1	1.0	1.0
4′	72.0	1.0	1.0	1.0	1.0
5′	78.2	1.1	1.1	1.1	0.9
6′	63.2	0.8	0.9	0.8	1.0

Enrichment ratios were normalized to the signal intensity of C-4′ as 1.0.
* Enrichment signal.

Fig. 2. Biosynthetic origin of carbon skeleton for **3**.



carbons at C-3, C-4a, C-6, C-9, C-13, C-15 and C-17. From these results described above, the biosynthesis of the aglycon part was revealed. The sugar moiety of **3** was considered to be derived from D-glucose. To confirm this hypothesis, incorporation of [1-^{13}C]D-glucose was performed and the enrichment carbon was measured by ^{13}C NMR. The signal intensity of C-1′ in the sugar moiety was enhanced at the relative value of 2.2 by [1-^{13}C]D-glucose. The reason of low incorporation was supposed that the glucose was metabolized by the producing strain.

All of these results, aglycon of **3** was derived from decaketide intermediate which was condensed by three moles of acetate and seven moles of propionate, and sugar moiety of **3** was derived from one mole of D-glucose as summarized in Fig. 2.

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