ANTIBACTERIAL ACTIVITY OF SOME ACETYL DERIVATIVES OF KUJIMYCIN A

Sir:

The structure of kujimycin (KJM)-A[1] was previously elucidated to be 4''-desacetyl lankamycin[2,3,4]. Here, we report on the structure-activity relationship among some acetyl derivatives of KJM-A.

KJM-A and KJM-B (lankamycin) were acetylated with acetic anhydride-pyridine for 1 hour at 23°C. The reaction mixture gave 8 spots from KJM-A and 4 spots from KJM-B with sulfuric acid on a silica gel TLC plate developed in chloroform - acetone (5 : 2). Each spot from KJM-A was numbered KJM-I~VIII from the top to bottom of the plate. The four spots from KJM-B gave identical Rf values to those of KJM I, II, III and V. KJM I and V had the same Rf values as triacetyl KJM-A and KJM-B[1], while unreacted KJM-A was spot 8.

In colorization with *p*-anisaldehyde reagent[5], KJM I, II, III and V showed purple and KJM IV, VI, VII and VIII showed violet. These results suggested that the C-4 hydroxyl group of arcanose was acetylated in KJM I, II, III and V but not in KJM IV, VI, VII and VIII since methyl arcanoside, and 4-O-acetyl methyl arcanoside, obtained from the methanolysate of KJM-A and B[1], showed respectively violet and purple color with *p*-anisaldehyde reagent.

Each component was eluted from the silica plate with ethylacetate following preparative TLC in the above system.

Data from the NMR spectra of KJM I~VIII are presented in Table 1.

The above data indicate that a methoxyl signal (s, 3H, 3.41 p.p.m.) of lankavose shifts to higher field by acetylation of neighbouring C-2 hydroxyl group of lankavose. The signal at 2.11~2.14 p.p.m. can be assigned to the acetyl proton at C-4 position of arcanose and the signal at 2.05~2.08 p.p.m. is assigned to the acetyl proton at C-11 and/or C-15 position of lankolide and/or at C-2 position of lankavose[1]. Accordingly, structures for KJM I~VIII are proposed as shown in Fig. 1.

KJM I, III, IV and VII were found to be inactive against *Staphylococcus aureus* FDA 209P but KJM II and VI were as active as KJM V (KJM-B) and VIII (KJM-A). Furthermore, KJM VI and VIII were found to be more active than KJM II and V in the induction of macrolide resistance in some staphylococci[6]. Therefore, we attempted to recover the antimicrobial activity of KJM I, III, IV and VII by enzymatic desacetylation[7] with an esterase, newly isolated from the fermentation broth filtrate of *Streptomyces* TPR 7-8. After incubation at 37°C for 1 hour in the solution of phosphate buffer, pH 7, KJM I, III, IV and VII were found to be bio-active and changed to KJM II, V, VI and VIII by TLC and NMR analysis respectively. The result indicated that the enzyme eliminated specifically an acetyl group at the C-2 position of lankavose.

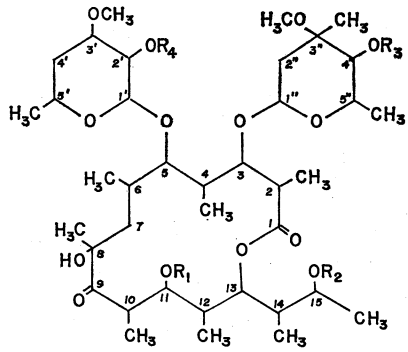
The esterase was partially purified by fractional precipitation with ammonium sulfate, followed by column chromatography on Sephadex G-75 and DEAE-cellulose. The enzyme obtained here was most active at pH 7 and at 40°C respectively. After incubation of the enzyme at 33°C for 30 minutes, more than 80 % of the activity remained between pH 5.0 and 10.0, while a remarkable decrease in activity was observed at pH 3.0 and 11.0. Heating of the enzyme at 60°C for 10 minutes at pH 7.0 led to 100 % inactivation of the enzyme.

From this study, it is suggested that a

Table 1. Analysis of NMR spectra of KJM I~VIII

	C-3' $-OCH_3$ (s)		C-4'' $-OCOCH_3$(s)	C-11, 15, 2' $-OCOCH_3$(s)
	3.30~3.33 p.p.m.	3.40~3.43 p.p.m.	2.11~2.14 p.p.m.	2.05~2.08 p.p.m.
KJM I	3H	—	3H	9H
KJM II	—	3H	3H	6H
KJM III	3H	—	3H	6H
KJM IV	3H	—	—	9H
KJM V	—	3H	3H	3H
KJM VI	—	3H	—	6H
KJM VII	3H	—	—	6H
KJM VIII	—	3H	—	3H

Fig. 1. The structure of partial acetylation products of KJM-A.



	R_1	R_2	R_3	R_4
KJM I	Ac	Ac	Ac	Ac
KIM II	Ac	Ac	Ac	H
KJM III	Ac	H	Ac	Ac
KJM IV	Ac	Ac	H	Ac
KJM V	Ac	H	Ac	H
KJM VI	Ac	Ac	H	H
KJM VII	Ac	H	H	Ac
KJM VIII	Ac	H	H	H

hydroxyl group at C-2 of lankavose is important for the revelation of antimicrobial activity with the KJMs. Similar findings have been reported by TARDREW *et al.*[8] for erythromycin esters. The hydroxyl group at C-4 of arcanose is related to the degree of macrolide-resistance inducibility. The enzymatic hydrolysis of 2'-ester in KJM indicates that the environment around 2'-hydroxyl group is suitable at least for binding with some protein. This suggests the possibility of a similar complex formation in the binding of KJM with ribosome. Our study on the functional relation between this enzyme and the bacterial ribosome is being continued.

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