MICROBIAL TRANSFORMATION OF ANTIBIOTICS

II. ADDITIONAL TRANSFORMATION PRODUCTS OF MARIDOMYCIN III

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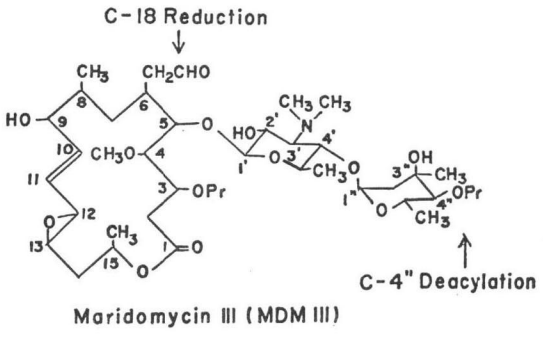
Streptomyces sp. strain No. K-245 was found to transform maridomycin III into four derivatives (A_1, A_2, A_3 and A_4) in addition to the transformation products reported previously. Isolation of the main product A_1 was carried out by column chromatography on silica gel developed with $CHCl_3$ - MeOH (19: 1). From the partial investigation of the structure of A_1, it proved to have a C 18-aldehyde group and C 4″-propionyl group but no antimicrobial activity. The relationships between A group's derivatives and known derivatives of maridomycin III are also discussed.

The studies on microbial transformation of antibiotics have been carried out in order to improve the antimicrobial activity of existing ones, to make them less-toxic or more effective against resistant strains. In the studies on microbial transformation of 16-membered macrolide antibiotics, 4″-deacylation[1~4], 3″-hydroxylation[5,6], 14-hydroxylation[7] and reduction of aldehyde[1,8,9] have been reported.

In our previous paper[1], *Streptomyces lavendulae* strain No. K-122 was shown to transform maridomycin (MDM) III[10,11] into three derivatives. Of these three transformation products, 18-dihydro-MDM III and 4″-depropionyl-MDM III were identical with those previously reported by NAKAHAMA *et al.*[9,2,3], whereas 18-dihydro-4″-depropionyl-MDM III was a new transformation product. The relationship between MDM III and its transformation products was considered to be as shown in Fig. 1; that is, 4″-depropionyl-MDM III was formed by deacylation of MDM III, and 18-dihydro-MDM III by reduction of MDM III. 18-Dihydro-4″-depropionyl-MDM III was formed either from 4″-depropionyl-MDM III by reduction or from 18-dihydro-MDM III by deacylation.

Streptomyces sp. strain No. K-245 was found to transform MDM III into four derivatives in addition to the transformation products described above. This paper deals with the isolation of the transformation products formed by strain No. K-245 and the relationship between MDM III and these derivatives.

Fig. 1. Transformation of MDM III into its derivatives by *Streptomyces lavendulae* strain No. K-122.



Materials and Methods

Antibiotics

Maridomycin (MDM) III (cf. Fig. 1), and 4″-depropionyl MDM III were kindly supplied by Takeda Chemical Industries, Ltd.. 18-Dihydro-MDM, 4″-depropionyl-MDM III (in part) and 18-dihydro-4″-depropionyl-MDM III were prepared by the transformation of MDM III by *S. lavendulae* strain No. K-122.[1]

Cultural Conditions

Transformation was carried out in 20~25 ml of a medium in 200-ml Erlenmeyer flasks. The seed culture medium containing 2% glucose, 3% soluble starch, 1% soybean flour, 1% corn steep liquor, 0.5% Polypepton, 0.3% NaCl and 0.5% $CaCO_3$ (pH 7.0), was inoculated with spores from the slant culture. *Streptomyces* sp. strain No. K-245 was grown for 24 hours at 28°C on a rotary shaker (180 rpm, 5 cm radius).

This culture was used to inoculate the transformation medium (GC medium) containing 5% glucose, 0.5% Polypepton, 2% cornsteep liquor, 0.3% NaCl and 0.5% $CaCO_3$ (pH7.0). After 48 hours of cultivation a conc. solution of MDM III in methanol was added to the culture (final concentration of MDM III: 1~2 mg/ml medium), and then the fermentation was continued for an additional 48 hours.

Thin-layer Chromatography (TLC)

The filtered broth was extracted with $CHCl_3$ at pH 8.2 and the solvent layer was chromatographed on silica gel G (Merck) plates using $CHCl_3$-MeOH (17: 3, v/v) as solvent. The position of the products was detected by heating the plates after spraying with 10% H_2SO_4.

Detection of O-Propionylmycarose[12,18]

The samples were hydrolyzed with 0.5 N HCl for 24 hours at room temperature, extracted with ether at pH 4.0, and the solvent layer was chromatographed (TLC) using 1-butanol - acetic acid - water (4: 1: 5, upper layer). O-Acylmycarose was detected by heating for 10 minutes at 90°C after spraying with vanillin-perchloric acid reagent[14].

Results

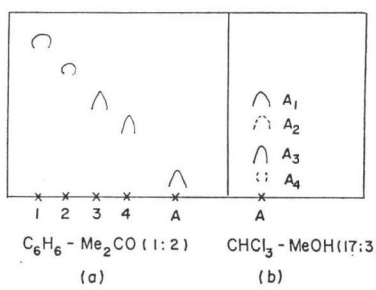
Transformation of MDM III by Growing and Washed Cells

Streptomyces sp. strain No. K-245 transformed MDM III into four derivatives in GC medium, whereas the other three derivatives formed by *S. lavendulae*[1] were also detected in glucose-bouillon and similar media. Typical thin-layer chromatograms of the products produced in GC medium are shown in Fig. 2. At first this strain was found to produce an unknown derivative, designated temporarily as A in Fig. 2(a). By changing the solvent system to $CHCl_3$-MeOH (17: 3), A proved to consist of four derivatives as shown in Fig. 2(b) and designated as A_1, A_2, A_3 and A_4 in the order of their decreasing Rf values on TLC. The same results were obtained with washed cells.

Isolation of Main Transformation Product A_1

Filtered broth was concentrated *in vacuo* by azeotropic distillation with 1-butanol, crude A complex (consisting of A_1, A_2, A_3 and A_4) was extracted with $CHCl_3$ at pH 8.2, and concentrated *in vacuo*. As shown in Fig. 3, crude A_1 was dissolved in $CHCl_3$, decolorized by activated charcoal and concentrated *in vacuo*. Purified A_1 was precipitated by the addition of *n*-hexane. The yield of A_1 from crude A complex was shown in Table 1. The mobility of purified A_1 and other derivatives was examined by TLC with results as shown in Table 2.

Fig. 2. Typical thin-layer chromatograms of MDM III transformation products by *Streptomyces* sp. strain No. K-245.
1: MDM III, 2: 18-dihydro-MDM III, 3: 4″-depropionyl-MDM III, 4: 18-dihydro-4″-depropionyl-MDM III, A: A complex.



Physicochemical Properties of A_1

A_1 is a white-colored, basic material and its melting point is 134~135°C. It showed only end absorption and the results of elementary analysis for A_1 were as follows: C 58.36, 58.39; H 8.21, 8.18; N 1.20, 1.17 (%). Its molecular

Fig. 3. Purification procedure of main transformation product A_1.

Culture filtrate
↓ adjustment of pH to 8.2
↓ extraction with $CHCl_3$
$CHCl_3$ layer
↓ concentration *in vacuo*
Crude A complex
↓ silica gel chromatography
↓ ($CHCl_3$ - MeOH, 19: 1)
A_1 fraction
↓ concentration *in vacuo*, decolorization with activated charcoal in $CHCl_3$
$CHCl_3$ layer
↓ concentration *in vacuo* precipitation with n-hexane
Purified A_1

Table 1. Yields of A_1 from crude A complex.

	Weight (mg)	Yield (%)
Crude A complex	720	100
A_1 fraction	145.8	20.3
A_1 after decolorization	123.5	17.2
Purified A_1	113.1	15.7

Table 2. Mobility of components $A_1 \sim A_4$ by thin-layer chromatography.

Component	Rf in the indicated solvent system			
	(1)	(2)	(3)	(4)
A_1	0.53	0.12	0.62	0.28
A_2	0.41	0.10	0.62	0.20
A_3	0.21	0.05	0.50	0.12
A_4	0.13	0.02	0.50	0.03

Solvent system:
(1) $CHCl_3$ - MeOH (17: 3)
(2) C_6H_6 - Me_2CO (1: 2)
(3) 1-BuOH - AcOH - H_2O (3: 1: 1)
(4) C_6H_6 - MeOH (4: 1)

weight is 860~890 by vapor pressure osmometry in $CHCl_3$. It is positive in the ELSON-MORGAN test and negative in the anthrone, FEHLING, erythromycin and carbomycin tests. Color reactions of A_1 and MDM III were similar.

Since A_1 degraded during mass spectrometry, A_1 was acetylated by acetic anhydride in pyridine according to the method described by KINUMAKI *et al.*[15] The NMR spectrum of acetylated A_1 is shown in Fig. 4.* A proton at δ 3.53 as C 4-methoxy group and a proton at δ 2.53 as a dimethylamino group of C 3 in mycaminose were still seen, which means there is no transformation on these positions. These protons were also seen in the NMR spectrum of free A_1. Moreover, simple depropionylation at C 4 in mycarose did not occur since the fragment ion peak at O-propionylmycarose (*m/e* 201) was seen in the mass spectrum of acetylated A_1. This fact is in good agreement with the result in the detection of O-propionylmycarose described below. Examination of the final structure of A_1 is now in progress and will be reported in a separate paper.

Transformation of MDM III into A_1, A_2, A_3 and A_4 and their Relationship to the Known Derivatives

Transformations of MDM III, 18-dihydro-MDM III, 4''-depropionyl-MDM III, 18-dihydro-4''-depropionyl-MDM III or A_1, by *Streptomyces* K-245 were examined. A_1 and A_3 were formed from MDM III, A_2 and A_4 from 18-dihydro-MDM III, A_3 from 4''-depropionyl-MDM III, A_4 from 18-dihydro-4''-depropionyl-MDM III and that A_3 was also formed from A_1.

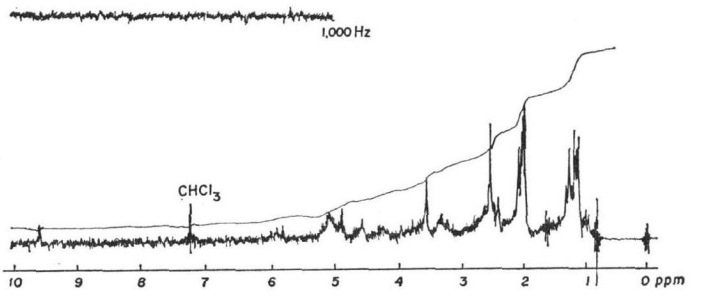
Moreover, O-propionylmycarose was detected in both MDM III and A_1 and not in 4''-depropionyl-MDM III and A_3. This result suggests that the transformation reaction of A_1 into A_3 is a deacylation reaction which was observed in the transformation of MDM III into 4''-depropionyl-MDM III.

From these results, the transformation pathway was diagrammed as shown in Fig. 5. In addition to the inner pathway previously reported[1], A_1, A_2, A_3 or A_4 was formed from MDM III, 18-dihydro-MDM III, 4''-depropionyl-MDM III or 18- dihydro-4''-depropionyl-MDM III, respectively, *via* trans-

* A proton at δ 9.62 which was recognized as an aldehyde proton in MDM III was still present.

formation at a common position. A_1 itself was transformed into A_2, A_3 and A_4 by reduction, deacylation or both in the same pathway as the inner one.

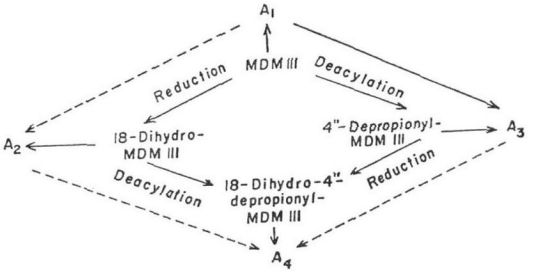
Fig. 4. NMR spectrum of acetylated A_1.



Discussion

Streptomyces sp. strain No. K-245 transformed MDM III into four derivatives. These derivatives were thought to be transformed *via* common transformation from MDM III, 18-dihydro-, 4″-depropionyl-, or 18-dihydro-4″-depropionyl-MDM III, respectively.

Fig. 5. Transformation of MDM III into its derivatives by *Streptomyces* K-245.



Although the antimicrobial spectrum is not shown in this paper, main product A_1, for example, lost all activity (retained 0.1% or less of activity of MDM III). It is interesting in the relationship between structure and antimicrobial activity that A_1 still has a C 18-aldehyde group which is one of the essential groups for antimicrobial activity of the macrolide antibiotics[1,3,16,17].

A-group's derivatives may be unknown or new microbial transformation products since the position transformed is in the other position than C 18-aldehyde and 4″-propionyl group. The final structure will be reported in the future.

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