MICROBIAL TRANSFORMATION OF ANTIBIOTICS III. REACYLATION OF 4″-DEPROPIONYL MARIDOMYCIN III INTO MARIDOMYCIN V (MARIDOMYCIN K) BY *STREPTOMYCES* SP. STRAIN NO. K-342

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In our previous paper[1], *Streptomyces lavendulae* strain No. K-122 was shown to transform maridomycin (MDM) III[2] into three derivatives; 18-dihydro MDM III (by reduction), 4″-depropionyl MDM III (by deacylation) and 18-dihydro-4″-depropionyl MDM III (by reduction and deacylation). Moreover, *Streptomyces* sp. strain No. K-245 was found to transform MDM III into four derivatives (A_1, A_2, A_3 and A_4)[3] in addition to the transformation products described above, and their transformation pathway was also discussed.

In the course of the screening for reacylation product of 4″-depropionyl MDM III, *Streptomyces* sp. strain No. K-342 was found to reacylate it. The present communication deals with the isolation and the structure of reacylation product, designated as MDM K.

To obtain MDM K, strain No. K-342 was subcultured in S-52 medium containing 5% glycerin, 0.5% Polypepton, 2% corn steep liquor and 0.3% NaCl in a 20 ml medium/200 ml Erlenmeyer flask for 1 day at 28°C on a rotary shaker. The seed culture thus obtained, was inoculated with the inoculum size of 10% into the same medium (10 ml/200 ml Erlenmeyer flask) and incubated at 28°C on a rotary shaker. After 16 hours of incubation, a concentrated solution of 4″-depropionyl MDM III in 20% methanol and phosphate buffer (pH 6) were added to the culture (final concentration of 4″-depropionyl MDM III and buffer were 1 mg/ml medium and 0.05 M, respectively). The incubation was continued further for 24 hours. MDM K, reacylation product, and residual, untransformed 4″-depropionyl MDM III were extracted from the culture filtrate with ethyl acetate at pH 8.2. The extract was concentrated *in vacuo* and the concentrate was designated temporarily as crude MDM K which contained about 70% of untransformed 4″-depropionyl MDM III. In addition, it was confirmed that MDM K was never formed from an acylated compound, MDM III, but only from 4″-depropionyl MDM III.

Crude MDM K was purified by silica gel column chromatography using chloroform-methanol as solvent system. The column was developed first with chloroform - methanol (98: 2, v/v), then with the same solvent pair (97: 3) and finally with methanol. Typical thin-layer chromatograms after column chromatography are summarized in Fig. 1. After removal of minor component (β) with the first solvent system, the column was developed with the second solvent system. Fractions corresponding to MDM K (fraction Nos. 16 to 50) were collected and dried *in vacuo*. After decolorization with activated charcoal in chloroform, *n*-hexane was added to the concentrate and the mixture was kept overnight in a cold room (5°C), thus affording needle crystals of purified MDM K.

To prove that MDM K is a real reacylation product, the presence of O-acyl mycarose moiety in MDM K was ascertained. MDM K and other related compounds such as MDM III, and 4″-depropionyl MDM III were hydrolyzed independently with 0.5 N HCl for 24 hours at room temperature. The mycarose moiety in the hydrolyzate was extracted with ether at pH 4.0 and the solvent layer was chromatographed and detected by heating for 10 minutes at 90°C after spraying with vanillin-perchloric acid reagent[4]. The corresponding O-acyl mycarose was detected in MDM K and derivatives other than 4″-depropionyl MDM III. Thus crude O-acyl mycarose from the extract was purified by column chromatography on silica gel using ethyl acetate - benzene (6: 4, v/v). The IR spectrum of O-acyl mycarose thus obtained, was identical with that of O-acetyl mycarose by WATANABE *et al.*[5] So far, it was supposed that acetyl group was introduced into 4″-depropionyl MDM III to form MDM K (identical with MDM V as a result).

From the above result, MDM K was compared with MDM V in biological and physicochemical properties. First of all, the antibacterial activity of MDM K was compared with those of MDM derivatives including MDM V.

Fig. 1. Typical thin-layer chromatograms after column chromatography of transformed products on silica gel.
Running conditions: Column size, 2×55 (cm); Adsorbent, Silica gel (Merck); Solvent system, described in the figure; Flow rate, 20~25 ml/hour; Fraction size, 10 ml. TLC: silica gel G (Merck); Solvent, benzene - acetone (1: 2).

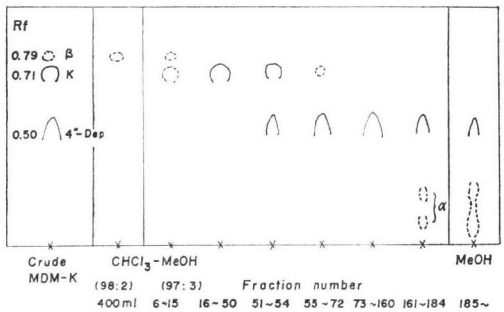

Table 1. Comparison of MDM-K with MDM-V

	MDM-K	MDM-V
m.p. (°C)(decomp.)	139~143	144~148
Molecular wt. Mass M^+ (*m/e*)	815	815
Elementary analysis (%)		
Found C	58.46	57.61
H	8.15	8.10
N	1.59	1.68
Rf values on TLC		
Benzene - MeOH (3: 1)	0.51	0.51
" (10: 1)	0.84	0.84
$CHCl_3$ - MeOH - NH_4OH (40: 3: 20)	0.48	0.48
Benzene - acetone (1: 1)	0.50	0.50
" (3: 1)	0.79	0.79
$CHCl_3$ - MeOH (50: 1)	0.77	0.77

The activity was lower than that of MDM III and higher than that of its starting material, 4″-depropionyl MDM III, and was almost the same as that of MDM V. Some physicochemical properties of MDM K and V were summarized in Table 1. They showed the same molecular weight and the same behavior on thin-layer chromatograms using various solvent systems. Moreover, the identity of MDM K with MDM V was also confirmed by IR, Mass and NMR spectral data.

In conclusion, MDM K is identical with MDM V in which acetyl group was introduced into the C 4-position of mycarose moiety of 4″-depropionyl MDM III.

Discussion

As for the biological reacylation, *Streptomyces hygroscopicus* No. B-5050, a maridomycins' producer, was found to reacylate 4″-depropionyl MDM III to form MDM V[6]. However, there have been no report described on biological reacylation of C 4-position by other strains than the producer. When various functional groups including fatty acids have been introduced into the deacylation derivatives by microbial conversion, antibiotics with improved properties will be obtained. Examinations about acyl transferase of this strain may reveal whether it will be realized or not.

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