MICROBIAL CONVERSION OF ANTIBIOTICS. IV

REDUCTION OF MARIDOMYCIN

KAZUO NAKAHAMA and SEIZI IGARASI

Central Research Division, Takeda Chemical Industries, Ltd.,
Osaka, Japan

(Received for publication April 22, 1974)

Maridomycin III, a macrolide antibiotic, was reduced at the aldehyde group to 18-dihydromaridomycin III by culture broth of *Nocardia mexicana* IFO 3927. 9-Propionylmaridomycin III and josamycin were also reduced to 18-dihydro-9-propionylmaridomycin III and 18-dihydrojosamycin, respectively, by the same strain. The structures of the reduction products were elucidated from the results of their IR, NMR and mass spectra. Antimicrobial activities of the reduction products against Gram-positive bacteria were about 1/10 of their substrates.

As described in previous papers, maridomycin III (MDM III), a macrolide antibiotic, was hydrolyzed to 4''-depropionylmaridomycin III by several bacteria[1] and actinomycetes.[2,3]

In the studies on screening of microorganisms which convert MDM III to other products, it was found that MDM III was reduced at the aldehyde group to 18-dihydromaridomycin III by *Nocardia mexicana* IFO 3927.

Although aldehyde group of several macrolide antibiotics could be reduced chemically,[4,5,6] the reduction by microorganisms has been reported only in the case of tylosin.[7]

This paper deals with the reduction of MDM III, 9-propionylmaridomycin III (PMDM III) and josamycin (JM) to 18-dihydromaridomycin III, 18 dihydro-9-propionylmaridomycin III and 18-dihydrojosamycin, respectively, by *Nocardia mexicana* IFO 3927. Antimicrobial activities of the reduction products are also presented.

Materials and Methods

Antibiotics

MDM III[8] and PMDM III[9] were prepared in our research laboratories. JM[10,11] was extracted from the commercial preparations.

Microorganism and culture conditions

Nocardia mexicana IFO 3927 was obtained from the Institute for Fermentation, Osaka. The strain was grown in 200 ml of a medium in a 1-liter Erlenmeyer flask at 28°C for 72 hours on a rotary shaker. The medium (pH 7.2) contained 2% glucose, 1% glycerol, 2% soluble starch, 1% soy bean flour, 1% corn steep liquor, 1% cottonseed meal, 0.5% peptone, 0.3% NaCl and 0.5% $CaCO_3$ in deionized water.

Thin-layer chromatography (TLC)

Samples were chromatographed on silica gel TLC plates (Merck) using chloroform-40% dimethylamine solution-water (20:3:30, v/v, lower layer) as the solvent system. The spots of antibiotics were detected by heating the plates after spraying 10% H_2SO_4.

Minimum inhibitory concentration (MIC)

MIC was determined by agar dilution method using glucose bouillon agar (pH 7.0). Test organisms were grown at 37°C for 18 hours, except that *Mycobacterium* sp. ATCC 607 was grown at 37°C for 2 days.

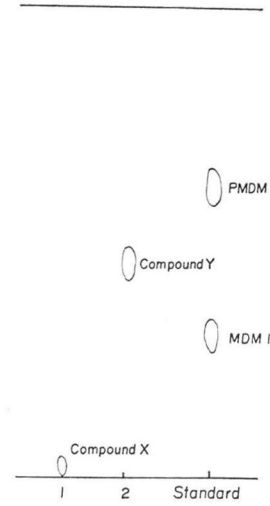
Results

Conversion of MDM III

MDM III (2 g) dissolved in methanol (40 ml) was added to culture broth of *Nocardia mexicana* IFO 3927 (2 liters), and the culture broth was incubated at 28°C for 72 hours with shaking. Thin-layer chromatogram of the culture broth indicated that MDM III added was completely converted to a new compound (compound X) (Fig. 1).

Fig. 1. Thin-layer chromatogram of culture broth after incubation.

After the incubation, the culture broth containing MDM III (1) or PMDM III (2) was extracted with ethyl acetate at pH 8.5, and then the extract was chromatographed on TLC plate.



The culture broth (2 liters) was adjusted to pH 8.5 with dil. NaOH and extracted with ethyl acetate. The extract was washed with water and extracted with 0.05 M citrate buffer (pH 3.0). The buffer layer was reextracted with ethyl acetate at pH 8.5. The extract was washed with water and concentrated *in vacuo*. The concentrate was chromatographed on a silica gel column using a solvent system of benzene-acetone (2:1, v/v), and the eluate containing compound X was concentrated *in vacuo* to give compound X (331 mg) as white powder. $[\alpha]_D^{25}$ −74.5° (*c* 1.02, EtOH).

Anal. Calcd. for $C_{41}H_{69}NO_{16}\cdot 1/2H_2O$: C, 58.55; H, 8.38; N, 1.66.
Found: C, 58.65; H, 8.47; N, 1.74.

Structure of Compound X

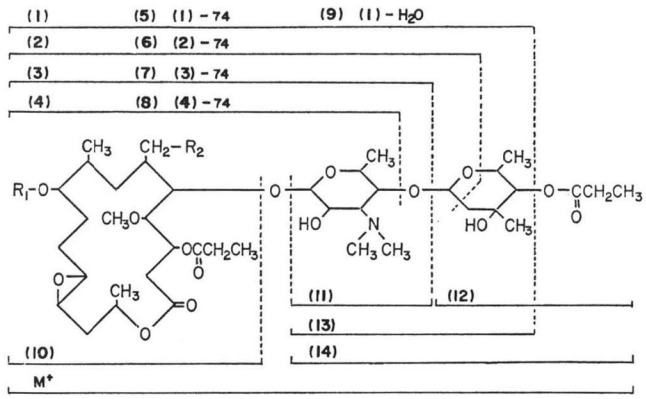
The IR spectrum of compound X was similar to that of MDM III, except that a band at 2730 cm^{-1} (-CHO) of MDM III disappeared in the spectrum of compound X.

The NMR spectrum of compound X was similar to that of 18-dihydromaridomycin II,[6] showing the signals assigned to $-N(CH_3)_2$ at δ 2.52 (6H, s), $-OCH_3$ at δ 3.53 (3H, s), $C_{4''}$ proton at δ 4.59 (1H, d), C_{11} olefine proton at δ 5.57 (1H, dd) and C_{10} olefine proton at δ 6.07 (1H, dd), which were observed in the spectrum of MDM III. A singlet at δ 9.62 assigned to -CHO of MDM III disappeared in the spectrum of compound X. A signal assigned to methylene protons at C_{17} position of compound X was observed at δ 1.63.

The mass spectrum of compound X showed the molecular ion peak at *m/e* 831, which was 2 mass units greater than that of MDM III. The fragment ion peaks reasonable for the structure were observed at *m/e* 441 (macrolactone), *m/e* 174 (mycaminose) and *m/e* 201 (propionylmycarose). The fragment ions involving the macrolactone moiety shifted to higher region by 2 mass units, compared with the corresponding ions of MDM III (Fig. 2).

These results indicated that compound X was 18-dihydromaridomycin III, a reduced derivative of MDM III at the aldehyde group. All of these data were reasonable by comparison with those of 18-dihydromaridomycin II which was obtained from maridomycin II by $NaBH_4$ reduction.[6]

Fig. 2. Mass fragmentation patterns of MDM III, compound X, PMDM III and compound Y.



	R_1	R_2
MDM III	H	CHO
Compound X	H	CH_2OH
PMDM III	$COCH_2CH_3$	CHO
Compound Y	$COCH_2CH_3$	CH_2OH

	M^+	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
MDM III	829	756	658	628	613	682	584	554	539	738	439	174	201	300	374
Compound X	831	758	660	630	615	684	586	556	541	740	441	174	201	300	374
PMDM III	885	812	714	684	669	738	640	610	595	794	495	174	201	300	374
Compound Y	887	814	716	686	671	740	642	612	597	796	497	174	201	300	374

Compound X: 18-dihydromaridomycin III $C_{41}H_{69}NO_{16}$
Compound Y: 18-dihydro-9-propionylmaridomycin III $C_{44}H_{73}NO_{17}$

Conversion of PMDM III

PMDM III (2 g) dissolved in methanol (40 ml) was added to culture broth of *Nocardia mexicana* IFO 3927 (2 liters), and the culture broth was incubated at 28°C for 72 hours with shaking. Thin-layer chromatogram of the culture broth indicated that PMDM III added was completely converted to a new compound (compound Y) (Fig. 1).

Purification of compound Y was carried out in the same manner as that of compound X to give compound Y (736 mg) as white powder. $[\alpha]_D^{25}$—66.6° (*c* 1.03, EtOH).

Anal. Calcd. for $C_{44}H_{73}NO_{17}$: C, 59.51; H, 8.29; N, 1.58.
Found: C, 59.45; H, 8.33; N, 1.33.

Structure of Compound Y

The IR spectrum of compound Y was similar to that of PMDM III, except that a band at 2730 cm^{-1} (-CHO) of PMDM III disappeared in the spectrum of compound Y.

The NMR spectrum of compound Y showed the signals assigned to $-N(CH_3)_2$ at δ 2.50 (6H, s), $-OCH_3$ at δ 3.53 (3H, s), $C_{4''}$ proton at δ 4.59 (1H, d) and olefine protons at δ 5.75 (1H, dd) and at δ 6.02 (1H, dd), which were observed in the spectrum of PMDM III. A singlet at δ 9.60 assigned to -CHO of PMDM III disappeared in the spectrum of compound Y. A signal assigned to methylene protons at C_{17} position of compound Y was observed at δ 1.68.

The mass spectrum of compound Y showed the molecular ion peak at *m/e* 887, which was 2 mass units greater than that of PMDM III. The fragment ion peaks reasonable for the struc-

ture were observed at *m/e* 497 (macrolactone), *m/e* 174 (mycaminose) and *m/e* 201 (propionylmycarose). The fragment ions involving the macrolactone moiety shifted to higher region by 2 mass units, compared with the corresponding ions of PMDM III (Fig. 2).

These results indicated that compound Y was 18-dihydro-9-propionylmaridomycin III, a reduced derivative of PMDM III at the aldehyde group.

Preparation of 18-Dihydrojosamycin

JM (850 mg) was added to culture broth of *Nocardia mexicana* IFO 3927 (850 ml), and the culture broth was incubated at 28°C for 72 hours with shaking. JM added was completely converted to 18-dihydrojosamycin during the incubation.

The culture broth was adjusted to pH 8.5 and extracted with ethyl acetate. The extract was washed with water and extracted with 0.05 M citrate buffer (pH 3.0). The buffer layer was reextracted with ethyl acetate at pH 8.5. The extract was washed with water and concentrated *in vacuo* to give 18-dihydrojosamycin (342 mg) as white powder.

The IR spectrum of 18-dihydrojosamycin was similar to that of JM, except that a band at 2740 cm^{-1} (-CHO) of JM disappeared in the spectrum of 18-dihydrojosamycin.

The NMR spectrum of 18-dihydrojosamycin showed the signals assigned to $-N(CH_3)_2$ at δ 2.48 (6H, s), $-OCH_3$ at δ 3.52 (3H, s), $C_{4''}$ proton at δ 4.58 (1H, d), C_{10} olefine proton at δ 5.55 (1H, dd), C_{11} olefine proton at δ 6.44 (1H, dd), C_{12} olefine proton at δ 6.01 (1H, dd), which were observed in the spectrum of JM. A singlet at δ 9.61 assigned to -CHO of JM disappeared in the NMR spectrum of 18-dihydrojosamycin.

The mass spectrum of 18-dihydrojosamycin showed the molecular ion peak at *m/e* 829, which was 2 mass units greater than that of JM. The fragment ion peaks reasonable for the structure were observed at *m/e* 411 (macrolactone), *m/e* 174 (mycaminose) and *m/e* 229 (isovalerylmycarose). The fragment ions involving the macrolactone moiety shifted to higher region by 2 mass units, compared with the corresponding ions of JM.

Antimicrobial Activity

Antimicrobial activities of 18-dihydromaridomycin III and 18-dihydro-9-propionylmaridomy-

Table 1. Antimicrobial spectra

Test organism	MIC (μg/ml)			
	MDM III	Compound X	PMDM III	Compound Y
Staphylococcus aureus FDA 209 P	1	100	1	>100
S. aureus Terajima	2	100	2	>100
S. aureus No. 87*	>100	>100	>100	>100
S. aureus OE-R*	100	>100	>100	>100
Micrococcus flavus IFO 3242	0.2	20	0.5	20
Sarcina lutea PCI 1001	0.1	10	0.1	20
Bacillus subtilis PCI 219	0.5	100	0.5	100
Pseudomonas aeruginosa IFO 3080	>100	>100	>100	>100
Escherichia coli NIHJ	>100	>100	>100	>100
Proteus vulgaris IFO 3045	>100	>100	>100	>100
Mycobacterium sp. ATCC 607	50	100	20	>100

Compound X: 18-dihydromaridomycin III.
Compound Y: 18-dihydro-9-propionylmaridomycin III.
* Macrolide resistant strains.

cin III were about 1/10 of MDM III and PMDM III, respectively (Table 1). The activity of 18-dihydrojosamycin was also much less than that of JM (data not shown).

Discussion

As described in this paper, MDM III was reduced at the aldehyde group to 18-dihydromaridomycin III by *Nocardia mexicana* IFO 3927. Of 526 strains of actinomycetes tested, 24 strains had the ability to reduce MDM III. Among these actinomycetes, all species of *Nocardia* tested (4 species, 6 strains) had the ability. It has been reported that tylosin is also reduced to relomycin by *Nocardia corallina*.[7] Of 670 strains of bacteria tested, only 2 strains had the ability to reduce MDM III. Fungi and yeasts tested did not reduce MDM III. It is interesting that the ability to reduce macrolide antibiotics at their aldehyde group is specifically distributed among species of *Nocardia*.

ADAMSKI *et al.*[12] reported that reduction of spiramycin at the aldehyde group to dihydrospiramycin decreased the antimicrobial activity. Reduction of leucomycin at the aldehyde group also remarkably decreased the activity.[5] As described in this paper, antimicrobial activities of 18-dihydromaridomycin III and 18-dihydro-9-propionylmaridomycin III were about 1/100 of their substrates (MDM III and PMDM III). Then, the importance of the aldehyde group for showing the maximum activity was also shown in MDM III and PMDM III.

As the reduction of macrolide antibiotics at their aldehyde group remarkably decreases their antimicrobial activities, the physiological role of the reduction by microorganisms may be detoxication of the antibiotics.

Acknowledgments

We thank Dr. R. TAKEDA and Dr. A. MIYAKE for their continued interest and encouragement throughout our work. We also thank Dr. T. KISHI and Mr. M. MUROI for their helpful discussions and Mr. T. FUGONO for determination of MIC.

References

1) NAKAHAMA, K.; M. IZAWA, M. MUROI, T. KISHI, M. UCHIDA & S. IGARASI: Microbial conversion of antibiotics. I. Deacylation of maridomycin by bacteria. J. Antibiotics 27: 425~432, 1974
2) NAKAHAMA, K.; T. KISHI & S. IGARASI: Microbial conversion of antibiotics. II. Deacylation of maridomycin by actinomycetes. J. Antibiotics 27: 487~488, 1974
3) NAKAHAMA, K.; T. KISHI & S. IGARASI: Microbial conversion of antibiotics. III. Hydroxylation of maridomycin I and josamycin. J. Antibiotics 27: 433~441, 1974
4) WHALEY, H.A.; E. L. PATTERSON, A.C. DORNBUSH, E. J. BACKUS & N. BOHONOS: Isolation and characterization of relomycin, a new antibiotic. Antimicr. Agents & Chemoth. -1963: 45~48, 1964
5) ŌMURA, S. & M. TISHLER: Relationship of structures and microbiological activities of the 16-membered macrolides. J. Med. Chem. 15: 1011~1015, 1972
6) MUROI, M.; M. IZAWA, H. ONO, E. HIGASHIDE & T. KISHI: Isolation of maridomycins and structure of maridomycin II. Experientia 28: 878~880, 1972
7) FELDMAN, L. I.; I. K. DILL, C.E. HOLMLUND, H. A. WHALEY, E. L. PATTERSON & N. BOHONOS: Microbiological transformations of macrolide antibiotics. Antimicr. Agents & Chemoth. -1963: 54~57, 1964
8) MUROI, M.; M. IZAWA, M. ASAI, T. KISHI & K. MIZUNO: Maridomycin, a new macrolide antibiotic. II. Isolation and characterization. J. Antibiotics 26: 199~205, 1973
9) HARADA, S.; M. MUROI, M. KONDO, K. TSUCHIYA, T. MATSUZAWA, T. FUGONO, T. KISHI & J. UEYANAGI: Chemical modification of maridomycin, a new macrolide antibiotic. Antimicr. Agents & Chemoth. 4: 140~148, 1973
10) OSONO, T.; Y. OKA, S. WATANABE, Y. NUMAZAKI, K. MORIYAMA, H. ISHIDA & K. SUZAKI: A new antibiotic, josamycin. I. Isolation and physico-chemical characteristics. J. Antibiotics 20: 174~180, 1967
11) OMURA, S.; Y. HIRONAKA & T. HATA: Chemistry of leucomycin. IX. Identification of leucomycin A_3 with josamycin. J. Antibiotics 23: 511~513, 1970
12) ADAMSKI, R. J.; H. HEYMANN, S. G. GEFTIC & S. S. BARKULIS: Preparation and antibacterial activity of some spiramycin derivatives. J. Med. Chem. 9: 932~934, 1966