ANTIBACTERIAL ACTIVITIES OF 9- OR 4''- ACYL DERIVATIVES OF MIDECAMYCIN (SF-837) AND 4''-DEPROPIONYLMIDECAMYCIN*

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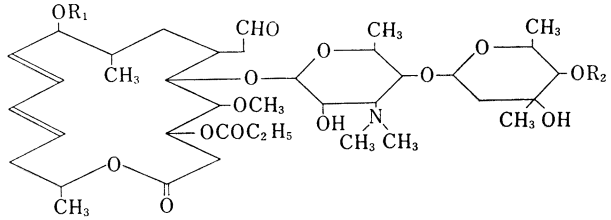
A new macrolide antibiotic, midecamycin (SF-837), which is mainly active against gram-positive bacteria[1], was isolated from a culture filtrate of *Streptomyces mycarofaciens* nov. sp.[2,3] The antibiotic consists of the 16-membered lactone ring, mycaminose and mycarose, having three free hydroxy groups at C-9, 2' and 3''(Fig. 1)[4]. Moreover, it contains two hydroxy groups at C-3 and C-4'' propionated biosynthetically. Of the two propionyl groups, one at C-4'' is easily removed enzymatically to rise to 4''-depropionylmidecamycin (SF-837 M_1) with the fourth free hydroxy group[5,6]. A large number of acyl derivatives substituted at these free hydoxy groups were prepared in our laboratories, to obtain better therapeutic agent. Of these, we report here the antibacterial activities of 9-acylmidecamycin, 9-acyl-4''-depropionylmidecamycin and 9-acetyl-4''-acyl-4''-depropionylmidecamycin(Table 1).

Fig. 1 Structure of midecamycin (1) and 4''-depropionylmidecamycin (18)



Midecamycin(1) R_1 : H, R_2 : COC_2H_5
4''-Depropionylmidecamycin(18) R_1 : H, R_2 : H

MATERIALS AND METHODS

Materials : Midecamycin was a product of Meiji Seika Kaisha, Ltd. 4''-Depropionylmidecamycin was prepared by the procedure already reported[5]. 9-Acylmidecamycin, 9-acyl-4''-depropionylmidecamycin and 9-acetyl-4''-acyl-4''-depropionylmidecamycin were prepared in our laboratories[7], and listed in Table 1 with structures.

Determination of MIC : The MIC of the derivatives were determined by the two-fold serial agar dilution method according to "the Standard Methods" recommended by the Japan Society of Chemotherapy. Theree strains of *Staphylococcus aureus*, one strain each of *Staphylococcus albus*, *Bacillus subtilis*, *Sarcina lutea*, *Salmonella paratyphi* and two strains of *Escherichia coli* were used as test organisms (Table 2), and heart infusion agar (Difco) was used as the assay plate medium. These plates were incubated at 37°C for 20 hours for bioassay.

Therapeutic effect in mice : Four-weeks old male ICR-JCL mice, weighing 18 to 22 g, were infected intraperitoneally with 0.5 ml of a (1 : 1) mixture of 10% gastric mucin and the overnight culture of *Staphylococcus aureus* 209-P that was diluted 5 times with the brain heart infusion broth. The challenge dose in each experiment was about oral administration (350 mg/kg), and the survival rate of the animals on 7 th day after administration was determined.

Blood concentration in mice : Four-weeks old male ICR-JCL mice, weighing 18 to 22 g, were used. Each of the derivatives, suspended in 2% gum arabic solution (0.5 ml/mouse), was administered orally, and blood samples were withdrawn directly from eyes at 0.25, 0.5, 1.0 and 2.0 hours. Blood concentration of the samples was determined by the paper disk method by using *S. lutea* as a test organism and the respective derivatives as standards.

RESULTS

I) Effect of acyl groups substituted at position

* This work was presented orally at the 22 nd meeting of Japan Society of Chemotherapy in Niigata, Sept., 1975.

Table 1. Structure of derivatives of midecamycin

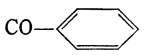
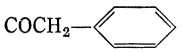
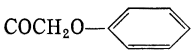
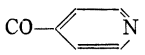
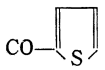
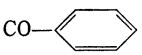
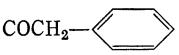
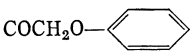
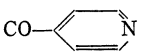
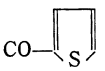
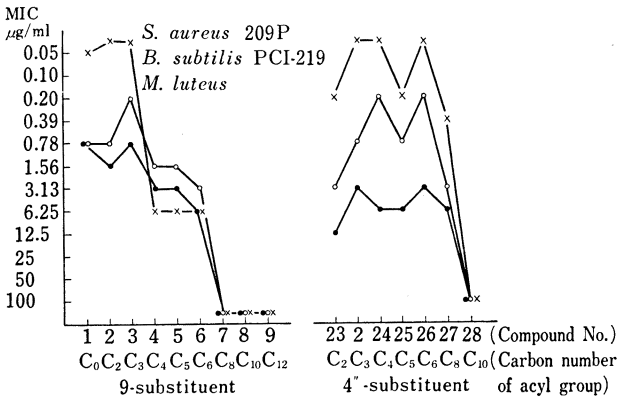
No.	Compound	R_1	R_2
1.	Midecamycin	H	$COCH_2CH_3$
2.	9-Acetylmidecamycin	$COCH_3$	〃
3.	9-Propionyl 〃	$COCH_2CH_3$	〃
4.	9-n-Butyryl 〃	$CO(CH_2)_2CH_3$	〃
5.	9-n-Valeryl 〃	$CO(CH_2)_3CH_3$	〃
6.	9-n-Caproyl 〃	$CO(CH_2)_4CH_3$	〃
7.	9-n-Octanoyl 〃	$CO(CH_2)_6CH_3$	〃
8.	9-n-Decanoyl 〃	$CO(CH_2)_8CH_3$	〃
9.	9-Lauroyl 〃	$CO(CH_2)_{10}CH_3$	〃
10.	9-iso-Butyryl 〃	$COCH(CH_3)_2$	〃
11.	9-iso-Valeryl 〃	$COCH_2CH(CH_3)_2$	〃
12.	9-Pivaroyl 〃	$COC(CH_3)_3$	〃
13.	9-Benzoyl 〃		〃
14.	9-Phenylacetyl 〃		〃
15.	9-Phenoxyacetyl 〃		〃
16.	9-Isonicotinyl 〃		〃
17.	9-Thenoyl 〃		〃
18.	4''-Depropionylmidecamycin	H	H
19.	9-Acetyl-4''-depropionylmidecamycin	$COCH_3$	〃
20.	9-Propionyl 〃	$COCH_2CH_3$	〃
21.	9-n-Butyryl- 〃	$CO(CH_2)_2CH_3$	〃
22.	9-iso-Butyryl- 〃	$COCH(CH_3)_2$	〃
23.	9-Acetyl-4''-acetyl-4''-depropionylmidecamycin	$COCH_3$	$COCH_3$
2.	9-Acetyl-4''-propionyl- 〃 (=9-Acetylmidecamycin)	〃	$COCH_2CH_3$
24.	9-Acetyl-4''-n-butyryl- 〃	〃	$CO(CH_2)_2CH_3$
25.	9-Acetyl-4''-n-valeryl- 〃	〃	$CO(CH_2)_3CH_3$
26.	9-Acetyl-4''-n-caproyl- 〃	〃	$CO(CH_2)_4CH_3$
27.	9-Acetyl-4''-n-octanoyl- 〃	〃	$CO(CH_2)_6CH_3$
28.	9-Acetyl-4''-n-decanoyl- 〃	〃	$CO(CH_2)_8CH_3$
29.	9-Acetyl-4''-iso-butyryl- 〃	〃	$COOH(CH_3)_2$
30.	9-Acetyl-4''-iso-valeryl- 〃	〃	$COCH_2CH(CH_3)_2$
31.	9-Acetyl-4''-pivaroyl- 〃	〃	$COC(CH_3)_3$
32.	9-Acetyl-4''-benzoyl- 〃	〃	
33.	9-Acetyl-4''-phenylacetyl- 〃	〃	
34.	9-Acetyl-4''-phenoxyacetyl- 〃	〃	
35.	9-Acetyl-4''-isonicotinyl- 〃	〃	
36.	9-Acetyl-4''-thenoyl- 〃	〃	

Table 2. Test microorganisms for MIC

a.	*Staphylococcus aureus* Rosenbach FDA-209-P JC-1*
b.	*Staphylococcus aureus* Terashima
c.	*Staphylococcus aureus* Smith S-424
d.	*Staphylococcus albus* 1200-A
e.	*Bacillus subtilis* PCI-219
f.	*Sarcina lutea*
g.	*Salmonella paratyphi* A Minami
h.	*Escherichia coli* communis
i.	*Escherichia coli* NIHJ JC-2

* microorganism used both for *in vitro* and *in vivo* test

Fig. 2 Relationship of the carbon length of the straight chain aliphatic fatty acid esters and *in vitro* activity (MIC)



9 of the lactone ring of midecamycin.

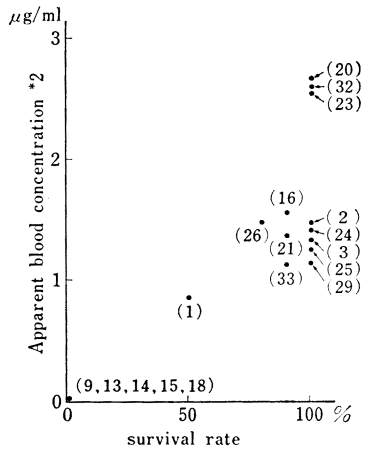
i) *In vitro* activities : The MIC of 9-acylmidecamycin against six gram-positive and three gram-negative bacteria are shown in Table 3. None of the 9-acyl derivatives showed significant *in vitro* activity against gram-negative bacteria, except for *Salmonella*, for which compounds 2, 4, 5, 16 and 17 exhibited weak activities. Therefore, the following discussion was confined to the activity against gram-positive bacteria. As shown in Fig. 2, *in vitro* antibacterial activities of straight-chain aliphatic fatty acid esters decreased in reverse to the length of carbon chain. Activities of lower aliphatic fatty acid esters (2, 3) were almost equal to that of the parent antibiotic (1), and as the chain length increased above C_4, fatty acid esters (4, 5, 6, 7, 8, 9) decreased their activity. The higher fatty acid esters above C_8 (7, 8, 9) were essentially inactive. Among the aliphatic acyl derivatives with C_5 to C_6 branched carbon chain, 9-isobutyryl derivative (10) was strongest followed by 9-isovaleryl (11) and 9-pivaroyl (12) derivatives in that order.

In a case of acyl esters having a benzen ring, the MIC value increased in the order of 9-benzoyl (13), 9-phenylacetyl (14) and 9-phenoxyacetylmidecamycin (15). 9-Isonicotinyl (16) and 9-thenoyl (17) derivatives that contained a pyridin or thiophen ring showed lower MIC value than the ester (13) having a benzen ring.

ii) Therapeutic effect and blood concentration : Among the aliphatic fatty acid esters, 9-acetyl (2) and 9-propionylmidecamycin (3) showed stronger therapeutic effect than midecamycin (1) against *Staphylococcus* infection in mice. *In vivo* activity of higher fatty acid derivatives such as 9-lauroylate (9) was weaker than midecamycin (1). 9-Isobutyrylate (10) having branched chain showed the same survival rate as midecamycin (1). 9-Benzoyl (13), 9-phenylacetyl (14), 9-phenoxyacetyl (15) and 9-thenoyl (17) derivatives were uneffective at the dose given, in spite of good *in vitro* activity. However, the therapeutic effect of 9-isonicotinylate (16) was exceptionally better than that of midecamycin (1) (Table 3).

9-Acetyl (2), 9-propionyl (3) and 9-isonicotinyl (16) derivatives, all of which showed high survival rate, gave good blood concentration as well, and the derivatives such as 9-lauroyl (9), 9-benzoyl (13), 9-phenylacetyl

Fig. 3 Relationship of blood concentrations and survival rates of acyl derivatives of midecamycin*1



*1 Figures in parenthesis indicate compound No. of derivatives.

*2 Blood concentration averaged for 2 hours after oral administration.

Table 3. *In vitro* and *in vivo* antibacterial activities of derivatives of midecamycin

Organism / Derivatives	MIC (μg/ml)									Survival rate*
	a	b	c	d	e	f	g	h	i	
1	0.78	1.56	0.78	1.56	0.78	0.05	50	>100	>100	50%
2	1.56	1.56	1.56	1.56	0.78	<0.05	100	>100	>100	100
3	0.78	0.78	0.78	0.39	0.20	<0.05	>100	>100	>100	100
4	3.13	1.56	1.56	1.56	1.56	6.25	50	>100	>100	—
5	3.13	3.13	3.13	3.13	1.56	6.25	100	>100	>100	—
6	6.25	3.13	6.25	3.13	3.13	6.25	>100	>100	>100	—
7	>100	>100	>100	>100	>100	>100	>100	>100	>100	—
8	>100	>100	>100	>100	>100	>100	>100	>100	>100	—
9	>100	>100	>100	>100	>100	>100	>100	>100	>100	0
10	0.78	0.39	0.39	0.20	0.10	<0.05	>100	>100	>100	50
11	3.13	3.13	3.13	3.13	3.13	3.13	>100	>100	>100	—
12	6.25	6.25	6.25	3.13	3.13	6.25	>100	>100	>100	—
13	12.5	12.5	12.5	12.5	6.25	0.39	>100	>100	>100	0
14	6.25	6.25	6.25	3.13	1.56	0.20	>100	>100	>100	0
15	3.13	3.13	3.13	1.56	0.78	0.10	>100	>100	>100	0
16	1.56	1.56	1.56	0.78	0.78	0.05	50	>100	>100	90
17	1.56	1.56	1.56	12.5	0.20	0.05	100	>100	>100	10
18	12.5	12.5	12.5	12.5	1.56	0.20	>100	>100	>100	10
19	12.5	12.5	12.5	12.5	1.56	0.39	>100	>100	>100	100
20	12.5	12.5	12.5	12.5	1.56	0.39	>100	>100	>100	100
21	12.5	12.5	12.5	12.5	1.56	1.56	>100	>100	>100	90
22	12.5	12.5	12.5	12.5	1.56	1.56	>100	>100	>100	70
23	12.5	12.5	12.5	6.25	3.13	0.20	>100	>100	>100	100
2	1.56	1.56	1.56	1.56	0.78	0.05	100	>100	>100	100
24	3.13	1.56	1.56	0.39	0.20	0.05	100	>100	>100	100
25	3.13	3.13	3.13	3.13	0.78	0.20	>100	>100	>100	100
26	1.56	1.56	1.56	0.78	0.20	0.05	>100	>100	>100	80
27	3.13	3.13	3.13	3.13	1.56	0.39	>100	>100	>100	—
28	>100	>100	>100	>100	>100	>100	>100	>100	>100	—
29	1.56	1.56	1.56	0.78	0.39	<0.05	100	>100	>100	100
30	1.56	1.56	1.56	1.56	0.39	<0.05	100	>100	>100	90
31	3.13	3.13	3.13	3.13	1.56	0.20	100	>100	>100	100
32	1.56	1.56	1.56	0.78	0.39	0.05	100	>100	>100	100
33	1.56	1.56	3.13	1.56	0.78	0.10	>100	>100	>100	100
34	6.25	6.25	6.25	6.25	0.78	0.10	>100	>100	>100	90
35	3.13	3.13	3.13	3.13	0.78	0.10	100	>100	>100	100
36	3.13	6.25	3.13	3.13	1.56	<0.05	>100	>100	>100	90

* Survival rate against *S. aureus* 209-P with oral administration of 350 mg/kg

(14), 9-phenoxyacetyl (15) and 9-thenoyl (17) derivatives exhibiting little *in vivo* activity showed essentially no recognizable blood concentration, suggesting poor oral absorption (Fig. 3).

II) Effect of acyl group at position 9 of the lactone ring of 4''-depropionylmidecamycin

i) *In vitro* activities: Aliphatic fatty acid esters of carbon number of 2 to 4 (19, 20, 21, 22) were tested. It was shown that the antibacterial spectra of 9-acyl derivatives were almost equal to that of the starting compound (18), indicating little influence of acyl groups to *in vitro* activity (Table 3).

ii) Therapeutic effect and blood concentration:

All derivatives so far examined, showed higher survival rate and good blood concentration than SF-837 M_1 (18) and midecamycin (1) (Table 3 and Fig. 3). Blood concentration in function of time of 9-acyl-4''-depropionyl derivatives (20, 21) gave a sharp maximum around 30 minutes after oral administration, and then rapidly decreased.

III) Effect of acyl groups at position 4'' of mycarose of 9-acetyl-4''-depropionylmydecamycin

i) *In vitro* activities : Except for 9-acetyl-4''-n-decanoylate (28), antibacterial spectra of 13 members of 4''-acyl derivatives were almost the same to 9-acetylmidecamycin (2), regardless of the structural variety of acyl groups. That is, 13 members of the derivatives were active against gram-positive bacteria, but less active against gram-negative bacteria, just as seen in 9-acylmidecamycins. In a case of straight chain aliphatic fatty esters, MIC of 4''-acylates of carbon number 3 to 8 including 9-acetylmidecamycin (2) were similar. 4''-Acetylate (23) showed higher MIC, and much more higher MIC was observed for 4''-decanoylate (28) (Table 3 and Fig. 2). Aliphatic fatty esters with branched chain showed MIC almost equal to the corresponding ones with straight chain (Table 3).

Substitution with acyl groups having a benzen ring resulted in raise in MIC in the order of benzoyl (32), phenylacetyl (33) and phenoxyacetyl (34) derivatives. In a case of acyl groups having a heterocyclic ring (35, 36), MIC values were almost equal to the benzoylate (32) (Table 3).

ii) Therapeutic effect and blood concentration : 4''-Acyl derivatives showed, without exception, good *in vivo* effect (Table 3). The effect of compounds No. 23 to No. 26 were indistinguishable under the experimental condition from that of 9-acetylmidecamycin (2) having 4''-propionyl group at C-4''. In contrast to 9-aromatic acyation that resulted in marked lowering in survival rate, 4''-aromatic acylation retained high survival rate. With regard to blood concentration, all of 9-acetyl-4''-acyl derivatives so far examined exhibited longer-lasting blood concentration, as compared to 9-acetyl-4''-depropionyl derivatives, and 4''-acetylate (23) and 4''-benzoylate (32) gave the highest blood concentration among them (Fig. 3).

DISCUSSION

Among the 16-membered macrolides related to midecamycin, OMURA *et al.*[8,9] studied the structure-activities relationship of leucomycin homologues and found that *in vitro* activity increased in parallel to carbon numbers of acyl groups at C-4'', but *in vivo* activity was not precisely followed *in vitro* activity. Acylation of 2-hydroxy group with acetyl to capryl groups resulted in a drop *in vitro* and *in vivo* activity.

HARADA *et al.*[10] examined antibacterial activities of mono, di and tri-acyl derivatives at C-9, 13 and 2' of maridomycin, and lower fatty acid esters such as acetate and propionate were found to have the best therapeutic effect. The present paper was provided wit hthe first examples of the structure-activity relationship of 9-acyl-4''-deacylmacrolides, in addition to that of 9-acyl-macrolides. It was of interest to see that 9-acylation of 4''-depropionylmidecamycin exhibited little influence on *in vitro* activity, but a profound effect on *in vivo* activity. Another interesting feature in this work was the behaviors of acyl esters containing an aromatic ring. When substituted at C-9 of the lactone ring, the acylates, except for isonicotinylate, exhibited poor *in vivo* activity, in spite of good *in vitro* activity. However, when substituted at C-4'' of the neutral sugar (mycarose), the acylates showed not only good *in vitro* effect but also better *in vivo* activity. The discrepancy between *in vitro* and *in vivo* activities 9-aromatic acylates could be explained by the difference in oral absorbability, because the derivatives showing lower survival rate did not give any significant blood concentration.

SUMMARY

The structure-activity relationship was studied on 9-acyl derivatives of midecamycin, and 9-acyl and 9-acetyl-4''-acyl derivatives of 4''-depropionylmidecamycin. Acyl substitution at position 9 of the lactone ring of midecamycin resulted in general in a decrease of *in vitro* activity, but some of derivatives were superior in *in vivo* activity. 9-Acyl substitution of 4''-depropionylmidecamycin retained *in vitro* bioactivity equal to that of the starting material, and showed improved *in vivo* activity.

4''-Acyl substitution of 9-acetyl-4''-depropionylmidecamycin resulted in an equal or slight decrease of *in vitro* activity, but an increase of *in vivo* activity. High survival rates of acyl derivatives were correlated with high blood concentration.

REFERENCES

1) NOMIYA, B.; K. MIYAUCHI, K. KAWAHARAJO, M. ONODA & T. WATANABE : Bacteriological study on SF-837, a new macrolide antibiotic. Chemotherapy 20 : 603～611, 1972

2) NIIDA, T.; T. TSURUOKA, N. EZAKI, E. AKITA & S. INOUYE : A new antibiotic, SF-837. J. Antibiotics 24 : 319～320, 1972
3) TSURUOKA, T.; T. SHOMURA, N. EZAKI, H. WATANABE, E. AKITA, S. INOUYE & T. NIIDA: Studies on antibiotic SF-837, a new antibiotic. I. J. Antibiotics 26 : 452～459, 1971
4) INOUYE, S.; T. TSURUOKA, T. SHOMURA, S. OMOTO & T. NIIDA : Studies on antibiotic SF-837, a new antibiotic II. Chemical structure of antibiotic SF-837. J. Antibiotics 26 : 460～475, 1971
5) TSURUOKA, T.; S. INOUYE, T. SHOMURA, N. EZAKI & T. NIIDA : Studies on antibiotic, SF-837, a new antibiotic. IV. Structure of antibiotic SF-837 A_2, A_3 and A_4. J. Antibiotics 26: 526～536, 1971
6) KAWAHARAJO, K.; T. YOSHIDA, T. WATANABE, K. MIYAUCHI, B. NOMIYA, S. TADA & S. KUWAHARA : Bacteriological studies on two metabolites (M_1, M_2) of SF-837. Chemotherapy 20 : 632～637, 1972
7) IWAMATSU, K.; S. OMOTO, S. INOUYE & T. NIIDA : The details of preparation of these derivatives will be reported elsewhere.
8) OMURA, S.; M. KATAGIRI, I. UMEZAWA, K. KOMIYA, T. MAEKAWA, K. SEKIKAWA. A. MATSUMAE & T. HATA : Structure-biological activities relationship among leucomycins and their derivatives. J. Antibiotics 21 : 532～538, 1968
9) OMURA, S.; M. KATAGIRI, A. NAGASAKI, H. YAMADA, I. UMEZAWA, K. KOMIYAMA & T. HATA : Studies on the chemical structure and some biological properties of leucomycin (Kitasamycins) and their related macrolides. Progress Antimicrobial Anticancer Chemotherapy 2 : 1043, 1971
10) 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