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Structure-activity Relationship of the Antimycoplasma Antibiotic Micacocidin—a Preliminary Study

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Mycoplasmas are known to be a caustic agent for infectious diseases in a wide variety of creatures. Antibiotics such as tetracycline and macrolides have been used for the treatment of those diseases. Because of the spread of the resistant strains, new medicines have been demanded. Furthermore, in view of recent reports that mycoplasmas are closely related to the proliferation of HIV,[1~3] the need to develop novel antimycoplasma agents become more urgent.

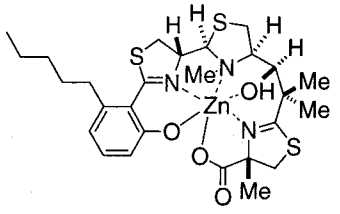
In our continuing studies to exploit a novel antimycoplasma antibiotic, we recently reported the isolation, determination of structure,[4~8] and total synthesis[9~11] of micacocidin (**1**), which corresponds to the metal-free ligand of micacocidin A (**2**). In this paper, we describe a preliminary study on the antimycoplasma activities of micacocidin derivatives[12] to further understand the structure-activity relationship.

Materials and Methods

Micacocidin A (**2**), the initial substance for derivation was obtained from the fermentation of *Pseudomonas* sp. No. 57-250,[5] and the ligand micacocidin (**1**) was prepared from **2** by treatment with dilute acid to release the zinc ion.[8]

The minimal inhibitory concentration (MIC) was determined by the modified agar or liquid dilution method designated by the Society of Chemotherapy in Japan.[8,13]

Fig. 1. Structure of micacocidin A.



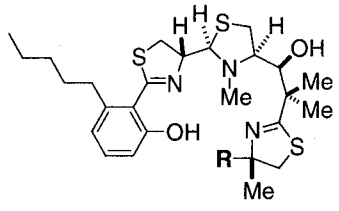
micacocidin A (**2**)

Results

At first, we examined the role of terminal carboxylic acid moiety of micacocidin (**1**) for antimycoplasma activity. Thus the carboxylic acid moiety was modified to the ester and amide groups then reduced to an alcohol function. Antimycoplasma activities of those derivatives are shown in Table 1.

Methyl ester **3** showed almost equal activities to **1**, but modification to a bulky ester group reduced the activity (compound **4**). For the amide derivatives, substitution on the amide nitrogen atom reduced the activity. However, alcohol **8** retained rather potent activity. These results suggested that the lack of bulkiness of the substituents as well as the presence of hydrogen atom required for forming

Table 1. Antimycoplasma activities of micacocidin derivatives of modified terminal carboxylic acid moiety.



Compound No.	R	MIC (μg/mL) *Mg*	*Mp*	*Mh*
1	CO_2H	0.10	≤0.00625	0.025
3	CO_2Me	0.20	≤0.00625	≤0.025
4	CO_2PCB	>3.13	0.39	0.10
5	$CONH_2$	0.39	0.20	0.20
6	CONHMe	0.78	0.78	0.05
7	$CONMe_2$	6.25	0.78	6.25
8	CH_2OH	0.39	0.025	0.05

Mg: *Mycoplasma gallisepticum*, *Mp*: *M. pneumoniae*, *Mh*: *M. hyopneumoniae*, PCB: *p*-chlorobenzyl

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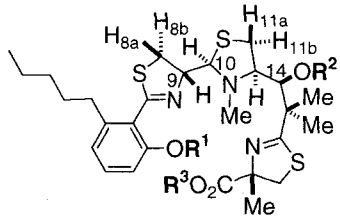
Table 2. Antimycoplasma activities of micacocidin derivatives of modified three functional groups, with partial ^{1}H-NMR data.



Compound No.	substituents			^{1}H-NMR data			MIC (μg/mL)		
	R^1	R^2	R^3	$\Delta\delta_{8a-b}$	$\Delta\delta_{11a-b}$	$J_{9,10}$	*Mg*	*Mp*	*Mh*
2	(Zn)	H	(Zn)	0.63	0.45	10.4	0.10	0.00625	0.025
1	H	H	H	0.31	0.32	9.1	0.10	≤0.00625	0.05
3	H	H	Me	0.31	0.32	9.2	0.20	≤0.00625	≤0.025
9	Me	H	H	0.30	0.25	9.3	1.56	0.10	0.10
10	H	Me	H	0.18	NC	8.1	>6.25	>3.13	>6.25
11	Me	H	Me	0.18	0.37	8.3	3.13	1.56	0.20
12	H	H	MOM	0.32	0.33	9.5	0.10	≤0.00625	0.025
13	H	MOM	MOM	0.19	NC	8.5	6.25	0.39	1.56
14	MOM	MOM	MOM	0.14	0.13	5.4	6.25	1.56	6.25

NC: Not calculated due to the overlapped signals.

hydrogen bond are crucial for exhibiting the activity.

Next, a methyl or methoxymethyl (MOM) group was introduced to three functional groups in **1**, *i.e.* the phenolic (R^1=H), the secondary alcoholic (R^2=H) and the carboxylic (R^3=H) hydroxyl groups. In these derivations, regioselective introductions were achieved by judicious choices of the starting substance (**1** or **2**) or of the reagents [(trimethylsilyl)diazomethane or diazomethane]. In case of **2**, the C-14 secondary alcohol reacted first among three functional groups, probably due to the chelation occurring between both phenolic and carboxylic groups with zinc ion. Thus, treatment of **2** with diazomethane gave mono-methylated product **10**. Antimycoplasma activity of those derivatives thus obtained are shown in Table 2 with some partial ^{1}H-NMR data.

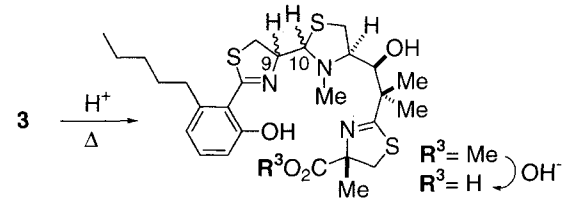
As described above, esterification of the carboxylic acid moiety in **1** had little effect on the activity (compounds **3**, **12**). However, introduction of methyl or MOM groups to the phenolic moiety resulted in the reduction of activity (compounds **9**, **11**). More strikingly, modification of the C-14 secondary alcohol moiety significantly reduced the activity (compounds **10**, **13**). Overall, activity and the number of introduced substituents were shown in inverse correlation.

Moreover, an interesting correlation was observed: the smaller the differences between the chemical shifts of C-8 and C-11 methylene protons ($\Delta\delta_{8a-b}$, $\Delta\delta_{11a-b}$; ppm) (**9**, **10**, **11**, **13**, **14**) and/or the coupling constants for C9-H and C10-H ($J_{9,10}$; Hz) (**10**, **11**, **13**, **14**) became, the weaker the activities were observed. These findings may indicate that the derivatives preserving potent activities respectively possess a similar spatial conformation to that of micacocidin (**1**) and consequently micacocidin A (**2**).

Since the importance of the spatial conformation for exhibiting activity was shown, our attention was then directed to examine the relationship between configuration and activity. Not only C-10 but also C-9 chiral centers of micacocidin methyl ester **3** were readily isomerized by heating under reflux in toluene with a catalytic amount of *p*-toluenesulfonic acid. The configurations of these stereo-centers were determined by the independent synthesis of the C-9 isomers.[11] Isomers thus obtained were separated by HPLC to compare their activities against *M. gallisepticum* (Table 3).

Regardless of the C-9 configuration, 10*R* isomers preserved potent activities, while 10*S* isomers were shown to have reduced activities. Molecular model study revealed that both [9*R*, 10*R*] and [9*S*, 10*R*] isomers may readily form

Table 3. Antimycoplasma activities of C-9 and C-10 isomers.



Compound No.	R^3	configuration C-9	configuration C-10	HPLC Rt (min.)	MIC (μg/mL) *Mg*
1	H	*R*	*R*	34.3	0.10
15		*R*	*S*	29.7	0.78
16		*S*	*R*	31.5	0.20
17		*S*	*S*	(34.3)	NT
3	Me	*R*	*R*	64.1	0.20
18		*R*	*S*	44.6	12.5
19		*S*	*R*	51.0	0.05
20		*S*	*S*	60.8	0.78

HPLC: ODS HG-5 (20x50mm + 20x250mm) 81%MeOH+1mM phosphate buffer (pH=7), 7.5 mL/min, det. 254 nm, NT: Not tested

respective metal complexes, however, the two 10*S* isomers may not.

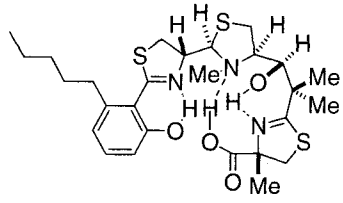
Discussion

The spatial structure of micacocidin (**1**) in solution is held with three intramolecular hydrogen bonds to take a folded conformation resembling that of micacocidin A (**2**) (Figure 2).[14] The almost equally potent activities of **1** and **2** presumably ascribe to their similar spatial conformations.

As described above, it is suggested that the potency of activities largely depend on the ability adopt folded conformation. For instance, modification of three hydroxyl functional groups, removing the intramolecular hydrogen bonds, may hinder the formation of the folded conformation and result in reduced activity. Weak activity observed upon introduction of a bulky substituent to the carboxylic acid moiety may be explained as the result of a conformational change. Furthermore, significant change of the activity caused by modification of the C-14 secondary alcohol moiety seems due to its central location in the molecule. Reduced activities of 10*S* isomers are also rationalized by the difficulty in taking folded conformation.

Finally, it is emphasized that the evidence accumulated here will clarify the action mechanism of the antimycoplasma activity by micacocidin, and facilitate the development of new antimycoplasma agents.

Fig. 2. Folded conformation of **1**.



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