RIBOSOME-BINDING ACTIVITIES AND ANTIMICROBIAL ACTIVITIES OF TYLOSIN AND ITS RELATED COMPOUNDS

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Structure-activity relationships of tylosin and related compounds were evaluated in terms of their antimicrobial and ribosome-binding activities. Demycarosyl derivatives, demycarosyltylosin and 20-deoxydemycarosylrelomycin, were slightly weaker than tylosin and 20-deoxyrelomycin, respectively, both in antimicrobial activity and in affinity to ribosomes. The corresponding demycarosyl-demycinosyl derivatives had weaker antimicrobial activities despite their relatively high affinities to ribosomes. A 23-deoxy-demycarosyl-demycinosyl derivative, 20-oxo-5-*O*-mycaminosylprotylonolide, had a higher affinity to ribosomes than that of tylosin and was equivalent to tylosin in antimicrobial activity against Gram-positive bacteria. These results suggest that the mycinose moiety increases the ability of the molecule to enter bacterial cells. Among the derivatives tested, a 23-iodo derivative, 20-deoxy-23-iodo-5-*O*-mycarosyltylonolide, had the highest affinity for ribosomes as well as the highest antimicrobial activity.

Tylosin is a 16-membered macrolide antibiotic produced commercially by a strain of *Streptomyces fradiae*[1,2]. Recently, microbial production and chemical modification of tylosin-related compounds have been reported from a few laboratories[3~6], and structure-activity relationships have been evaluated. However, there are no reports concerning their ribosome-binding activity except that CORCORAN *et al.*[7] reported the affinities of tylosin, relomycin, demycarosyltylosin (desmycosin) and macrosin to ribosomes from *Bacillus subtilis* relative to [^{14}C]erythromycin A, and that TSUCHIYA *et al.*[8] showed the binding activity of 3-*O*-acetyl-4″-*O*-isovaleryltylosin to ribosomes from a resistant strain of *Staphylococcus aureus*.

PESTKA *et al.*[9] have established a method to evaluate the affinities of macrolide antibiotics to *Escherichia coli* ribosomes by determining their ability to compete with [^{14}C]erythromycin for binding to ribosomes. PESTKA *et al.*[10] and ŌMURA *et al.*[11] studied the structure-activity relationships of leucomycins by comparison of their antimicrobial activities with their affinities for ribosomes. Recently, we established a method to evaluate the affinities of 16-membered macrolide antibiotics to ribosomes with [10,11,12,13-^{3}H]tetrahydroleucomycin A_3[12].

In this paper, we describe the ribosome-binding activities and antimicrobial activities of tylosin-related compounds which were prepared during the investigation of tylosin biosynthesis in *S. fradiae*, and discuss the structure-activity relationships including the effects of mycaminose, mycarose and mycinose moieties, and of CHO, CH_3 and CH_2OH groups at C-20.

Materials and Methods

All of the tylosin-related compounds other than 20-deoxyrelomycin were synthesized from tylosin provided from Eli Lilly & Co. (Indianapolis, USA), as described previously[3]. 20-Deoxyrelomycin was prepared from 20-deoxydemycarosylrelomycin by bioconversion with the tylosin-producing organism *S. fradiae* in the presence of cerulenin, an inhibitor of polyketide biosynthesis[13]. [10,11,12,13-^{3}H]Tetrahydroleucomycin A_3 (4.45 Ci/mmol) was prepared from leucomycin A_3[12]. The determination

of the affinities of tylosin-related compounds to ribosomes was performed by the filter assay method with *E. coli* ribosomes as described previously[12].

Results and Discussion

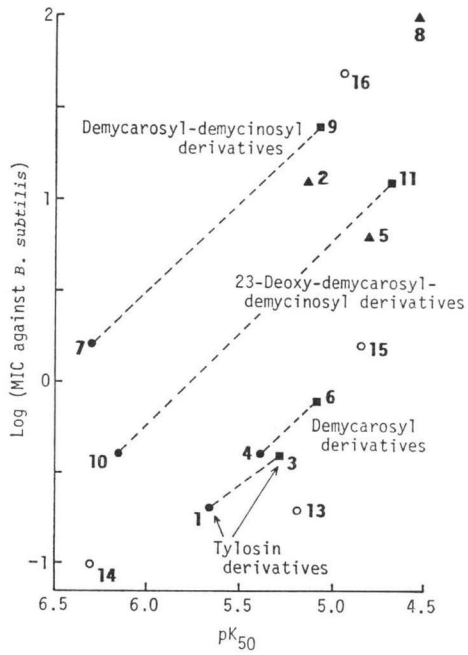
The antimicrobial activities and ribosome affinities of tylosin-related compounds prepared in our laboratory[3,4] were assayed. Table 1 shows the MIC values against 4 species of bacteria and the ID_{50} values for each in competition with [^{3}H]tetrahydroleucomycin A_3 for binding to *E. coli* ribosomes. Fig. 1 shows log (MIC against *B. subtilis*) as a function of pK_{50} ($-\log ID_{50}$).

The MIC and ID_{50} values of tylosin were similar to those of leucomycin A_3 (0.31 μg/ml against *B. subtilis* and 1.8 μM, respectively)[12]. With tylosin (**1**), 20-deoxyrelomycin (**3**), demycarosyltylosin (**4**, desmycosin) and 20-deoxydemycarosylrelomycin (**6**), their antimicrobial activities and affinities to ribosomes were well correlated each other as shown in Fig. 1. Compounds **4** and **6** were somewhat weaker than **1** and **3**, respectively, both in antimicrobial activity against Gram-positive bacteria and in affinity to ribosomes. Conversely, with the other compounds tested, there was poor correlation between antimicrobial activity and affinity to ribosomes (Fig. 1). 20-Hydroxymethyl derivatives (**2**, **5** and **8**), demycarosyl-demycinosyl derivatives (**7** and **9**), and 23-deoxy-demycarosyl-demycinosyl derivatives (**10** and **11**) had relatively weak antimicrobial activities against Gram-positive bacteria despite their relatively high affinities to ribosomes. This suggests that the bacterial cells are less permeable to these compounds. For example, 5-*O*-mycaminosyltylonolide (**7**) and 20-oxo-5-*O*-mycaminosylprotylonolide (**10**) had greater affinities for ribosomes than did tylosin, however the antimicrobial activities of **7** and **10** against Gram-positive bacteria were lower than or equivalent to that of tylosin. These results suggest that the mycinose moiety increases the ability of these compounds to enter the bacterial cells, or to accumulate within the cells as CORCORAN *et al.*[7] speculated.

Fig. 1. Log (MIC) as a function of pK_{50} of [^{3}H]-tetrahydroleucomycin A_3 binding to ribosomes for tylosin and its related compounds.

The MIC values against *B. subtilis* (Table 1) were used. The pK_{50} is the negative log of ID_{50} (Table 1).

The symbols ●, ▲, and ■ indicate the derivatives possessing aldehyde, hydroxymethyl, and methyl groups, respectively, at C-20. Open circles show other derivatives.

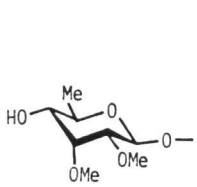


An intermediate of tylosin biosynthesis, protylonolide (**12**), showed no antimicrobial activity and no affinity for ribosomes. On the other hand, 5-*O*-mycaminosylprotylonolide (**11**) and all of the tested compounds having mycaminose moiety were active in both assay systems. This suggests that the mycaminose moiety is necessary for antimicrobial and ribosome-binding activities of tylosin derivatives.

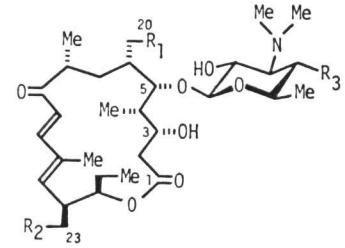
Among the derivatives tested, 23-deoxy-23-iodo-5-*O*-mycaminosyltylonolide (**14**) had the

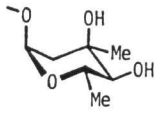
Table 1. Affinities to ribosomes and antimicrobial activities of tylosin and its related compounds.

Concentration (ID_{50}) of a compound for 50% inhibition of [^{3}H]tetrahydroleucomycin A_8 binding to ribosomes and the minimal inhibitory concentration (MIC) were assayed as described in the text.



Mycinose



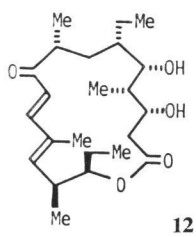


Mycarose

No.	Compound	R_1	R_2	R_3	ID_{50} (μM)	MIC (μg/ml)			
						BS*	SA*	ML*	EC*
1	Tylosin	CHO	Mycinose	Mycarose	2.2	0.2	0.8	<0.1	50
2	Relomycin	CH_2OH	Mycinose	Mycarose	7.5	12.5	6.3	0.8	100
3	20-Deoxyrelomycin	CH_3	Mycinose	Mycarose	5.4	0.4	1.6	0.8	>100
4	Demycarosyltylosin (Desmycosin)	CHO	Mycinose	OH	4.1	0.4	1.6	<0.03	25
5	Demycarosyl-relomycin	CH_2OH	Mycinose	OH	16.4	6.3	6.3	0.2	25
6	20-Deoxyde-mycarosyl-relomycin	CH_3	Mycinose	OH	8.6	0.8	0.8	<0.03	50
7	5-*O*-Mycaminosyl-tylonolide	CHO	OH	OH	0.5	1.6	1.6	0.1	25
8	5-*O*-Mycaminosyl-relonolide	CH_2OH	OH	OH	30.0	>100	>100	12.5	100
9	20-Deoxy-5-*O*-mycaminosyl-relonolide	CH_3	OH	OH	8.7	25	6.3	6.3	25
10	20-Oxo-5-*O*-mycaminosyl-protylonolide	CHO	H	OH	0.7	0.4	0.4	0.1	1.6
11	5-*O*-Mycaminosyl-protylonolide	CH_3	H	OH	21.5	12.5	3.1	1.6	25
12	Protylonolide**				>1,000	>100	>100	>100	>100
13	20-Deoxy-20-iodo-demycarosylrelo-mycin	CH_2I	Mycinose	OH	6.6	0.2	0.1	<0.1	50
14	23-Deoxy-23-iodo-5-*O*-mycaminosyl-tylonolide	CHO	I	OH	0.5	0.1	0.1	<0.1	3.1
15	20,23-Dideoxy-20,23-diiodo-5-*O*-mycaminosyl-relonolide	CH_2I	I	OH	14.5	3.1	3.1	0.8	50
16	8,20-Cyclo-20-hydroxy-5-*O*-mycaminosyl-tylonolide**				11.5	50	25	6.3	100

* BS, *Bacillus subtilis* PCI 219; SA, *Staphylococcus aureus* FDA 209P; ML, *Micrococcus luteus* PCI 1001; EC, *Escherichia coli* NIHJ.

** The structures are as follows.



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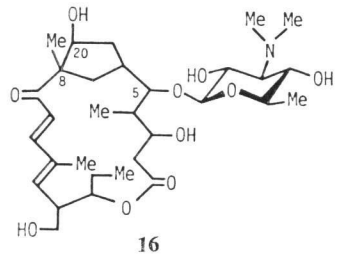

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highest affinity for ribosomes and the highest antimicrobial activity. Compounds **10** and **14** had relatively high antimicrobial activities against a Gram-negative bacterium, *E. coli*. The high antimicrobial activities against *E. coli* are of interest in relation to the poor permeability of the outer membrane of this organism.

As reported in a previous paper, demycarosylleucomycin A_3 is much lower than leucomycin A_3 in both antimicrobial and ribosome-binding activity. On the other hand, as described above, demycarosyltylosin (**4**), 5-*O*-mycaminosyltylonolide (**7**), and 20-oxo-5-*O*-mycaminosylprotylonolide (**10**) have relatively high antimicrobial activities and ribosome-binding activities. Especially, **7** and **10** have higher affinities for ribosomes than that of tylosin. Thus, it would be worthwhile to use these compounds as starting materials for chemical modification. Recently, chemical modification of **4** and **7** were attempted[5,14].

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