NOTE

STRUCTURE ACTIVITY RELATIONSHIP IN SIXTEEN MEMBERED MACROLIDE ANTIBIOTICS

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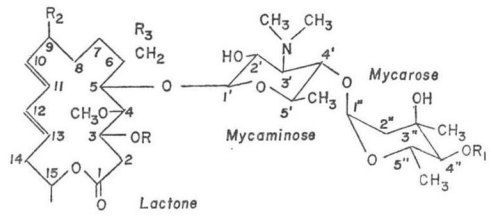
Leucomycin (Kitasamycin) is a macrolide antibiotic having activity against gram-positive bacteria and certain *Mycoplasma* strains. Comparison of antimicrobial activities of the variuos leucomycin congeners (**I**～**X**) indicates a clear structure-activity relationship among them. The acylation of the 3-hydroxy group somewhat reduces the *in vitro* activity while the acylation of the 4″-hydroxy group of mycarose produces higher activity. The activity is dependent on the length of the acyl group and follows the order, isovaleryl > butyryl > propionyl > acetyl > free[$A_1 > A_5 > A_7 > A_9 > V$][1]. Work done on the structural modifications of leucomycins and their analogues by ŌMURA[2] and also in our laboratory has established certain requirements in the structure of these sixteen-membered macrolides for biological activity. Thus reduction of the double bonds was shown not to affect the activity. As expected, oxidation of the 9-hydroxy group thus producing analogues of carbomycin B (**XI**) did not affect the activity. Reduction of the aldehyde to the corresponding carbinol (**XII**, **XIII**) almost completely abolished the antimicrobial activity. Thiosemicarbazide or a SHIFF base[1] obtained from the macrolide retained their activity probably because of their facile *in situ* conversion into the original aldehyde function. The dimethyl acetal (**XVI**) obtained from niddamycin by treatment with methanol in presence of mild acid, was found to be devoid of activity. Acylation of 2′-hydroxy group of mycaminose of niddamycin (**XV**) did not affect the activity appreciably. Removal of the neutral sugar mycarose by mild acidic treatment yielded the corresponding demycarosyl leucomycin or demycarosyl carbomycin B (**XVII**) that was devoid of any antimicrobial property.

It would seem from the comparison of activity of various leucomycin congeners, having different acyl substituents on mycarose as in A_1, A_5, A_7 and A_9, that the polarity of the molecule plays an important role in the structure activity relationship at least *in vitro*.

The present study was undertaken in an attempt to understand the role of various functional groups of the leucomycin molecule, at a molecular level. Since the macrolide antibiotics are known to manifest their activity by combining with the ribosome, thus inhibiting the biosynthesis of proteins, experiments were designed to ascertain their role in protein biosynthesis in the whole cell system as well as in a cell free system. If a compound does not inhibit the biosynthesis of protein in a whole cell system but is active in a cell-free preparation, it is probably because it is unable to enter the cell due to some subtle structural peculiarity. The inability of an analogue to inhibit protein biosynthesis in either system would indicate the requirement of a particular functional group in the molecule for biological activity. As already mentioned, the aldehyde group seemed to be important for *in vitro* activity. That this group plays a vital role at the molecular level for antimicrobial activity, was established by the fact that the dihydro derivatives (**XII**, **XIII**) did not inhibit the biosynthesis of protein in intact cells of *Bacillus subtilis*. They also did not have any effect in a cell-free system, whereas the original antibiotic showed appreciable activity in both systems. This would indicate that the aldehyde group of the sixteen-membered macrolides are somehow involved in complexing with the ribosome.

Although the solubility and the polarity of carbomycin and 2′-isovaleryl demycarosyl carbomycin (**XVIII**) were found to be very similar, the latter compound was devoid of any antimicrobial activity. It did not inhibit the biosynthesis of protein either in a whole cell or in a cell-free system, indicating that acylated mycarose has also other roles than that for the transport of the antibiotic into the cell. The importance of mycarose moiety



	Components	R	R_1	R_2	R_3
I	Leucomycin A_1	H	$COCH_2CH(CH_3)_2$	OH	CHO
II	Leucomycin A_3	$COCH_3$	$COCH_2CH(CH_3)_2$	OH	CHO
III	Leucomycin A_4	$COCH_3$	$COCH_2CH_2CH_3$	OH	CHO
IV	Leucomycin A_5	H	$COCH_2CH_2CH_3$	OH	CHO
V	Leucomycin A_6	$COCH_3$	$COCH_2CH_3$	OH	CHO
VI	Leucomycin A_7	H	$COCH_2CH_3$	OH	CHO
VII	Leucomycin A_8	$COCH_3$	$COCH_3$	OH	CHO
VIII	Leucomycin A_9	H	$COCH_3$	OH	CHO
IX	Leucomycin U	$COCH_3$	H	OH	CHO
X	Leucomycin V	H	H	OH	CHO
XI	Carbomycin B	$COCH_3$	$COCH_2CH(CH_3)_2$	=O	CHO
XII	Dihydro-leucomycin A_3	$COCH_3$	$COCH_2CH(CH_3)_2$	OH	CH_2OH
XIII	Dihydro-niddamycin	H	$COCH_2CH(CH_3)_2$	=O	CH_2OH
XIV	Niddamycin	H	$COCH_2CH(CH_3)_2$	=O	CHO
XV	2′-Acetyl niddamycin	H	$COCH_2CH(CH_3)_2$	=O	CHO
XVI	Niddamycin acetal	H	$COCH_2CH(CH_3)_2$	=O	$\langle^{OCH_3}_{OCH_3}$
XVII	Demycarosyl carbomycin B	$COCH_3$		=O	CHO
XVIII	2′-Isovaleryl demycarosyl carbomycin B				

was further established by the fact that other demycarosyl derivatives (**XVII**, **XVIII**) were found to be non-inhibitory in a cell-free system. Leucomycin fraction V (**X**) that is deacylated on mycarose has about 1/30th the activity of fraction A_1 (**I**), whereas both of them were found to inhibit the synthesis of protein in cell-free system[4]. These results indicate that the lipophylic acyl groups on mycarose are merely needed for the transport of the molecule into the cell. This is also indicated from the comparative biological activities of various leucomycin fractions[1], the higher biological activity being related to the number of carbon atoms of the acyl group.

Experimental

2′Acetyl niddamycin (**XV**).

To a solution of 200 mg of niddamycin in 50 ml acetone, 1 ml of acetic anhydride was added and the solution was kept at room temperature for 2 hours. The solution was concentrated to a small volume and poured into ice water, the pH was adjusted to 8 by adding sodium bicarbonate and extracted with ethyl acetate. Ethyl acetate extract was washed with water, dried over Na_2SO_4. Removal of solvent gave 205 mg of product that was crystallized from ether, hexane, m.p. 97~101°C. λ_{max}^{MeOH} 280 nm $E_{1\,cm}^{1\%}$ 496. N.M.R. δ $CDCl_3$, 2.41 (6H typical shift of $N\langle^{CH_3}_{CH_3}$ from 2.52 due to acylation of adjacent hydroxy function) 9.7 (1H-CHO).

Niddamycin dimethyl acetal (**XVI**).

A solution of 100 mg of niddamycin in 5 ml of 0.25% methanolic HCl was left at room temperature for 1 hour. After this, the pH was adjusted to 8, and poured into ice-water and extracted with ethyl acetate. Ethyl acetate was washed with water, dried over Na_2SO_4. Removal of solvent gave 100 mg solids that was recrystallized from ether, hexane m.p. 85~86°C. λ_{max}^{MeOH} 280 nm. $E_{1cm}^{1\%}$ 495. N.M.R. δ $CDCl_3$ 3.1, 3.2, 3.4 ($3\text{-}OCH_3$), no aldehyde proton.

Demycarosyl carbomycin B (**XVII**).

This was prepared from carbomycin B as described by OMURA *et al.*[7] in case of leucomycin A_3. The product was purified by chromatography over silica gel (Merck) using 20% acetone in benzene as solvent system. M.p. 109~111°C. λ_{max}^{MeOH} 280 nm, $E_{1\,cm}^{1\%}$ 500. N.M.R. δ $CDCl_3$ 2.52 (6H $N\langle^{CH_3}_{CH_3}$) 9.5 (1H CHO).

2′Isovaleryl-demycarosyl carbomycin B (**XVIII**).

This was prepared by acid hydrolysis of 2′isovaleryl carbomycin B. M.p. 110~112°C. λ_{max}^{MeOH} 280 nm, $E_{1\,cm}^{1\%}$ 495. N.M.R. δ $CDCl_3$ 9.5 (1H CHO), 2.4 [6H, $N\langle^{CH_3}_{CH_3}$ typical shift due to 2′ acylation].

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Table 1. MIC of various macrolide derivatives and their effect on protein biosynthesis

Compound	Inhibition of protein synthesis				M.I.C.[a]	
	Inhibition of ^{14}C leucine[c] incorporation in *B. subtilis*		Poly-U phenylalanine[d] incorporation in a cell-free *E. coli* system		*B. subtilis* (ATCC 11774) μg/ml	*S. pyogenes* μg/ml
	at 10 μg/ml (%)	ED_{50} (μg/ml)[b]	% inhibition at 10 μg/ml	ED_{50} (μg/ml)		
I	>98	1.0	63	2.0	0.16	1.25
XI	93	4.0	68	5.4	0.62	5.0
XII	12	>100	8.4	—	>10	100
XIII	17	29.0	26	—	>10	50.0
XIV	>98	1.6	58	6.0	0.62	1.25
XV	86.2	0.94	35.8	>20	1.6	12.5
XVI	8	35.0	12	—	>10	25~50
XVII	33	15.0	None	—	>10	25~50
XVIII	None		None		>10	>100

[a] The compounds were dissolved in 1 % citric acid and the solution was diluted with water or buffer to the required concentration. Minimum inhibitory concentration (M.I.C.) was determined by using the serial dilution method. *Staphylococcus pyogenes* strain used was sensitive to leucomycin and resistant to penicillin G.

[b] For determination of the concentration required to cause 50 % inhibition (ED_{50}) the assay was done in the presence of at least 4 different concentrations of the compound. Percent inhibition was plotted against concentration and ED_{50} read from the curve.

[c] Incorporation of ^{14}C-leucine into *Bacillus subtilis*: *Bacillus subtilis* ATCC 11774 was cultivated in 2 % glucose-1 % yeast extract medium at 30°C. This growth was used to inoculate fresh medium and the cells in logarithmic phase of growth were collected by centrifugation. The cells were suspended in 0.4 % glucose-0.2 % yeast extract to give an absorbancy of 3.0~3.5 at 660 nm. The effect of the compound on incorporation cf ^{14}C-leucine in *B. subtilis* cells was examined under the conditions described below.

Total volume of the reaction mixture was 1.0 ml, containing 0.38 ml of 0.4 % glucose-0.2 % yeast extract, 0.1 ml of antibiotic and 0.5 ml of cell suspension. After 15-minute incubation 20 μl of ^{14}C-leucine (0.1 μc) was added and the mixture incubated for 2 hours at 37°C. Protein synthesis was measured by the incorporation of ^{14}C-leucine into cold trichloracetic acid-precipitable material isolated and washed on Whatman glass fibre paper. Radioactivity was measured in a liquid scintillation spectrometer.

[d] Protein synthesis in cell-free extracts: Cell-free protein synthesizing extract of *Escherichia coli* Q 13 was prepared according to the procedure of NIRENBERG[5]. *E. coli* extract S-30 was not preincubated. The buffer used for extraction contained 10 mM Tris, pH 7.8, 14 mM magnesium acetate, 60 mM ammonium chloride and 0.1 mM dithiothreitol. The S-30 fraction was dialyzed for 16 hours at 4°C against the buffer used for extraction (dithiothreitol concentration increased to 1 mM). Poly-U-directed incorporation of ^{14}C-phenylalanine into polypeptide by S-30 fraction was measured according to the procedure of NIRENBERG[5].

Poly-U-directed incorporation of ^{14}C-phenylalanine: Total volume of the reaction mixture was 0.25 ml, containing 0.1 ml of S-30 fraction (about 2 mg protein), 0.33 mM ATP, 0.03 mM GTP, 12.5 mM phosphoenolpyruvate, 7 μg pyruvate kinase, 0.4 μc ^{14}C-phenylalanine, 0.2 mM of the other 19 amino acids, poly-U, 10 μg, tris (pH 7.8), 100 mM, magnesium acetate, 14 mM and KCl, 50 mM; incubation: 30 minutes at 37°C. Polyphenylalanine formation was measured by the incorporation of ^{14}C-amino acid into hot trichloracetic acid-precipitable material using the filter paper disc technique discribed by BOLLUM[6].

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