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Quantitative Structure–Activity Relationships of *O*-Acyl Derivatives of Leucomycin for Antimicrobial and Ribosome-Binding Activities

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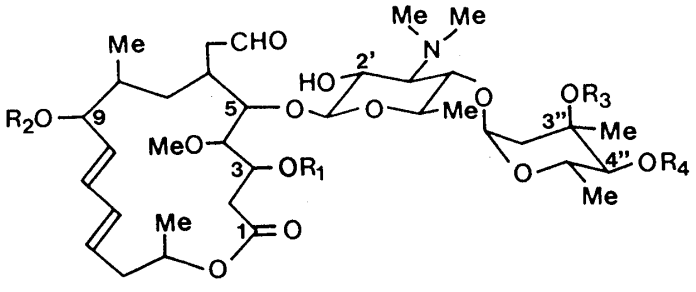
Quantitative structure–activity relationships analyses of *O*-acylleucomycins for antimicrobial and ribosome-binding activities were carried out based on partition coefficient values. The analyses indicate that the antimicrobial activity against gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus*) and the ribosome-binding activity are parabolically related to the hydrophobic character, and that the local hydrophobicity of the 3″-*O*-acyl group which is attached to the tertiary hydroxyl function is especially significant for the antimicrobial action. The correlation of antibacterial activity against gram-positive bacteria with ribosome-binding activity is also significant, but that in the case of gram-negative *Escherichia coli* is very poor. This discrepancy is considered to arise because the antimicrobial activity depends strongly on the ability of the drugs to permeate through the outer membranes (gram-positive bacteria do not have such membranes).

Keywords——quantitative structure–activity relationships analysis; regression analysis; partition coefficient; *O*-acylleucomycin; antimicrobial activity; ribosome-binding activity

Macrolide antibiotics inhibit protein synthesis in procaryotic cells by binding to the 50S ribosome subunits and thereby exhibit antibacterial activity.[1,2] Structure–activity relationships have been studied for the antibacterial and ribosome-binding activities of the 16-membered macrolide leucomycin and its acyl derivatives.[3,4] In those studies, affinity to ribosomes was assayed by using [^{14}C]erythromycin, as reported by Pestka.[5] Recently, Ōmura *et al.*[6] established a new analytical method using [^{3}H]tetrahydroleucomycin A_3 and examined the affinities to ribosomes of leucomycins and 3″-*O*-acyl derivatives in relation to their antimicrobial activity.

The present paper deals with quantitative structure–activity relationships (QSAR) of leucomycins and their *O*-acyl derivatives, including newly synthesized ones, for antimicrobial activity and affinity to ribosomes.



Experimental

Materials——3″-*O*-Isovalerylleucomycin A_5 and leucomycin A_{13} were generous gifts from Dr. Sakakibara of

Toyo Jozo Co., Ltd.

Assays——Antimicrobial activity (minimal inhibitory concentration, MIC) and affinity to ribosomes (concentration for 50% inhibition of [^{3}H]tetrahydroleucomycin A_3 binding, ID_{50}) were assayed as described by Ōmura *et al.*[6] The MIC values (μg/ml) of 3″-*O*-isovalerylleucomycin A_5 and leucomycin A_{13} against *Bacillus subtilis* PIC 219, *Staphylococcus aureus* FDA 209P, *Micrococcus luteus* PCI 1001, and *Escherichia coli* NIHJ were 0.63, 0.31, 0.16 and >10; and 0.16, 0.16, 0.08 and >10, respectively. Their ID_{50} values were 3.2 and 1.2 μM, respectively. The data for leucomycins and their *O*-acyl derivatives except for 3″-*O*-isovalerylleucomycin A_5 and leucomycin A_{13} were obtained by Ōmura *et al.*[6]

The above MIC values (in μg/ml) were converted into molar concentrations. The values in terms of log 1/MIC and pI_{50} ($\log 1/ID_{50}$) were used for the QSAR study.

Parameters——For lipophilicity, the logarithm of the partition coefficient of substituents ($\Delta \log P$, the difference between $\log P$ for *O*-acyl and that for OH) calculated by the fragment method established by Hansch and Leo[7] was used. P_1, P_2, P_3 and P_4 indicate the partition coefficients of the substituents R_1, R_2, R_3 and R_4, respectively. The indicator variables, I_1 and I_3 for R_1 = acyl and R_3 = acyl, respectively, were also used in the analysis.

Calculations——Correlations of log 1/MIC and pI_{50} were studied by multiple regression analysis. Computation was performed with an OKITAC system 50V model 65 computer using a specially written Fortran program.

Results and Discussion

Correlation between Antibacterial Activity and Affinity to Ribosomes of Leucomycin *O*-Acyl Derivatives

As previously reported by Pestka *et al.*[3] and Ōmura *et al.*,[4] correlations between affinity to ribosomes and antimicrobial activity against gram-positive bacteria of leucomycins and their 9-*O*-acyl derivatives were observed. However, the binding to ribosomes of 2′-*O*-acyl derivatives was poor compared to their relatively strong antimicrobial activities, suggesting that they exhibit antimicrobial activity as a result of gradual hydrolysis during antimicrobial assays.

We have formulated Eqs. 1—6 (Table II) for the correlation between the antibacterial activity and affinity to ribosomes of *O*-acylleucomycins, with special attention to 3″-*O*-acyl derivatives, based on the data in Table I.

As shown in Table II, the correlation between affinity to ribosomes and antimicrobial activity was not high. Pestka *et al.*[3] have reported that natural leucomycins (3 and 4″-*O*-acyl derivatives) show a good correlation between antimicrobial and ribosome-binding activities. However, as Ōmura *et al.*[6] reported, 3″-*O*-acyl derivatives exhibited stronger antimicrobial activities against gram-positive bacteria than their mother compounds without any increase in affinity to ribosomes, suggesting that the 3″-*O*-acyl derivatives are more able to permeate into the gram-positive bacterial cells. Therefore, the low correlation between antimicrobial and ribosome-binding activities is considered to arise because the data used for calculation included those for many 3″-*O*-acyl derivatives. In fact, by the use of indicator variables, Eqs. 2 and 5 with relatively high correlation coefficients were obtained. In these two equations, the term I_3 was highly significant ($p<0.01$).

On the other hand, the antimicrobial activity against the gram-negative bacterium *Escherichia coli* did not correlate to the affinity to ribosomes (Eq. 6). In general, macrolide antibiotics are known not to permeate well through the outer membranes, which are contained only in gram-negative bacteria, and this may explain the rather poor antimicrobial activity against *E. coli*.

Correlation between Antimicrobial Activity or Affinity to Ribosomes and Partition Coefficient

Equations 7—10 (Table III) were formulated for the correlation between the antibacterial activity and partition coefficient based on the data in Table I. The partition coefficient was presented as $\Delta \log P$, calculated by the fragment method.[7] The correlation was relatively good. The highest correlation was observed between antimicrobial activity against *Bacillus subtilis* and partition coefficient. The equations indicate that hydrophobic character is

TABLE I. Parameters Used in the Formulation of the Equations for the Antibacterial Activity and Affinity to Ribosomes of Leucomycin *O*-Acyl Derivatives

No.	R_1[a]	R_2[a]	R_3[a]	R_4[a]	pI_{50}[b]	log 1/MIC[c]				Δ log P[d]				Indicator variable	
						BS	SA	ML	EC	R_1	R_2	R_3	R_4	I_1	I_3
1[e]	H	H	H	H	5.40	5.45	5.73	6.64	4.15	0	0	0	0	0	0
2	H	H	Bu	Ac	6.00	7.01	6.71	7.91	4.21	0	0	2.00	0.92	0	1
3	H	Ac	Bu	Ac	5.89	6.73	6.44	7.33	4.23	0	0.92	2.00	0.92	0	1
4	H	Ac	iso-Va	Ac	5.85	6.74	6.14	7.94	3.94	0	0.92	2.41	0.92	0	1
5[e]	H	H	H	Pr	5.57	5.99	5.99	6.88	4.48	0	0	0	1.46	0	0
6	H	H	Pr	Pr	6.05	6.71	6.71	7.61	4.91	0	0	1.46	1.46	0	1
7	H	Ac	Bu	Pr	5.92	6.74	7.04	7.04	4.24	0	0.92	2.00	1.46	0	1
8	H	Pr	Bu	Pr	5.85	6.74	6.74	7.65	3.95	0	1.46	2.00	1.46	0	1
9[e]	Pr	H	H	Pr	—	6.42	6.42	7.31	4.21	1.46	0	0	1.46	1	0
10	Pr	Ac	Ac	Pr	5.80	6.75	7.05	7.65	4.25	1.46	0.92	0.92	1.46	1	1
11[e]	H	H	H	Bu	5.92	6.68	6.68	7.59	4.77	0	0	0	2.00	0	0
12	H	H	Ac	Bu	5.89	7.01	7.31	7.91	4.80	0	0	0.92	2.00	0	1
13	H	H	Pr	Bu	5.96	7.32	7.02	7.92	4.52	0	0	1.46	2.00	0	1
14	H	H	Bu	Bu	5.89	6.72	7.02	7.62	4.53	0	0	2.00	2.00	0	1
15	H	H	iso-Va	Bu	5.50	6.13	6.44	6.73	4.93	0	0	2.41	2.00	0	1
16	H	Ac	Ac	Bu	5.75	6.73	6.44	7.63	4.54	0	0.92	0.92	2.00	0	1
17	H	Ac	Pr	Bu	5.92	7.04	7.34	7.64	4.54	0	0.92	1.46	2.00	0	1
18	H	Ac	Bu	Bu	6.00	7.04	7.04	7.95	3.95	0	0.92	2.00	2.00	0	1
19[e]	Ac	H	H	Bu	5.89	6.42	6.42	7.31	4.51	0.92	0	0	2.00	1	0
20	Ac	H	Ac	Bu	5.89	7.03	6.73	7.33	4.54	0.92	0	0.92	2.00	1	1
21	Ac	Ac	H	Bu	5.75	6.73	6.73	7.33	4.23	0.92	0.92	0	2.00	1	0
22	Ac	Ac	Ac	Bu	5.89	7.35	6.75	7.95	3.95	0.92	0.92	0.92	2.00	1	1
23[e]	H	H	H	iso-Va	5.96	6.69	6.99	6.94	4.78	0	0	0	2.41	0	0
24	H	H	Ac	iso-Va	5.89	6.71	7.02	7.62	4.52	0	0	0.92	2.41	0	1
25	H	H	Pr	iso-Va	6.05	6.72	7.02	7.62	4.93	0	0	1.46	2.41	0	1
26[e]	Ac	H	H	iso-Va	5.75	6.43	6.43	7.32	4.52	0.92	0	0	2.41	1	0
27	Ac	H	Ac	iso-Va	5.77	6.74	7.04	7.63	4.24	0.92	0	0.92	2.41	1	1
28[e]	H	H	H	*n*-Ca	5.92	6.69	6.69	6.99	4.89	0	0	0	3.08	0	0

a) Ac, acetyl; Pr, propionyl; Bu, butyryl; iso-Va, isovaleryl; *n*-Ca, caproyl. *b*) log $1/ID_{50}$ (concentration of a compound for 50% inhibition of [^{3}H]tetrahydroleucomycin A_3 binding to ribosomes.[6]) *c*) Minimal inhibitory concentration (represented in molar concentrations). BS, *Bacillus subtilis*; SA, *Staphylococcus aureus*; ML, *Micrococcus luteus*; EC, *Escherichia coli*. *d*) Increase of the logarithm of partition coefficient by acylation, calculated by the fragment method of Hansch and Leo.[7] *e*) These are leucomycin components: **1**, V; **5**, A_7; **11**, A_5; **19**, A_4; **23**, A_1; **26**, A_3; **28**, A_{13}.

TABLE II. Equations for the Antibacterial Activity and Affinity to Ribosomes of Leucomycin *O*-Acyl Derivatives

$$\log 1/\mathrm{MIC} = k_1 \mathrm{pI}_{50} + k_2 I_1 + k_3 I_3 + k_0$$

Equation No.	log 1/MIC against	k_1	k_2	k_3	k_0	n[a]	r[b]	s[c]	F[d]
1	BS	1.944 [$t=6.23$[g]]			−4.662	27	0.780	0.247	38.78
2	BS	1.719 [$t=6.39$[g]]	0.223 [2.49[e]]	0.298 [3.30[f]]	−3.096	27	0.874	0.200	24.69
3	SA	1.621 [$t=4.34$[g]]			−2.752	27	0.655	0.296	18.83
4	ML	1.544 [$t=3.93$[g]]			−1.556	27	0.618	0.311	15.43
5	ML	1.183 [$t=3.24$[f]]		0.358 [2.94[f]]	0.659	27	0.738	0.272	14.38
6	EC	0.232 [$t=0.57$]			3.088	27	0.113	0.322	0.33

a) Number of data points. The data for all the compounds in Table I except **9** were used. *b*) Correlation coefficient. *c*) Standard deviation from the regression equation. *d*) *F* statistics. *e*) $p<0.05$. *f*) $p<0.01$. *g*) $p<0.001$.

TABLE III. Equations Showing the Correlation between the Antimicrobial Activity and Partition Coefficient of Leucomycin *O*-Acyl Derivatives

$$\log 1/\mathrm{MIC} = k_1 \Delta \log P_3 + k_2 (\Delta \log P_3)^2 + k_3 \Delta \log P_4 + k_4 \Sigma \Delta \log P_{1-4}{}^{a)} + k_5 (\Sigma \Delta \log P_{1-4})^2 + k_0$$

Equation No.	log 1/MIC against	k_1	k_2	k_3	k_4	k_5	k_0	n[b]	r	s	F
7	BS	0.611 [$t=3.01$[d]]	−0.265 [3.13[d]]		0.558 [3.74[d]]	−0.069 [2.72[c]]	5.512	28	0.817	0.240	11.55
8	SA	0.797 [$t=4.31$[e]]	−0.295 [3.47[d]]	0.325 [4.01[e]]			5.829	28	0.800	0.243	14.20
9	ML	0.870 [$t=3.77$[e]]	−0.328 [3.14[d]]				7.146	28	0.631	0.308	8.25
10	EC	0.195 [$t=3.07$[d]]		0.399 [5.80[e]]		−0.042 [5.19[e]]	4.079	28	0.812	0.195	15.53

a) The sum of $\Delta \log P$ values for R_1, R_2, R_3 and R_4. *b*) The data for all the compounds in Table I were used. *c*) $p<0.05$. *d*) $p<0.01$. *e*) $p<0.001$.

parabolically related to the antimicrobial activity, though the antimicrobial activity against different microorganisms varies somewhat depending on the position of the acyl group. The maximum activity against *B. subtilis* (BS), $\log 1/\mathrm{MIC}\ (\mathrm{BS})_{\max}$, is expected at $\Delta \log P_3 = 1.15$ and $\Sigma \Delta \log P_{1-4} = 4.04$. The $\log 1/\mathrm{MIC}\ (\mathrm{SA})_{\max}$ and $\log 1/\mathrm{MIC}\ (\mathrm{ML})_{\max}$ are expected at $\Delta \log P_3 = 1.35$ and 1.33, respectively. As shown in Eq. 11, the average antimicrobial activity against the gram-positive bacteria, BS, *S. aureus* (SA) and *M. luteus* (ML), Av. log 1/MIC (BS, SA, ML), correlates well to the partition coefficient ($r=0.837$).

$$\begin{aligned}\text{Av. log 1/MIC (BS, SA, ML)} = {} & 0.694 \Delta \log P_3 - 0.294 (\Delta \log P_3)^2 \\ & [t=4.06_{(p<0.001)}] \quad [4.13_{(p<0.001)}] \\ & + 0.410 \Sigma \Delta \log P_{1-4} - 0.051 (\Sigma \Delta \log P_{1-4})^2 + 6.004 \\ & [3.27_{(p<0.01)}] \quad [2.37_{(p<0.05)}]\end{aligned} \tag{11}$$

$n=28$; $r=0.837$; $s=0.201$; $F=13.46$

The Av. log 1/MIC (BS, SA, ML)$_{max}$ of *O*-acylleucomycins is expected at $\Delta\log P_3 = 1.18$ and $\Sigma\Delta\log P_{1-4} = 4.02$. Av. log 1/MIC (BS, SA, ML) also correlates well to the partition coefficients of the substituent groups R_3 and R_4 as shown in Eq. 12, derived by neglecting the small contributions of R_1 and R_2.

$$\begin{aligned}\text{Av. log 1/MIC (BS, SA, ML)} = {} & 0.754\Delta\log P_3 - 0.285(\Delta\log P_3)^2 \\ & [t = 4.57_{(p<0.001)}] \quad [3.85_{(p<0.001)}] \\ & + 0.643\Delta\log P_4 - 0.148\,(\Delta\log P_4)^2 + 6.058 \\ & [2.75_{(p<0.05)}] \quad [2.04]\end{aligned} \tag{12}$$

$$n = 28;\ r = 0.824;\ s = 0.209;\ F = 12.12$$

From Eq. 12, Av. log 1/MIC (BS, SA, ML)$_{max}$ is expected at $\Delta\log P_3 = 1.32$ and $\Delta\log P_4 = 2.17$. These values are almost equal to the $\Delta\log P$ values of propionyl (1.46) and butyryl (2.00), respectively. The compound 3″-*O*-propionylleucomycin A_5 (**13**) having propionyl and butyryl groups at 3″- and 4″-positions is currently under development for medical use.

It should be noted that, in Eqs. 11 and 12, the terms $\Delta\log P_3$ and $(\Delta\log P_3)^2$ are highly significant at $p < 0.001$. A remarkable characteristic of R_3 is that it is attached to a tertiary hydroxyl function; the other substituents are attached to secondary hydroxyl functions. Moreover, there is a methyl group adjacent to R_3. Apart from the positional effect, these structural features may distinguish R_3 from the other substituents in terms of stability to enzymatic hydrolysis, bulkiness of the hydrophobic moiety, and other steric influences related to the permeability and interactions. In this study, the substituents were limited to aliphatic acyl functions. Details of the contribution of the R_3 group might be clarified if a wide variety of substituent groups could be introduced.

The standard deviations of the above regression Eqs. 11 and 12 were 0.201 and 0.209, respectively. These values are considered to be relatively good in view of the inevitable experimental errors because the theoretical accuracy of log 1/MIC is 0.30 ($=\log 2$), *i.e.* ± 0.15, when MIC is determined using sample solutions prepared by two-fold serial dilution. Moreover, the reason why the *r* values for Eqs. 11 and 12 (0.837 and 0.824, respectively) are not very high is considered to be at least partly due to the narrow range of log 1/MIC values.

We have also formulated Eq. 13 for the affinity to ribosomes of 3″-*O*-acylleucomycins.

$$\begin{aligned}pI_{50} = {} & 1.297\Delta\log P_3 - 0.416(\Delta\log P_3)^2 + 0.265\Sigma\Delta\log P_{1-4} - 0.040(\Sigma\Delta\log P_{1-4})^2 - 0.835\,I_3 + 4.579 \\ & [t = 3.66_{(p<0.01)}] \quad [3.69_{(p<0.01)}] \quad [4.11_{(p<0.001)}] \quad [3.60_{(p<0.01)}] \quad [3.20_{(p<0.01)}]\end{aligned} \tag{13}$$

$$n = 27;\ r = 0.808;\ s = 0.102;\ F = 7.89$$

The maximum pI_{50} is expected at $\Delta\log P_3 = 1.56$ and $\Sigma\Delta\log P_{1-4} = 3.31$. In this equation, the parameter for the local hydrophobicity of the 3″-*O*-acyl group is involved parabolically again, as is that for the total hydrophobicity. The physical meaning of I_3 is uncertain. The negative coefficient (-0.835) for I_3 might suggest the presence of steric hindrance at the 3″-position. Replacing I_3 by the van der Waals volume of R_3 produced a formula similar to Eq. 13. From the formula, however, the optimal $\Delta\log P_3$ was calculated to be 4.40, which is not considered to be a reasonable value. Other factor(s) must be investigated to clarify the meaning of I_3.

Since Jain and Gombar[8] showed by QSAR based on the data reported by Pestka *et al.*[3] and Ōmura *et al.*[4] that acylation at position 4″ increases affinity for ribosomes, we attempted to calculate the relationship using $\Delta\log P_4$ in place of $\Delta\log P_3$. However, this term was not significant.

Thus, the above QSAR work has revealed that the antimicrobial action and affinity to ribosomes of *O*-acylleucomycin derivatives are parabolically related to the hydrophobic character, and that the local hydrophobicity of the 3″-*O*-acyl group which is attached to the tertiary hydroxyl group is especially significant.

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