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Structure–Activity Relationships among the *O*-Acyl Derivatives of Leucomycin. Correlation of Minimal Inhibitory Concentrations with Binding to *Escherichia coli* Ribosomes

S. Ōmura, A. Nakagawa,

Kitasato University and The Kitasato Institute, 9-1, Shirokane 5 chome, Minato-ku, Tokyo, Japan

H. Sakakibara, O. Okekawa,

Research Laboratory, Tōyo Jōso Co., Ltd., Tokyo, Japan

R. Brandsch, and S. Pestka*

Roche Institute of Molecular Biology, Nutley, New Jersey 07110. *Received September 2, 1976*

The synthesis, antimicrobial activity, and binding to ribosomes of leucomycin and leucomycin derivatives are described. In general, the binding of the leucomycins and the leucomycin derivatives to ribosomes correlated with their antimicrobial activity. Some 2′-*O*-acyl derivatives apparently underwent gradual hydrolysis during antimicrobial assays, for their binding to ribosomes was poor compared to their relatively good antimicrobial activities. Correlation between antimicrobial activity and binding to ribosomes, their molecular site of action, provides some insight into the nature of the active molecular moieties.

We have studied the relationship between structure and microbial activity of 16-membered ring macrolide antibiotics, chiefly leucomycin and its derivatives.[1,2] It has been speculated that the presence of the aldehyde group on the lactone ring and the dimethylamino group on the mycaminose moiety may be essential for antimicrobial activity.[2,3] The combination of antimicrobial and cell-free assays permits direct evaluation of such hypotheses.

The macrolides bind to the 50S subunit of prokaryotic ribosomes and inhibit protein synthesis.[4] In addition, erythromycin derivatives and other macrolides compete for ribosomal binding sites.[5–7] Thus, the ability of a macrolide to compete with erythromycin for binding to ribosomes is related to its affinity for ribosomes. The ability to inhibit erythromycin binding in general paralleled the antibacterial activity of these derivatives.[3,7,8] However, where discrepancies between antibacterial activity and binding to ribosomes exist, other factors such as cellular permeability or modification of the antibiotic may be particularly relevant.[3,7,8]

In this paper, we describe the synthesis of acylated derivatives of leucomycin V. In addition, their antimicrobial activities (MIC) and their binding to ribosomes were evaluated.

Chemistry. The leucomycin complex is composed of ten components which differ in the groups (hydroxyl or acetyloxy) at the 3 position on the lactone ring and the acyl group at the 4″ position of mycarose.[9] These components were used as starting materials to obtain the various acyl derivatives. The acyl derivatives of leucomycin were obtained by the following general methods. The reaction of leucomycin V, 8 (LM-V), with acetic anhydride and pyridine yields tetraacetyl-LM-V, in which four hydroxyl groups (3 and 9 position on lactone ring, 2′ position on mycaminose, and 4″ position on mycarose) are acetylated in high yield. By treatment of the tetraacetate with methanol, the acetyl group at the 2′ position is selectively deacetylated because of the basicity of the dimethylamino group at the 3′ position adjacent to the hydroxyl group, yielding triacetyl-LM-V. Furthermore, instead of using acetic anhydride and pyridine, the reaction with acyl chloride in the presence of amines selectively yields only 9-*O*-acyl derivatives without acylation of the 2′-hydroxyl group. By controlling the reactivity of each hydroxyl group as described above, acetyl, propionyl, butyryl, monochloroacetyl, dichloroacetyl, 3-carboxypropionyl, 4-carboxybutyryl, and 2-methylbutyryl substitutions of the various hydroxyl groups of the leucomycin molecule were prepared. The structure of the leucomycin components and their acyl derivatives is shown in Table I. In addition, the mass spectral fragmentation peaks for each compound are summarized in Table I.

Results and Discussion

The concentrations at which [^{14}C]erythromycin binding was inhibited 50% by the leucomycin derivatives and the minimum inhibitory concentrations (MIC) are summarized in Table I. The minimal inhibitory concentration against *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 6633, and *Klebsiella pneumoniae* ATCC 10031 was determined by the agar dilution method.

As can be seen from Table I, both 2′-*O*-acetyl-LM-A_5 (**10**) and 2′-*O*-propionyl-LM-A_5 (**11**) exhibited approximately the same antimicrobial activities against *S. aureus*, *B. subtilis*, and *K. pneumoniae* as LM-A_5 (**4**) containing a free 2′-hydroxyl. However, **4** inhibited [^{14}C]erythromycin binding to ribosomes much better than did **10** and **11**: much higher concentrations of compounds **10** and **11** (14.8 and 13.8 μM, respectively) were required to inhibit [^{14}C]erythromycin binding to ribosomes 50% than of compound **4** (1.1 μM). Thus, the decrease in affinity of 2′-*O*-acyl derivatives for ribosomes suggests that the presence of the 2′-hydroxyl of mycaminose might play a role in binding to ribosomes or that acylation of the 2′-

hydroxyl interferes with binding of the derivative to ribosomes.

The 9-*O*-acyl-LM-A_3 derivatives **13–16** with an acyl group (acetyl, propionyl, butyryl, and isobutyryl groups) on the 9-hydroxyl of the lactone ring exhibited similar antimicrobial activities and concentrations for 50% inhibition of [^{14}C]erythromycin binding to ribosomes compared to that of LM-A_3 (**1**). A similar result was observed with the derivatives (**18–20**) of leucomycin A_5. This indicates that a free hydroxyl at the 9 position is not essential for binding to ribosomes. Of the 9-*O*-acyl derivatives, the antimicrobial activity of 9-*O*-acetyl-LM-A_5 (**18**) was approximately equivalent to that of **4**; and the concentrations for 50% inhibition of [^{14}C]erythromycin binding to ribosomes were approximately equivalent: 1.1 and 0.9 μM for **4** and **18**, respectively. The contribution of the 4″-hydroxyl of the mycarose moiety to antimicrobial activity and ability to inhibit [^{14}C]erythromycin binding to ribosomes was made by comparing LM-A_3 (**1**), -A_4 (**3**), -A_6 (**5**), -A_8 (**6**), and -U (**7**). It was found that their binding to ribosomes and antimicrobial activity decrease with decreasing size of the 4″-*O*-acyl group. For example, LM-U (**7**) (10.7 μM) had about one-ninth the activity of **1** (1.2 μM) in inhibiting [^{14}C]erythromycin binding to ribosomes. Of the 9-*O*-acyl derivatives, 4″-*O*-(2-methylbutyryl)-9-*O*-propionyl-LM-V (**31**) which contained a 4″-*O*-2-methylbutyryl group appeared to bind to ribosomes with the greatest affinity. Compound **31** (1.7 μM) was about 2.4-fold as active as 9-*O*-propionyl-LM-V (**30**) (4.0 μM) containing a 4″-hydroxyl. The high affinity of compound **31** for ribosomes is consistent with its high antimicrobial activity compared to that of compound **30** with an unsubstituted 4″ position. On the other hand, 9-*O*-chloroacetyl-LM-A_3 (**32**) and 9-*O*-(dichloroacetyl)-LM-A_3 (**33**) were similar to 9-*O*-acetyl-LM-A_3 (**13**) in antimicrobial activity and ribosomal binding. However, the concentration for 50% inhibition of erythromycin binding to ribosomes for 9-*O*-chloroacetyl-LM-A_5 (**34**) was 0.9 μM, approximately the same as that of **1**. Also, 2′-*O*-(3-carboxypropionyl)-LM-A_3 (**36**), obtained by esterification of **1** with succinic anhydride, was approximately equivalent to **1** in antimicrobial activity but was only about one-third as effective as **1** in inhibiting [^{14}C]erythromycin binding to ribosomes. Similarly 2′-*O*-acetyl-LM-A_3 (**9**) exhibited approximately the same antimicrobial activity as **1** but was $^1/_{28}$th as active as **1** in inhibiting erythromycin binding to ribosomes.

Correlation between the concentration (pK_{50}) for 50% inhibition of [^{14}C]erythromycin binding to ribosomes and log (MIC) values for the acyl derivatives is summarized in Figure 1. There was a good correlation between the concentration (pK_{50}) and the log (MIC) values for the majority of compounds (solid circles, Figure 1). These included the leucomycin components **1–8** and the 9-*O*-acyl derivatives **13–16** and **30**. However, despite their good antimicrobial activities, the 2′-*O*-acyl derivatives **9–12** competed for erythromycin binding to ribosomes poorly (open circles, Figure 1). Analogously, compounds **22**, **24**, **26**, **27**, and **41**, containing a substituted 2′-hydroxyl, exhibited good antimicrobial activity but relatively poor binding to ribosomes (Table I; open circles, Figure 1). These results suggest that the 2′-*O*-acyl group (on mycaminose) undergoes hydrolysis during the incubation for determination of MIC. However, compounds **36**, **37**, **39**, and **40**, with a 3-carboxypropionyl group on the 2′-hydroxyl, exhibited antimicrobial activity which correlated with their binding to ribosomes (Table I; closed circles, Figure 1). Thus, substitution of an acidic group on the 2′

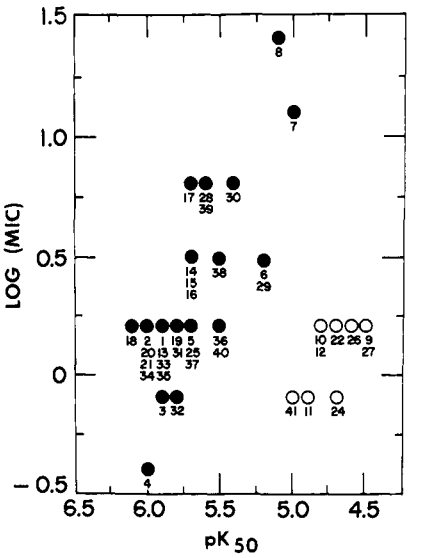


Figure 1. Log (MIC) as a function of concentration for 50% inhibition of erythromycin binding to ribosomes for leucomycins and leucomycin derivatives. The log of the MIC in micrograms per milliliter for each of the compounds as determined against *S. aureus* (Table I) was plotted as a function of the pK_{50} (Table I) for these same compounds. The numbers in the figure refer to the compounds as numbered in Table I. Points where antimicrobial activity and pK_{50} values correlated with each other are given as solid circles. Open circles represent points deviating from the general trend. The strain, *S. aureus* ATCC 6538P, used in this study differed from the strain, *S. aureus* FDA 209P, used in a previous report.[3]

position permits a relatively good interaction with ribosomes. Such correlations between antimicrobial activity (MIC) and ability to bind to ribosomes might provide significant directions for synthesizing more active derivatives.

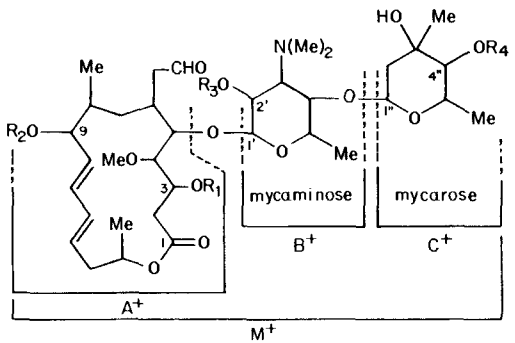
Experimental Section

Melting points (uncorrected) were determined on a MRK Prism scope melting point apparatus. Mass spectra were obtained on a JMS D-100 mass spectrometer. Nuclear magnetic resonance spectra were determined in $CDCl_3$ (Me_4Si) solution on a JEOL MH-100 spectrometer. The structural assignments of various acylleucomycin derivatives were made by analysis of molecular ion peaks for the derivatives and of each fragmentation pattern of aglycon, mycaminose, and mycarose moieties on a high-resolution mass spectrometer.[11] Also, the number and the position of acyl groups in leucomycin derivatives were determined from the nuclear magnetic resonance and mass spectra.[12,13] The syntheses and characteristics of some leucomycin derivatives have been reported.[1]

2′-*O*-Acetyl-LM-A_5 (11). To a solution of **4** (1 g) in dry acetone (10 mL) was added acetic anhydride (0.13 mL). After standing for 3 h at room temperature, the reaction mixture was concentrated. Water (50 mL) was added to the residue and the resulting precipitate was extracted three times with $CHCl_3$ (30 mL). The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. Compound **11** was obtained as an amorphous powder: mp 123–126 °C; NMR ($CDCl_3$) δ 2.04 ppm (2′-$OCOCH_3$). Anal. ($C_{40}H_{67}NO_{14}$).

2′-*O*-(3-Carboxypropionyl)-LM-A_3 (36) and 2′-*O*-(3-Carboxypropionyl)-LM-A_5 (37). To a solution of 1 g of LM-A_3, compound **1**, in dry acetone (10 mL) was added succinic anhydride (282 mg) while cooling. The reaction mixture was allowed to stir overnight at room temperature. A large amount of water was added to the solution and then the reaction mixture was extracted three times with $CHCl_3$ (30 mL). The $CHCl_3$ layer was dried and evaporated to obtain a white powder, **36** (1.1 g): mp 112–116 °C; NMR ($CDCl_3$) δ 2.30 ppm (3-$OCOCH_3$). Anal. ($C_{45}H_{71}NO_{17}$). Compound **37** [mp 124–129 °C; anal. ($C_{41}H_{67}NO_{16}$)] was prepared by a procedure similar to the synthesis of compound **36**. The

Table I. Antimicrobial Activities and Concentration for 50% Inhibition of [^{14}C]Erythromycin Binding to Ribosomes for Leucomycins and Their *O*-Acyl Derivatives



Compd	Compd no.	R_1	R_2	R_3	R_4	Molecular and fragmentation ion (*m/e*)[a] H^+	A^+	B^+	C^+	MIC, μg/mL: *S. aureus* ATCC 6538P	*B. subtilis* ATCC 6633	*K. pneumoniae* ATCC 10031	Concn for 50% inhibn of [^{14}C]erythromycin binding to ribosomes, μM	pK_{50}
Erythromycin unlabeled										0.4	0.2	6.3	0.75	6.1
Leucomycin (LM) A_3	1	$COCH_3$	H	H	$COCH_2CH(CH_3)_2$	827	409	174	229	1.6	0.4	25	1.2	5.9
LM-A_1	2	H	H	H	$COCH_2CH(CH_3)_2$	785	367	174	229	1.6	0.4	25	1.0	6.0
LM-A_4	3	$COCH_3$	H	H	$COCH_2CH_2CH_3$	813	409	174	215	0.8	1.6	50	1.2	5.9
LM-A_5	4	H	H	H	$COCH_2CH_2CH_3$	771	367	174	215	0.4	0.8	12.5	1.1	6.0
LM-A_6	5	$COCH_3$	H	H	$COCH_2CH_3$	799	409	174	201	1.6	3.1	50	1.9	5.7
LM-A_8	6	$COCH_3$	H	H	$COCH_3$	785	409	174	187	3.1	6.3	100	6.7	5.2
LM-U	7	$COCH_3$	H	H	H	743	409	174	145	12.5	6.3	100	10.7	5.0
LM-V	8	H	H	H	H	701	367	174	145	25	12.5	100	7.4	5.1
2'-*O*-Acetyl-LM-A_3	9	$COCH_3$	H	$COCH_3$	$COCH_2CH(CH_3)_2$	869	409	216	229	1.6	0.8	25	33.9	4.5
2'-*O*-Acetyl-LM-A_5	10	H	H	$COCH_3$	$COCH_2CH_2CH_3$	813	367	216	215	1.6	0.8	12.5	14.8	4.8
2'-*O*-Propionyl-LM-A_5	11	H	H	$COCH_2CH_3$	$COCH_2CH_2CH_3$	827	367	230	215	0.8	1.6	12.5	13.8	4.9
2'-*O*-Propionyl-LM-A_4	12	$COCH_3$	H	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$	869	409	230	215	1.6	0.8	25	17.8	4.8
9-*O*-Acetyl-LM-A_3	13	$COCH_3$	$COCH_3$	H	$COCH_2CH(CH_3)_2$	869	451	174	229	1.6	1.6	50	1.3	5.9
9-*O*-Propionyl-LM-A_3	14	$COCH_3$	$COCH_2CH_3$	H	$COCH_2CH(CH_3)_2$	883	465	174	229	3.1	0.4	100	1.9	5.7
9-*O*-Butyryl-LM-A_3	15	$COCH_3$	$COCH_2CH_2CH_3$	H	$COCH_2CH(CH_3)_2$	897	479	174	229	3.1	1.6	100	1.9	5.7
9-*O*-Isobutyryl-LM-A_3	16	$COCH_3$	$COCH(CH_3)_2$	H	$COCH_2CH(CH_3)_2$	897	479	174	229	3.1	0.8	100	2.0	5.7
9-*O*-Acetyl-LM-A_9	17	H	$COCH_3$	H	$COCH_3$	785	409	174	187	6.3	3.1	50	2.1	5.7
9-*O*-Acetyl-LM-A_5	18	H	$COCH_3$	H	$COCH_2CH_2CH_3$	813	409	174	215	1.6	0.4	25	0.9	6.1
9-*O*-Propionyl-LM-A_5	19	H	$COCH_2CH_3$	H	$COCH_2CH_2CH_3$	827	423	174	215	1.6	0.8	50	1.7	5.8
9-*O*-Butyryl-LM-A_5	20	H	$COCH_2CH_2CH_3$	H	$COCH_2CH_2CH_3$	841	437	174	215	1.6	1.6	50	0.9	6.0
9-*O*-Acetyl-LM-A_1	21	H	$COCH_3$	H	$COCH_2CH(CH_3)_2$	827	409	174	229	1.6	0.8	50	1.1	6.0
2',9-Di-*O*-acetyl-LM-A_3	22	$COCH_3$	$COCH_3$	$COCH_3$	$COCH_2CH(CH_3)_2$	911	451	216	229	1.6	1.6	100	21.4	4.7
2',9-Di-*O*-propionyl-LM-A_3	23	$COCH_3$	$COCH_2CH_3$	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$	939	465	230	229	6.3	6.3	100	>100	
2',3-Di-*O*-acetyl-LM-A_5	24	$COCH_3$	H	$COCH_3$	$COCH_2CH_2CH_3$	855	409	216	215	0.8	0.8	100	20.9	4.7
3,9-Di-*O*-acetyl-LM-A_5	25	$COCH_3$	$COCH_3$	H	$COCH_2CH_2CH_3$	855	451	174	215	1.6	0.8	100	2.2	5.7
2',9-Di-*O*-acetyl-LM-A_5	26	H	$COCH_3$	$COCH_3$	$COCH_2CH_2CH_3$	855	409	216	215	1.6	0.8	100	23.4	4.6
2',3,9-Tri-*O*-acetyl-LM-A_5	27	$COCH_3$	$COCH_3$	$COCH_3$	$COCH_2CH_2CH_3$	897	451	216	215	1.6	1.6	100	33.1	4.5

9-*O*-Acetyl-LM-V	28	H	$COCH_3$	H	H	743	409	174	145	6.3	3.1	100	2.8	5.6
9-*O*-Acetyl-LM-U	29	$COCH_3$	$COCH_3$	H	H	785	451	174	145	3.1	1.6	50	6.3	5.2
9-*O*-Propionyl-LM-V	30	H	$COCH_2CH_3$	H	H	757	423	174	145	6.3	3.1	100	4.0	5.4
4″-*O*-(2-Methylbutyryl)-9-*O*-propionyl-LM-V	31	H	$COCH_2CH_3$	H	$COCH(CH_3)CH_2CH_3$	841	423	174	229	1.6	0.8	50	1.7	5.8
9-*O*-(Chloroacetyl)-LM-A_3	32	$COCH_3$	$COCH_2Cl$	H	$COCH_2CH(CH_3)_2$					0.8	0.4	50	1.7	5.8
9-*O*-(Dichloroacetyl)-LM-A_3	33	$COCH_3$	$COCHCl_2$	H	$COCH_2CH(CH_3)_2$					1.6	0.8	50	1.4	5.9
9-*O*-(Chloroacetyl)-LM-A_5	34	H	$COCH_2Cl$	H	$COCH_2CH_2CH_3$					1.6	0.4	25	0.9	6.0
9-*O*-(Dichloroacetyl)-LM-A_5	35	H	$COCHCl_2$	H	$COCH_2CH_2CH_3$					1.6	1.6	25	1.3	5.9
2′-*O*-(3-Carboxypropionyl)-LM-A_3	36	$COCH_3$	H	$COCH_2CH_2$-COOH	$COCH_2CH(CH_3)_2$					1.6	1.6	25	3.5	5.5
2′-*O*-(3-Carboxypropionyl)-LM-A_5	37	H	H	$COCH_2CH_2$-COOH	$COCH_2CH_2CH_3$					1.6	0.8	100	1.9	5.7
9-*O*-(3-Carboxypropionyl)-LM-A_3	38	$COCH_3$	$COCH_2CH_2$-COOH	H	$COCH_2CH(CH_3)_2$					3.1	3.1	100	3.4	5.5
2′,9-Bis-*O*-(3-carboxypropionyl)-LM-A_5	39	H	$COCH_2CH_2$-COOH	$COCH_2CH_2$-COOH	$COCH_2CH_2CH_3$					6.3	3.1	50	2.8	5.6
9-*O*-Acetyl-2′-*O*-(3-carboxypropionyl)-LM-A_5	40	H	$COCH_3$	$COCH_2CH_2$-COOH	$COCH_2CH_2CH_3$					1.6	1.6	25	3.3	5.5
2′-*O*-(4-Carboxybutyryl)-LM-A_5	41	H	H	$COCH_2CH_2$-CH_2COOH	$COCH_2CH_2CH_3$					0.8	0.8	25	9.3	5.0

[a] M^+, molecular ion; A^+, aglycon ion; B^+, mycaminose ion; C^+, mycarose ion; since mass spectral analysis of compounds 32–41 did not show clear fragment peaks, these were not included.

structural assignment of these compounds was confirmed by methanolysis of **36** and **37** which yielded 1 and **4**, respectively.

2′-*O*-(4-Carboxybutyryl)-LM-A_5 (41). In a manner similar to the preparation of compound **36**, 10.3 g of LM-A_5 (**4**) in dry acetone (20 mL) was acylated with glutaric anhydride (2.3 g) to obtain 2′-*O*-(4-carboxybutyryl)-LM-A_5, **41** (10.5 g), as an amorphous powder: mp 121–124 °C. Anal. ($C_{43}H_{69}NO_{16}$).

2′,9-Bis-*O*-(3-carboxypropionyl)-LM-A_5 (39), 9-*O*-(3-Carboxypropionyl)-LM-A_3 (38), and 9-*O*-Acetyl-2′-*O*-(3-carboxypropionyl)-LM-A_5 (40). To a solution of 1 g of LM-A_5, **4**, in dry acetone (10 mL), succinic anhydride (3.63 g) was added while cooling. After stirring for 3 days, a large volume of water was added to the reaction mixture. The mixture was extracted with $CHCl_3$ (60 mL). The extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness to obtain a white powder, 2′,9-bis-*O*-(3-carboxypropionyl)-LM-A_5 (**39**): mp 116–200 °C. Anal. ($C_{45}H_{71}NO_{19}$). Compound **38** (mp 129–135 °C) was prepared by a method similar to that used to prepare **39**. Starting with compound 1, 2′,9-bis-*O*-(3-carboxypropionyl)-LM-A_3 was obtained. Methanolysis of 2′,9-bis-*O*-(3-carboxypropionyl)-LM-A_3 yielded **38**. Compound **40** (mp 120–124 °C) was obtained by reaction of 9-*O*-acetyl-LM-A_5 (**18**) and succinic anhydride in a manner similar to that of **39**.

9-*O*-Acetyl-LM-V (28). To a solution of LM-V (**8**) (5 g, 0.007 mol) in dry pyridine (20 mL) was added acetyl chloride (1.23 g, 0.016 mol) while cooling. The reaction mixture was allowed to stand for 10 h at room temperature and then poured onto 300 mL of ice-cold water. The resulting precipitate was extracted three times with $CHCl_3$ (100 mL) at pH 8.0. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue (4.2 g) was chromatographed over silica gel G with benzene and acetone (4:1 to 3:1) as the eluent. Fractions were combined on the basis of silica gel thin-layer chromatography with benzene–acetone (1:1) as solvent. The solvent was removed to yield 9-*O*-acetyl-LM-V (**28**) as the main product and 3,4″,9-tri-*O*-acetyl-LM-V, 4″,9-di-*O*-acetyl-LM-V, and **8** as minor components. Compound **28** was obtained as an amorphous powder (1.8 g): mp 120–126 °C; NMR ($CDCl_3$) δ 2.06 ppm (9-$OCOCH_3$). Anal. ($C_{37}H_{61}NO_{13}$).

9-*O*-Propionyl-LM-V (30). To a solution of LM-V (**8**) (5 g, 0.007 mol) in dry pyridine (25 mL) was added propionyl chloride (1.45 g, 0.016 mol) at 0 °C. After standing for 10 h, the reaction mixture was poured onto 300 mL of ice-cold water and extracted three times with $CHCl_3$ (100 mL). The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue (4.8 g) was purified by silica gel column chromatography with benzene–acetone (7:1) to obtain 9-*O*-propionyl-LM-V (**30**) as the main product: mp 115–119 °C. Anal. ($C_{38}H_{63}NO_{13}$).

4″-*O*-(2-Methylbutyryl)-9-*O*-propionyl-LM-V (31). 9-*O*-Propionyl-LM-V (**30**) (1 g, 0.0013 mol) was dissoved in dry pyridine (5 mL). To the solution was added 2-methylbutyryl chloride (204 mg, 0.0017 mol). The reaction mixture was allowed to stand for 10 h at room temperature and then extracted three times with 20 mL of $CHCl_3$ at pH 8. The extract was evaporated to dryness. The residue (0.9 g) was purified by silica gel column chromatography with benzene–acetone (8:1) to yield an amorphous powder, **31**: mp 111–114 °C. Anal. ($C_{43}H_{71}NO_{15}$).

9-*O*-(Chloroacetyl)-LM-A_5 (34) and 9-*O*-(Dichloroacetyl)-LM-A_5 (35). LM-A_5 (**4**) (800 mg) was dissolved in dry pyridine (10 mL) and 0.12 mL of chloroacetyl chloride was gradually dropped into the solution which was maintained at a temperature below 5 °C. After stirring for 20 min, the reaction mixture was extracted with $CHCl_3$ (50 mL). The extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue (850 mg) was purified by silica gel column chromatography with benzene–acetone (2:1) to obtain 9-*O*-(chloroacetyl)-LM-A_5 (**34**) (480 mg) as an amorphous powder: mp 128–130 °C. Anal. ($C_{41}H_{66}NO_{14}Cl$). In a similar manner, 9-*O*-(dichloroacetyl)-LM-A_5 (**35**) was prepared.

The other compounds, 2′-*O*-acyl, 9-*O*-acyl, 2′,9-di-*O*-acyl, 3,9-di-*O*-acyl, and 2′,3-di-*O*-acyl derivatives, were synthesized by a similar procedure.

Determination of Binding of Leucomycin Acyl Derivatives to Ribosomes from *Escherichia coli*. Binding of [^{14}C]erythromycin to *E. coli* ribosomes was determined as described previously.[10] Ability of leucomycin and the related compounds to compete with [^{14}C]erythromycin for binding to

ribosomes was determined in 0.50-mL reaction mixtures which contained 0.01 M Tris–HCl (pH 7.2), 0.1 M KCl, 0.004 M $MgCl_2$, 0.01 M NH_4Cl, 13.6 A_{260} units of NH_4Cl-washed *E. coli* ribosomes, 1.2 μM [^{14}C]erythromycin A, and various concentrations of leucomycin or related compounds as indicated. Incubations were performed at 24 °C for 30 min. At the end of the incubation, reactions were stopped by diluting the reaction mixture with 3 mL of cold solution A (0.005 M $MgCl_2$, 0.15 M KCl, and 0.01 M Tris–HCl, pH 7.2). The diluted reaction mixture was filtered through a 25-mm diameter membrane filter (HAWP, Millipore Corp.); the tube and filter were immediately washed an additional three times with 3 mL of cold solution A. The filters were dried under an infrared lamp and radioactivity was determined with a scintillation spectrometer.[10] The concentration of each derivative which produced 50% inhibition of [^{14}C]erythromycin binding to ribosomes was determined as described in previous reports.[3,7]

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Book Reviews

Hormones, Behavior, and Psychopathology. Edited by Edward J. Sachar. Raven Press, New York, N.Y. 1976. xviii + 307 pp. 16.5 × 24 cm. $24.00.

For the last few years, the interrelations between the central nervous and endocrine systems have been intensely studied, and much progress has been made. Evidence is rapidly accumulating that the hormones derived from the brain (pituitary and hypothalamic peptides) and from the outer endocrine glands can exert direct brain effects, which may have behavioral consequences, entirely independent of their endocrine effects. Thus, increasing attention is being paid to the brain as a possible target organ for hormones. The brain, in turn, modulates its own environment inside the body via the pituitary gland. This timely book concerns the effects of hormones on brain function and the influences of the brain and behavior on endocrine function. The 24 chapters were presented at the 65th Annual Meeting of the American Psychopathological Association in March 1975.

The progress in integrating psychiatric and endocrine relations deserves recognition by all investigators involved in planning the therapy of mental diseases and many of the endocrine disorders. The symposium published in this book gathered a selection of topics and authors to illustrate many of the facets of psychoendocrinology.

Studies of the roles of hormones in behavior have been greatly aided by the isolation, purification, and, in some cases, synthesis of hypothalamic, pituitary, and peripheral hormones. Evidence that many of these hormones and their analogues may be psychoactive is presented and discussed. Furthermore, certain fragments and analogues of hormones, practically devoid of endocrine activity, have been shown to affect the brain. The effects of hormones and analogues on the brain have important implications; hormonal defects (with or without endocrine manifestations) may cause neuro- and psychopathology, and hormonal and antihormonal therapy may be applied to neurological and mental illnesses.

This book is a brief but good introduction to behavioral endocrinology. The first nine chapters (143 pp) mostly concern recent studies of psychotropic activities (in animals and man), and potential and experimental psychotherapeutic applications, of hormones and hormone-related drugs. Besides recent experiments, it has been known for a long time that natural, normal, or pathological changes in the endocrine system can effect mood and behavior, and the findings in this area—both old and recent—are reviewed, and their implications are well discussed, in several of these chapters. The next 14 chapters (145 pp) mostly concern the other aspect of the network—the influence of the central nervous system and psychotropic drugs on endocrine functions. The separation of these two types of psychoendocrine relations is only to group the chapters, and the *interdependence* of behavior and hormones is not at all lost sight of. For example, it is theorized that various hormones (e.g., the gonadal hormones) may affect the enzymes involved in monoamine metabolism in the brain; several chapters in the second half of the book concern studies of the roles of monoaminergic neurons in regulating the secretion of hypothalamic and pituitary hormones.

A substantial bonus of this book is that many of the chapters would be good material for medical, pharmacy, nursing, and allied health students to overcome the dichotomous view of mind separate from body. In this context, the final chapter, Dr. Shagass' Presidential Address, titled "The Medical Model in Psychiatry", should be read because it is a gem of an appropriate addition.

Each chapter is carefully written and edited. Some of the chapters are very concise, but all are well referenced. There is a thorough subject index at the end of the book.

Department of Pharmacology **Jerry Frankenheim**
Pharmaceutical Division
Pennwalt Corporation
Rochester, New York 14623

The Juvenile Hormones. Edited by Lawrence I. Gilbert. Plenum Press, New York and London. 1976. 17.5 × 26 cm. x + 572 pp. $45.00.

This volume includes the Proceedings of an International Symposium on the Chemistry, Metabolism, and Modes of Action of the Juvenile Hormones of Insects held at Lake Geneva, Wis., in Nov 1975. Despite the year's delay in publishing these papers, they are still quite up to date since they contain references to numerous papers published in 1975 and refer, in many cases, to original work whose reports are still in press.

Much of the data presented at the symposium have not been published previously, and this book thus serves very well to update Menn and Beroza's "Insect Juvenile Hormones: Chemistry and Action", which appeared in 1972. The volume is subdivided into five sections: I, Chemistry of the Juvenile Hormones and Juvenile Hormone Analogs (four papers); II, Biosynthesis and Metabolism of Juvenile Hormone (five papers); III, Juvenile Hormone Effects at the Cellular Level (eight papers); IV, Juvenile Hormone Effects