ACYL DERIVATIVES OF 16-MEMBERED MACROLIDES

I. SYNTHESIS AND BIOLOGICAL PROPERTIES OF 3''-*O*-PROPIONYLLEUCOMYCIN A_5 (TMS-19-Q)

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Using leucomycin A_5 (**1**), 3''-*O*-propionylleucomycin A_5 (**7**) was synthesized by the following synthetic route: 2''-*O*-acetylation, 3,9-di-*O*-trimethylsilylation, 3''-*O*-propionylation, 3,9-di-*O*-detrimethylsilylation and 2'-*O*-deacetylation. Acylation of the 3''-tertiary hydroxyl group of 2'-*O*-acetyl-3,9-di-*O*-trimethylsilylleucomycin A_5 with propionyl chloride in the presence of tribenzylamine at 70°C gave a 3''-*O*-propionyl derivative in 96% yield. The structure of the final compound, 3''-*O*-propionylleucomycin A_5 (**7**) was confirmed by means of mass, ^{1}H-NMR and ^{13}C-NMR spectrometry and chemical degradations.

3''-*O*-Propionylleucomycin A_5 (**7**) showed higher antibacterial activity *in vitro* and higher serum levels than its mother antibiotic. The biological properties of **7** also were compared with those of josamycin and midecamycin**.

Leucomycin A_5 (**1**) (Fig. 1) is one of the components of a 16-membered macrolide antibiotic complex, leucomycin, produced by *Streptomyces kitasatoensis*[1]. It shows strong antibacterial activity[2], and can be successfully obtained as single component by controlling the culture conditions[3]. Leucomycin A_5 (**1**) has secondary hydroxyl groups at the C-3, C-9, and C-2' positions and a tertiary hydroxyl group at the C-3'' position in the molecule. Acylation of the hydroxyl groups at the C-3, C-9, and C-2' positions has been performed by many workers. However, in most cases the antibacterial activity of the acyl derivatives was lower than that of the mother antibiotics[4]. Acylation of the hydroxyl group at the C-3'' position has rarely been performed, since it is a tertiary hydroxyl group and less reactive than the secondary ones at C-3, C-9, and C-2' positions. OMOTO *et al.*[5] reported the synthesis of 9,3''-di-*O*-acetylmidecamycin from midecamycin, but no one has described the acylation of the hydroxyl group at the C-3'' position alone. The authors have succeeded in selective acylation of the hydroxyl group at the C-3'' position in leucomycin A_5 by treatment with an acyl chloride with heating in the presence of tribenzylamine after protection by trimethylsilylation of the hydroxyl groups at the C-3 and C-9 positions and acetylation of the hydroxyl group at the C-2' position.

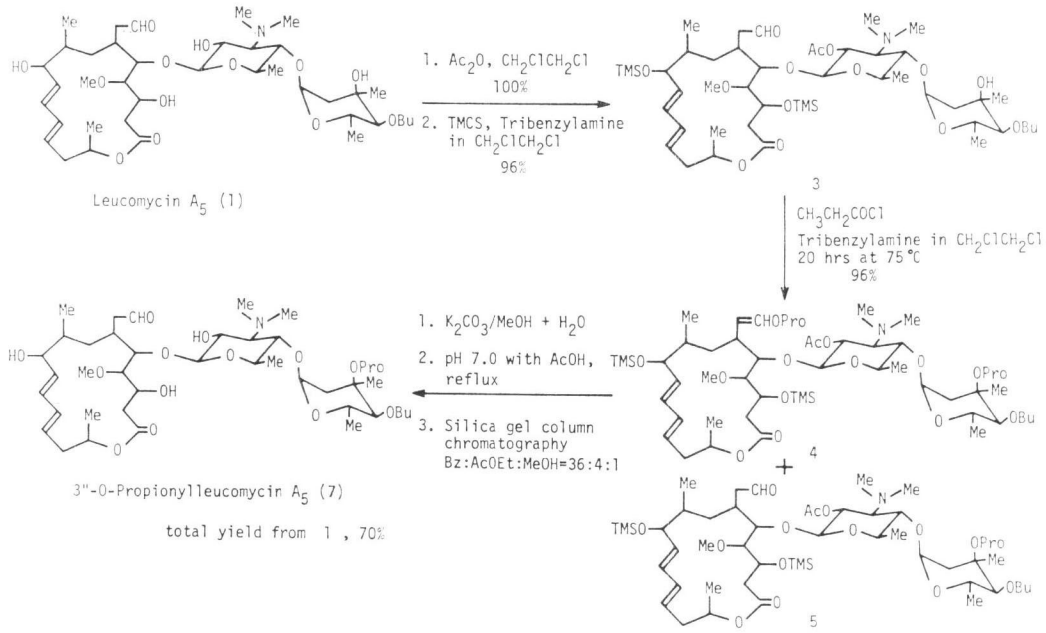
This paper describes the synthesis of 3''-*O*-propionylleucomycin A_5, which among 3''-*O*-acylleucomycin derivatives possesses the most potent antimicrobial activity and a high serum level. The succeeding paper will report the comparison of the biological properties of 3''-*O*-acylleucomycins including the 3''-*O*-propionyl derivative and midecamycin[6].

** Josamycin and midecamycin were purchased from Yamanouchi Pharmaceutical Co. Ltd. and Meiji Seika Kaisha Ltd., respectively, and their potencies were corrected with the standard samples from National Institute of Health of Japan.

Synthesis of 3″-*O*-Propionylleucomycin A_5 (7)

The synthesis of compound **7** is shown in Fig. 1. The hydroxyl group at the C-2′ position in leucomycin A_5 (**1**) was acetylated with acetic anhydride at room temperature to afford a quantitative yield of the 2′-*O*-acetyl derivative. Then, trimethylsilylation of both hydroxyl groups at the C-3 and C-9 positions in the 2′-*O*-acetyl derivative was effectively carried out by using trimethylchlorosilane and pyridine (or tribenzylamine). The 2′-*O*-acetyl-3,9-di-*O*-trimethylsilylleucomycin A_5 (**3**) thus obtained was allowed to react with propionyl chloride in the presence of tribenzylamine at 70°C to 90°C for 20 to 40 hours to give predominantly 2′-*O*-acetyl-17,18-enol-18,3″-di-*O*-propionyl-3,9-di-*O*-trimethylsilylleucomycin A_5 (**4**) and a small amount of 2′-*O*-acetyl-3″-*O*-propionyl-3,9-di-*O*-trimethylsilylleucomycin A_5 (**5**). Treatment of the mixture of compounds **4** and **5** with K_2CO_3 in aqueous methanol, resulted in the removal of the trimethylsilyl groups at the C-3 and C-9 positions and the enolpropionyl group at the C-18 position. After neutralization of the reaction mixture with acetic acid and heating, the acetyl group at the C-2′ position was hydrolyzed yielding 3″-*O*-propionylleucomycin A_5 (**7**).

Fig. 1. Synthesis of 3″-*O*-propionylleucomycin A_5 (**7**).



Structure of 3″-*O*-Propionylleucomycin A_5 (7)

The structure of 3″-*O*-propionylleucomycin A_5 (**7**) was supported by mass (Fig. 2), ^{1}H-NMR (Fig. 3), and ^{13}C-NMR (Fig. 4) spectral evidence. Compound **7** showed a molecular ion (M^+) larger than compound **1** by *m/z* 56 (CH_3CH_2CO minus 1) and each fragment peak due to the mycarose moiety increases by *m/z* 56. The fact that the fragment ion at *m/z* 197 (18%) (mycarose moiety minus COC_2H_5) was much more intense than that of the *m/z* 183 (2%) ion (mycarose moiety minus COC_3H_7) also indicated the introduction of a propionyl group at the C-3″ position[5]. In the ^{13}C-NMR spectrum of **7**, the signal of the C-3″ shifted to a lower magnetic field (δ 69.3[6] in **1**, δ 77.8 in **7**) by the introduction of the propionyl group at the C-3″ position, and new signals arising from the propionyl group appeared at δ 174.1, 28.8 and 9.2 as shown in Fig. 4. Omoto *et al.* reported that the α-carbon

Fig. 2. Diagnostic fragmentation of 3''-*O*-propionylleucomycin A_5 (**7**).

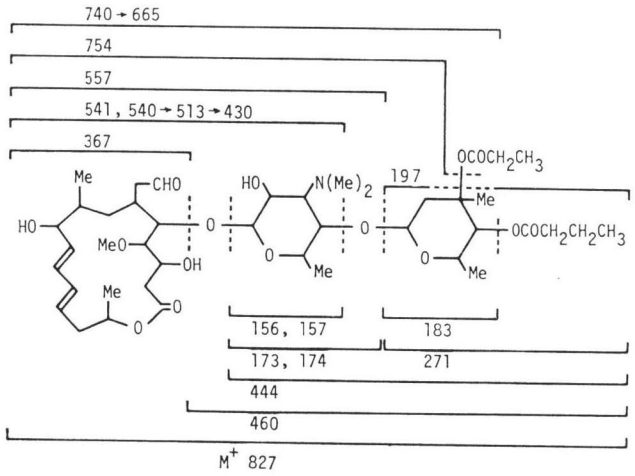
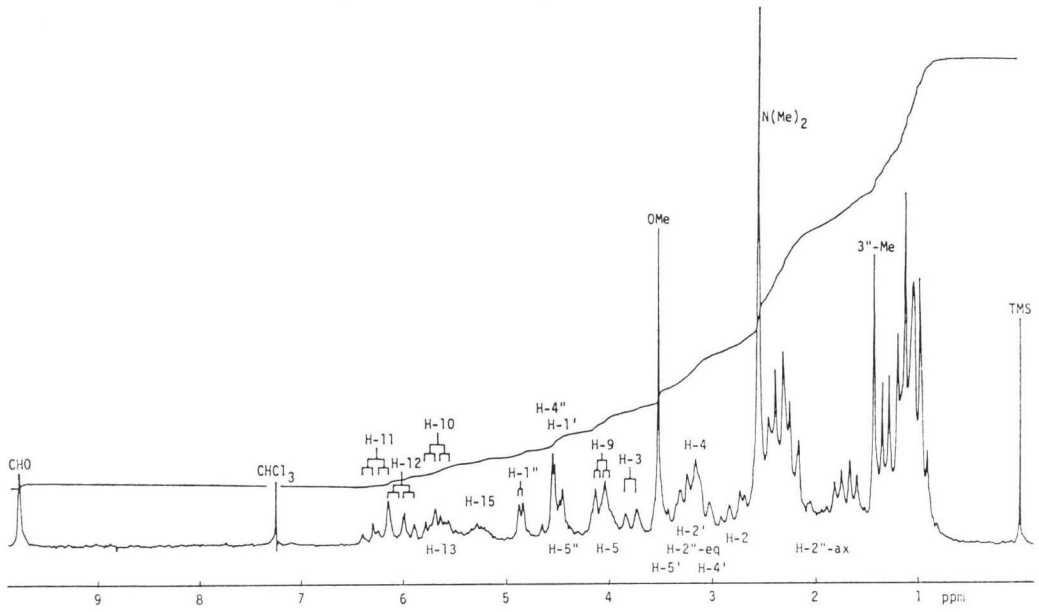
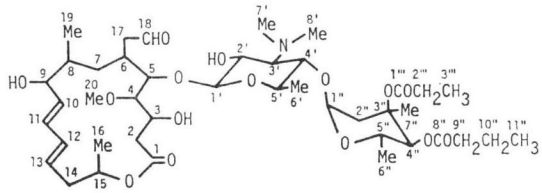


Fig. 3. 100 MHz ^{1}H-NMR spectrum of 3''-*O*-propionylleucomycin A_5 (**7**) in $CDCl_3$.



of a C-3''-*O*-propionyl group of a midecamycin derivative gave a signal at δ 28.8, while that of a C-4''-*O*-propionyl derivative gave one at δ 27.6[5]. Because a signal appeared at δ 28.8, the propionyl group in **7** was presumed to be at the hydroxyl group in the C-3'' position. Compound **7** was hydrolyzed with Amberlyst 15 resin in aqueous dioxane-methanol to yield 4-*O*-butyryl-3-*O*-propionyl-L-mycarose (**8**) and methyl-4-*O*-butyryl-3-*O*-propionyl-L-mycaroside (**9**). 3-*O*-Acetyl-4-*O*-propionyl-L-mycarose being an analog of the compounds **8** and **9**, and its methyl glycosides have been obtained from megalomicin C_2 by JARET *et al.*[8] and from 9,3''-diacetylmidecamycin by OMOTO *et al.*[5] ^{13}C-Chemical shift values of both acylmycarosides **8** and **9** are listed in Fig. 5. The α-carbon of the propionyl

Fig. 4. ^{13}C-Chemical shifts of leucomycin A_5 (**1**), leucomycin A_7 (LM-A_7) and 3″-*O*-propionylleucomycin A_5 (**7**) in deuteriochloroform.



Carbon	Chemical shift			Carbon	Chemical shift			Carbon	Chemical shift		
	1*	LM-A_7	**7**		**1**	LM-A_7	**7**		**1**	LM-A_7	**7**
1	173.9[a]	174.3[a]	173.7[a]	15	68.3[d]	68.2[d]	68.3[d]	1″	97.0	96.9	98.5
2	38.0	37.8	37.9	16	20.1	20.1	20.2	2″	41.9	41.9	36.7
3	71.7	71.6	70.7	17	43.1[c]	43.0[c]	43.0[c]	3″	69.3	69.4	77.8
4	79.2	79.0	80.3	18	202.7	202.7	202.8	4″	77.2	77.1	79.0
5	85.2	85.2	85.2	19	15.0	14.9	14.8	5″	63.5	63.4	63.4
6	30.7	30.5	30.4	20	61.7	61.8	61.9	6″	17.8[e]	17.8[e]	17.5[e]
7	30.7	31.0	31.1	1′	104.0	103.8	103.7	7″	25.3	25.3	22.5
8	34.1	33.8	33.9	2′	68.8[d]	68.7[d]	69.1[d]	8″	173.5[a]	174.0[a]	173.2[a]
9	73.0	72.9	73.2	3′	69.1[d]	69.1[d]	69.1[d]	9″	36.2	27.6	36.3
10	130.2[b]	129.8[b]	129.8[b]	4′	76.0	75.8	77.8	10″	18.5	9.3	18.6
11	134.1[b]	134.2[b]	134.5[b]	5′	73.0	72.9	73.1	11″	13.6		13.8
12	132.5[b]	132.4[b]	132.5[b]	6′	18.8[e]	18.9[e]	18.3[e]	1‴			174.1
13	131.5[b]	131.6[b]	131.7[b]	7′	41.9	41.9	41.6	2‴			28.8
14	41.9[c]	41.9[c]	41.9[c]	8′	41.9	41.9	41.6	3‴			9.2

*: Reference 6)

a, b, c, d, e: Assignments within each sample may be reversed.

group at the C-3 position appeared at δ 28.7. This chemical shift was easily assigned by selective $^{13}C\{^1H\}$ decoupling experiments.

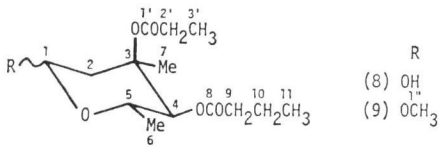
Antimicrobial Activity

The antimicrobial activity (MIC) of 3″-*O*-propionylleucomycin A_5 (**7**) was compared with that of josamycin (leucomycin A_3) and midecamycin. As shown in Tables 1 and 2, compound **7** showed an increase of antibacterial activity against Gram-positive bacteria compared with the other two 16-membered macrolides tested, both of which are currently used in the clinic. In the experiments with an inoculum size of 1×10^8 cells/ml (Table 2), compound **7** showed antimicrobial activities against *Staphylococcus aureus* 0116[9] and *Streptococcus pyogenes* 1022 which are both macrolide-resistant strains. Compound **7** exhibited a strong antimycoplasmal activity: the activity was twice or more higher than that of josamycin. Compound **7** and the two macrolides were inactive against Gram-negative bacteria except *Haemophilus influenzae* 1346.

Serum Levels

Compound **7** and josamycin were orally given to test animals and their serum levels compared as shown in Fig. 6. The serum levels of compound **7** and josamycin varied depending on the species of the test animals. In rats, the serum level of josamycin was higher than that of compound **7**, while

Fig. 5. ^{13}C-Chemical shifts of 4-*O*-butyryl-3-*O*-propionyl-α- and β-L-mycaroses (**8**) and methyl 4-*O*-butylyl-3-*O*-propionyl-α- and β-L-mycarosides (**9**).



Carbon	α-**8**	β-**8**	α-**9**	β-**9**
1	90.9	92.2	97.7	98.9
2	36.3	40.7	36.0	39.3
3	78.2	80.7	78.0	80.6
4	77.7	77.2	77.6	77.3
5	62.5	68.3	62.4	68.0
6	17.4	17.6	17.4	17.6
7	22.6	22.2	22.5	22.2
8	173.2	173.1	173.0	172.9
9	36.1	36.1	36.1	36.1
10	18.5	18.5	18.4	18.4
11	13.7	13.7	13.7	13.7
1′	174.2	173.3	174.0	173.0
2′	28.8	28.7	28.7	28.7
3′	8.9	9.2	8.9	9.2
1″	—	—	54.9	56.3

The assignment was supported by partial decoupling.

in other animals such as dogs, monkeys and chimpanzees the level of compound **7** was considerably higher.

Toxicity

Compound **7** was given to *dd*Y mice and Slc-Wister rats intraperitoneally, subcutaneously and orally, and the acute toxicity was examined (Table 3). As to acute, subacute and chronic toxicities in the rats and dogs, no appreciable abnormalities were noticed in the blood constituents and the pathological observations.

OMOTO *et al.* reported the synthesis of a midecamycin derivative having an acyl group introduced at the C-3″ hydroxyl group, using an acid anhydride which induced 4″ to 3″ acyl migration[5]. However, no methods have been known that enable introduction of the acyl group directly onto the C-3″ hydroxyl group in a high yield. At first the authors tried to use γ-collidine and an acyl chloride, conditions which are generally used for the acylation of a tertiary hydroxyl group. However, the reaction mixture was pigmented and the yield of the C-3″ acyl product was low. Among the various bases tested, tribenzylamine was found to be the best reagent for synthesizing a C-3″-*O*-acyl derivative with a high yield (96%). Although the reaction mechanism using tribenzylamine is not known yet, it is supposed that the insoluble hydrochloride salt of tribenzylamine precipitates during the reaction and is removed from the reaction system.

3″-*O*-Propionylleucomycin A_5 (**7**) prepared by the above method, was selected from a large number of synthetic acyl derivatives[7] in which acylation has taken place on the hydroxyl groups at the C-3, C-9, and C-3″ positions. It showed an *in vitro* antibacterial activity against Gram-positive bacteria about twice as high as that of leucomycin A_5 (**1**), josamycin and midecamycin. In the experiments with animals such as dogs, monkeys and chimpanzees, compound **7** showed serum levels twice or more as high as that of josamycin. Compound **7** did not show any particular acute toxicity, thus being similar to leucomycin and josamycin. These results suggest the usefulness of 3″-propionylleucomycin A_5 (**7**) as a clinical drug. *In vivo* evaluation of compound **7** is now in progress.

Experimental

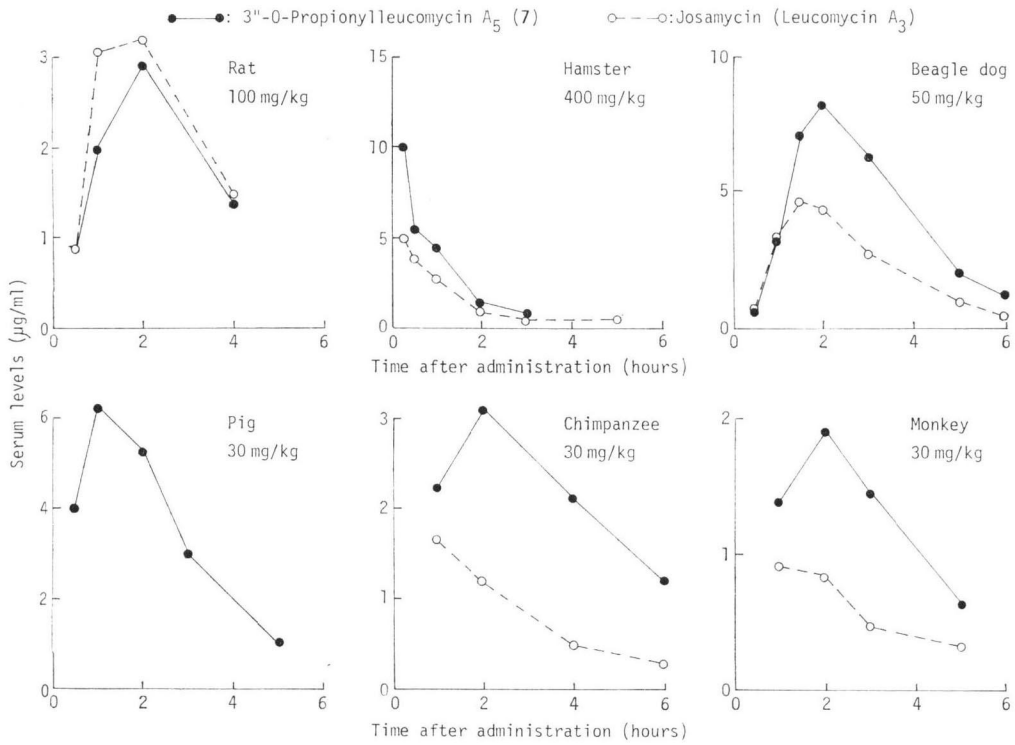
General Methods

Melting points were not corrected. Optical rotations were determined with a Jasco Model DIP-180 polarimeter. UV and IR spectra were recorded on Hitachi Model 323 UV and Model 260-50 IR spectrometers. NMR spectra were measured in $CDCl_3$ using a Jeol JNM-FX100 spectrometer, with

Fig. 6. Serum levels of 3″-*O*-propionylleucomycin A_5 (7) and josamycin in various animals after oral administration.

Determination: bioassay, paper-disk method (*M. luteus*)

Plotting: mean value±S.E. (n)



TMS as an internal standard. Mass spectra were obtained with a Jeol JMS-D300 mass spectrometer. Column chromatography was performed on Merck silica gel 60 (Art. 7734). Rf values were measured by the following thin-layer chromatography. Carrier: TLC plates of silica gel 60 (Art. 5721, E. Merck); developer: A; *n*-hexane - benzene - acetone - ethyl acetate - methanol (90: 80: 25: 60: 30, v/v), B; benzene - acetone (3: 1, v/v), C; benzene - acetone (6: 1, v/v), D; benzene - acetone (13: 1, v/v), E; benzene - ethyl acetate (2: 1, v/v). Spots were detected by spraying with sulfuric acid and heating.

Synthesis of 3″-*O*-Propionylleucomycin A_5 (7)

To leucomycin A_5 (**1**) (10 g) dissolved in dry 1,2-dichloroethane (50 ml) acetic anhydride (4.2 ml) was added and the mixture was stirred for half an hour at room temperature. The reaction mixture was added to ice-water (200 ml), adjusted to pH 10 by adding 7% aqueous ammonia, and then the mixture was extracted with 1,2-dichloroethane (50 ml). The extract was dried over anhydrous magnesium sulfate and concentrated to dryness *in vacuo* to obtain 2′-*O*-acetylleucomycin A_5 (**2**) (10 g, yield, 94.8%).

To 2′-*O*-acetylleucomycin A_5 (**2**) (10 g) dissolved in dry 1,2-dichloroethane (50 ml) tribenzylamine (10 g) and trimethylchlorosilane (6.6 ml) were added and the mixture was stirred for fifteen hours at 5~10°C. The reaction mixture was poured into water (200 ml), adjusted to pH 9.5 with 7% aqueous ammonia, and then extracted with 1,2-dichloroethane (50 ml). The extract was washed with water, dried over anhydrous magnesium sulfate and then evaporated *in vacuo* to obtain crude 2′-*O*-acetyl-3,9-di-*O*-trimethylsilylleucomycin A_5 (**3**) (12.7 g, yield, 96.1%).

To the above crude substance **3** dissolved in dry 1,2-dichloroethane (50 ml) tribenzylamine (31 g) was added, and then propionyl chloride (11.25 ml) was added to it dropwisely under ice-cooling. The

Table 1. Antibacterial spectra of 3''-*O*-propionylleucomycin A_5 (**7**), josamycin (JM) and midecamycin (MDM).

Test organism	10^8 cells/ml (MIC; μg/ml)		
	7	JM	MDM
S. aureus ATCC 6538P	0.8	3.1	1.6
S. aureus MS353	1.6	3.1	3.1
S. aureus MS353 C36	0.8	1.6	1.6
S. aureus 0126 (Mac^r B)*1	0.8	3.1	3.1
S. aureus MS353 AO (Mac^r A)	>100	>100	>100
S. aureus 0116 (Mac^r A)	>100	>100	>100
S. aureus 0119 (Mac^r A)	>100	>100	>100
S. aureus 0127 (Mac^r A)	>100	>100	>100
S. epidermidis sp-al-1	0.4	3.1	3.1
S. pyogenes N.Y. 5	≦0.05	0.1	0.4
S. pyogenes 1022 (Mac^r)	>100	>100	>100
S. agalactiae 1020	0.2	0.4	0.4
S. faecalis 1501	0.8	3.1	3.1
S. pneumoniae NCTC7465*2	0.1	0.2	0.2
M. luteus ATCC 9341	≦0.05	0.1	≦0.2
C. diphtheriae P.W. 8	≦0.05	≦0.05	≦0.2
B. subtilis ATCC 6633	0.4	0.8	1.6
E. coli NIHJ JC-2	>100	>100	>100
S. enteritidis Gaertner	>100	>100	>100
S. sonnei E33	>100	>100	>100
H. influenzae 1326*3	12.5	25	25
M. pneumoniae Mac*4	0.008	0.016	—
M. gallisepticum KP-13*4	0.008	0.032	—
M. hyopneumoniae J*4	0.02	0.16	—
M. pulmonis m53*4	0.4	6.3	—

Media: HIA (Difco)
*1: Reference 9)
*2: BHIA supplemented with 5% horse serum
*3: HIA supplemented with 2% Bacto-Fildes enrichment (Difco)
*4: PPLO broth supplemented with 20% horse serum

mixture was stirred at 75°C for 20 hours. The reaction mixture was poured into water (200 ml), adjusted to pH 9.5 with aqueous ammonia and extracted with 1,2-dichloroethane (100 ml). The extract was dried over anhydrous magnesium sulfate and evaporated to obtain a mixture of 2'-*O*-acetyl-17,18-enol-18,3''-di-*O*-propionyl-3,9-di-*O*-trimethylsilylleucomycin A_5 (**4**), and a small amount of 2'-*O*-acetyl-3''-*O*-propionyl-3,9-di-*O*-trimethylsilylleucomycin A_5 (**5**) and a large amount of tribenzylamine. Methanol (495 ml) and 8% aqueous K_2CO_3 (55 ml) were added thereto, and the mixture was stirred at room temperature for 1 hour yielding 2'-*O*-acetyl-3''-*O*-propionylleucomycin A_5 (**6**). The reaction mixture was adjusted to pH 7.5 with acetic acid and refluxed for 20 hours, and then evaporated *in vacuo*. The residue dissolved in 1,2-dichloroethane was washed once with 3% aqueous ammonia and twice with water, and then evaporated *in vacuo*. The residue was dissolved in cold methanol and the insoluble tribenzylamine was filtered off. The filtrate was evaporated *in vacuo* to obtain crude 3''-*O*-propionylleucomycin A_5 (**7**) (10.4 g, yield, 94.7%), which was dissolved in a small amount of benzene and applied to a silica gel column (1.5 cm×60 cm). Elution was carried out with a mixture of benzene - ethyl acetate - methanol (36: 4: 1, v/v). Each fraction (18 ml) was examined by silica gel thin-layer chromatography and the fractions showing a spot at Rf 0.57 (A) were collected and evaporated *in vacuo* to ob-

Table 2. Antibacterial spectra of 3''-*O*-propionylleucomycin A_5 (7), josamycin (JM) and midecamycin (MDM).

Test organism	10^6 cells/ml (MIC; μg/ml)		
	7	JM	MDM
S. aureus ATCC 6538P	0.2	0.4	0.8
S. aureus MS353	0.4	0.8	3.1
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S. aureus 0126 (Mac^r B)*1	0.4	0.4	0.8
S. aureus MS353 AO (Mac^r A)	>100	>100	>100
S. aureus 0116 (Mac^r A)	12.5	>100	>100
S. aureus 0119 (Mac^r A)	>100	>100	>100
S. aureus 0127 (Mac^r A)	>100	>100	>100
S. epidermidis sp-al-1	0.2	0.4	3.1
S. pyogenes N.Y. 5	≦0.05	≦0.05	≦0.2
S. pyogenes 1022 (Mac^r)	0.4	>100	>100
S. agalactiae 1020	0.2	0.2	0.2
S. faecalis 1501	0.4	0.8	3.1
S. pneumoniae NCTC 7465*2	—	—	—
M. luteus ATCC 9341	≦0.05	≦0.05	≦0.2
C. diphtheriae P.W. 8	≦0.05	≦0.05	≦0.2
B. subtilis ATCC 6633	0.2	0.4	1.6
E. coli HINJ JC-2	>100	>100	>100
S. enteritidis Gaertner	>100	>100	>100
S. sonnei E33	>100	>100	>100
H. influenzae 1326*3	6.3	12.5	12.5
M. pneumoniae Mac*4	—	—	—

Media: HIA (Difco)
*1: Reference 9)
*2: BHIA supplemented with 5% horse serum
*3: HIA supplemented with 2% Bacto-Fildes enrichment (Difco)
*4: PPLO broth supplemented with 20% horse serum

Table 3. Acute toxicity of 3''-*O*-propionylleucomycin A_5 (7).

		LD_{50} (mg/kg)		
		P.O.	S.C.	I.P.
Mice	male	>5,000	>2,000	>2,000
	female	>5,000	>2,000	>2,000
Rats	male	>2,000	>1,000	>1,000
	female	>2,000	>1,000	>1,000

tain the purified product (7.5 g, over all yield, 70%). Rf 0.57 (A), 0.14 (B), mp. *ca.* 116°C; $[\alpha]_D^{20}$ −71° (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} 232 nm (ε 28,000); IR, (in KBr) 3440, 2930, 1750 sh., 1730, 1720, 1425, 1370, 1260, 1190, 1170, 1150, 1120, 1080, 1025, 1020 and 990 sh. cm^{-1}. pKa 6.33 (in 50% EtOH). Mass data are shown in Fig. 2. ^{1}H-NMR, δ 1.44 (s, 3''-CH_3), 2.57 (s, $N(CH_3)_2$), 3.65 (s, OCH_3), 9.86 (s, CHO). ^{13}C-NMR data are shown in Table 1. *Anal.* Calcd. for $C_{42}H_{69}O_{15}N$ (828.00): C, 60.93; H, 8.40; N, 1.69. Found: C, 60.63; H, 8.58; N, 1.77.

Purification of 2'-*O*-Acetyl-3,9-di-*O*-trimethylsilylleucomycin A_5 (3)

A crude powder (5 g) of **3** was applied to a silica gel column (1 cm×60 cm) and eluted with benzene - acetone (20: 1, v/v). The fractions (18 ml) showing a spot at Rf 0.64 (C) were collected and evaporated *in vacuo* to obtain **3** (3.5 g). UV λ_{max}^{EtOH} 232 nm (ε 29,000). IR (in KBr), 3500, 2950, 1735, 1455, 1410, 1370, 1250, 1230, 1170, 1125, 1100, 1055, 1025, 1000, 900, 845, and 750 cm^{-1}. Mass, *m/z*

957 w (M^+), 870 m ($M^+ - C_3H_7COO$), 430 m, 331 w, 216 s, 174 m, 115 s and 109 s. ^{1}H-NMR, δ 0.07 (s, 9-TMS), 0.18 (s, 3-TMS), 2.05 (s, 2'-CH_3CO), 2.41 (s, $N(CH_3)_2$), 3.41 (s, OCH_3), 9.82 (s, CHO). *Anal.* Calcd. for $C_{47}H_{83}O_{15}NSi_2$: C, 58.91; H, 8.73; N, 1.46. Found: C, 59.04; H, 9.00; N, 1.42.

Isolation of 2'-*O*-Acetyl-17,18-enol-18,3''-di-*O*-propionyl-3,9-di-*O*-trimethylsilylleucomycin A_5 (**4**) and 2'-*O*-Acetyl-3''-*O*-propionyl-3,9-di-*O*-trimethylsilylleucomycin A_5 (**5**)

A mixture (3.0 g) of compounds **4** and **5** prepared by the procedure described above was purified by silica gel column chromatography (1.5×75 cm), developing with a mixture of benzene and acetone (40: 1, v/v). The fractions showing a spot at Rf 0.55 (D) were collected, and evaporated to give a chromatographically homogeneous substance **4** (1.0 g). UV λ_{max}^{EtOH} 232 nm (ε 27,600). IR (in KBr) 3470, 2950, 1740, 1458, 1375, 1255, 1235, 1200, 1155, 1135, 1100 sh., 1060, 1030 sh., 1000, 940, 905, 845 and 755 cm^{-1}. Mass, *m/z* 1069 w (M^+), 996 w ($M^+ - C_2H_5COO$), 982 w ($M^+ - C_3H_7COO$), 486 m, 389 w, 388 w, 331 w, 271 w, 183 w, 155 s and 109 s. ^{1}H-NMR, δ 0.05~0.22 (m, 3,9-di-TMS), 1.42 (s, 3''-CH_3), 2.03 (s, 2'-CH_3CO), 2.42 (s, $N(CH_3)_2$), 3.40 (s, OCH_3), no CHO signal. *Anal.* Calcd. for $C_{53}H_{91}O_{17}NSi_2$: C, 59.45; H, 8.57; N, 1.31. Found C, 59.43; H, 8.64; N, 1.16. The fractions showing Rf 0.46 (D) were collected, and evaporated to give compound **5** (0.6 g). UV λ_{max}^{EtOH} 232 nm (ε 27,600). IR (in KBr), 3450, 2950, 1735, 1450, 1370, 1250, 1230, 1200, 1170 sh., 1150, 1125, 1100 sh., 1055, 1030, 995 sh., 900, 842 and 750 cm^{-1}. Mass, *m/z* 1013 w, 940 w ($M^+ - C_2H_5COO$), 926 w ($M^+ - C_3H_7COO$), 486 m, 331 w, 271 w, 197 m, 155 s and 109 s. ^{1}H-NMR, δ 0.06 (s, 9-TMS), 0.18 (s, 3-TMS), 1.41 (s, 3''-CH_3), 2.04 (s, 2'-CH_3CO), 2.42 (s, $N(CH_3)_2$), 3.40 (s, OCH_3), 9.8 (s, CHO), *Anal.* Calcd. for $C_{50}H_{87}O_{16}NSi_2$: C, 59.20; H, 8.64; N, 1.38. Found: C, 59.45; H, 8.94; N, 1.43.

Isolation of 2'-*O*-Acetyl-3''-*O*-propionylleucomycin A_5 (**6**)

A solution of **4** (1 g) in methanol (50 ml) and 8% aqueous K_2CO_3 (5.5 ml) was allowed to stand for 1 hour at room temperature, and then evaporated to dryness. The residue was purified by silica gel column chromatography (1 cm×30 cm), developing with a mixture of benzene and acetone (20: 1, v/v, Rf 0.29 (B)) to yield a white powder of **6** (850 mg). UV λ_{max}^{EtOH} 232 nm (ε 29,400). IR (in KBr) 3480, 2980 sh., 2940, 1730, 1455, 1375, 1235, 1200, 1175 sh., 1155, 1130, 1055, 1030, 920 and 845 cm^{-1}. Mass, *m/z* 869 w (M^+), 796 w ($M^+ - C_2H_5COO$), 782 w ($M^+ - C_3H_7COO$), 599 w, 598 w, 583 w, 486 m, 367 w, 271 w, 197 m, 183 w, 155 s, 129 s and 109 s. ^{1}H-NMR, δ 1.41 (s, 3''-CH_3), 2.03 (s, 2'-CH_3CO), 2.42 (s, $N(CH_3)_2$), 3.46 (s, OCH_3), 9.77 (s, CHO). *Anal.* Calcd. for $C_{44}H_{71}O_{16}N$: C, 60.74; H, 8.22; N, 1.61. Found: C, 60.50; H, 8.44; N, 1.47.

Isolation of 4-*O*-Butyryl-3-*O*-propionyl-L-mycarose (**8**) and its Methyl Glycoside (**9**)

A solution of **7** (1 g) in water (20 ml), methanol (30 ml) and dioxane (10 ml) was treated with Amberlyst 15 (7 ml) at 40°C for 16 hours. The resin was removed by filtration, and the filtrate was concentrated to give a syrup. This was chromatographed on a silica gel column (1.5 cm×30 cm) and developed with benzene - ethyl acetate (4: 1, v/v). The fractions (5 ml) showing Rf 0.79 (E) were collected, and then evaporated to give compound **9** (157 mg). CI-Mass; *m/z* 320 w (MNH_4^+), 303 w (MH^+), 301 w ($M^+ - 1$), 285 w, 271 m ($M^+ - OCH_3$), 229 w ($M^+ - C_2H_5COO$), 215 w ($M^+ - C_3H_7COO$), 197 s ($M^+ - C_2H_5COO - CH_3OH$), 183 w ($M^+ - C_3H_7COO - CH_3OH$), 169 w, 110 m and 109 s (using ammonia as reactant gas). ^{1}H-NMR, α-L-anomer: δ 0.97 (t, 4-$CH_3CH_2CH_2CO$), 1.13 (d, 5-CH_3), 1.42 (s, 3-CH_3), 3.28 (s, 1-OCH_3), 4.18 (dq, H-5), 4.61 (d, H-4), 4.66 (d, H-1), β-L-anomer: δ 0.97 (t, 4-$CH_3CH_2CH_2CO$), 1.18 (d, 5-CH_3), 1.47 (s, 3-CH_3), 3.46 (s, 1-OCH_3), 3.92 (dq, (H-5), 4.58 (d, H-4), 4.47 (dd, H-1). ^{13}C-NMR data are shown in Fig. 5. The white powder obtained from the fractions showing Rf 0.49(E) was a mixture of 4-*O*-butyryl-3-*O*-propionyl-α- and β-L-mycarose (**8**) (140 mg). CI-Mass; *m/z* 306 w (MNH_4^+), 289 w (MH^+), 288 w (M^+), 287 w ($M^+ - 1$), 285 w, 271 m ($M^+ - OH$), 215 w ($M^+ - C_2H_5COO$), 201 w ($M^+ - C_3H_7COO$), 197 s ($M^+ - C_2H_5COO - H_2O$), 1.83 s ($M^+ - C_3H_7COO - H_2O$), 127 w and 109 s. ^{1}H-NMR, α-L-anomer: δ 0.98 (t, 4-$CH_3CH_2CH_2CO$), 1.12 (t, 3-CH_3CH_2CO), 1.13 (d, 5-CH_3), 1.45 (s, 3-CH_3), 1.70 (m, 4-CH_3CH_2-CH_2CO), 1.70 (H-2ax, overlap with 4-$CH_3CH_2CH_2CO$), 2.35 (q, 3-CH_3CH_2CO), 2.37 (t, 4-CH_3CH_2-CH_2CO), 2.66 (dd, 1-OH), 3.19 (dd, H-2 eq), 4.40 (dq, H-5), 4.62 (d, H-4), 5.24 (dd, H-1), $J_{1,2ax}$ 4.0 Hz, $J_{1,2eq}$ 0.7, $J_{2ax,eq}$ 15.0, $J_{4,5}$ 9.8, $J_{5,6}$ 6.1, $J_{1,OH}$ 4.1. β-L-anomer: δ 0.98 (t, 4-$CH_3CH_2CH_2CO$), 1.14 (t,

3-CH_3CH_2CO), 1.17 (d, 5-CH_3), 1.47 (s, 3-CH_3), 1.69 (m, 4-$CH_3CH_2CH_2CO$), 1.70 (H-2ax, overlap with 4-$CH_3CH_2CH_2CO$), 2.34 (q, 3-CH_3CH_2CO), 2.37 (t, 4-$CH_3CH_2CH_2CO$), 3.07 (d, 1-OH), 3.11 (dd, H-2eq), 3.97 (dq, H-5), 4.59 (d, H-4), 4.91 (ddd, H-1). $J_{1,2ax}$ 9.6 Hz, $J_{1,2eq}$ 1.8, $J_{2ax,eq}$ 14.8, $J_{4,5}$ 9.8, $J_{5,6}$ 6.1, $J_{1,OH}$ 6.9. ^{13}C-NMR data are shown in Fig. 5.

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