CHEMICAL MODIFICATION OF DELTAMYCINS
I. 4″-O-ACYL ANALOGS OF DELTAMYCINS

YASUTAKA SHIMAUCHI, KOSUMI HORI, MICHIKO SAKAMOTO (nee HASEGAWA), YOSHIFUMI MUTOH, YASUO FUKAGAWA, SENJI HORI and TOMOYUKI ISHIKURA

Central Research Laboratories, Sanraku-Ocean Co., Ltd., Fujisawa, Japan

JOSEPH LEIN

Panlabs, Inc., Fayetteville, New York, U.S.A.

(Received for publication August 28, 1979)

Using 4″-O-deacyldeltamycin as a starting material, various 4″-O-acyl derivatives were chemically synthesized by the following scheme: 2′-O-acetylation→4″-O-acylation→2′-O-deacetylation. 4″-O-Deacyldeltamycin is produced during the fermentation of deltamycin producing organisms or can be prepared by the biological deacylation of the deltamycins. 4″-O-Phenylacetyl-4″-O-deacyldeltamycin (PAD) showed good activity against various bacteria and mycoplasma. Among *para*-substituted PAD derivatives, NPAD (*p*-nitrophenylacetyl-) and SPAD (*p*-methylsulfonylphenylacetyl-) showed increased potency as compared to PAD against certain bacteria. The PAD was evaluated by *in vivo* experiments for plasma levels in mice and dogs and curative activity on the infected mice with *Staphylococcus aureus* Smith by subcutaneous or oral administration.

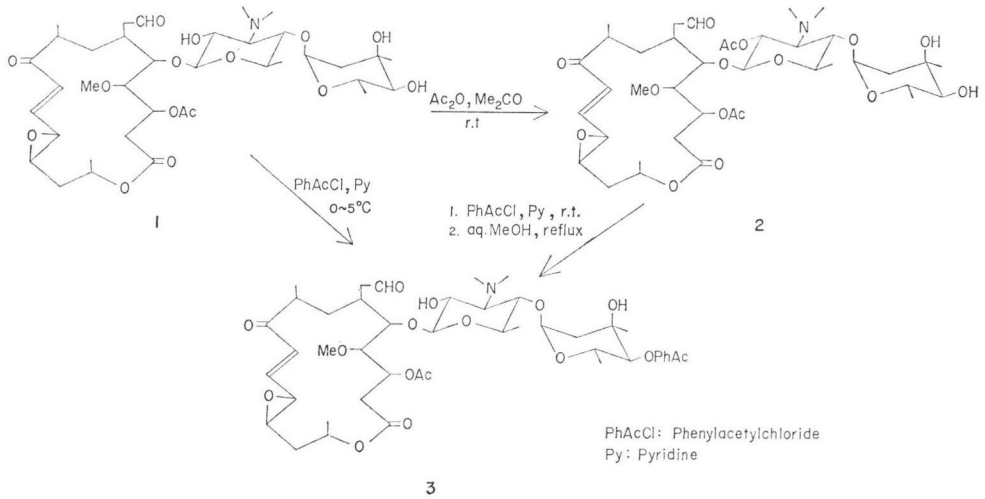
Deltamycins are basic 16-membered macrolide antibiotics produced by *Streptomyces halstedii* subsp. *deltae* KOUNO *et* ISHIKURA. They consist of four components, designated deltamycin (DLM) A_1, A_2, A_3 and A_4[1,2], and are active against Gram-positive bacteria. The chemical structures of these components differ in the acyl group attached to the 4″-O-position in the mycarose moiety[3]. Concerning the antimicrobial activity among DLMs, the most active is DLM A_4 having an isovaleryl ester as the acyl group, while DLM A_1, having an acetyl ester, is relatively weak. It has been previously reported that the 4″-O-ester in similar macrolides is easily hydrolyzed *in vivo*, and the activity is largely lost[4]. A number of DLM derivatives have been prepared by introducing other acyl groups at 4″-O-position. The purpose was to improve the antibiotic activity and to increase the stability of the 4″-O-ester of the original macrolide.

This paper deals with the preparation of DLM 4″-O-acyl analogs and their biological activities.

Preparation Methods of 4″-O-Acyl Derivatives

4″-O-Deacyl-DLM (**1**), a starting material for this series, was prepared from DLM complex by biological conversion with deacylase of *Corynespora cassiicola etc*[6] or obtained from the fermentation broth as one of DLM components under fermentation conditions suitable for 4″-O-deacyl-DLM production. Selective protection of 2′-hydroxyl group of **1** by acetylation was carried out with acetic anhydride in dry benzene, acetone or dichloromethane. After the starting material had been blocked, acylations at 4″-hydroxyl group of 2′-O-acetyl-4″-O-deacyl-DLM (**2**) was accomplished by using acyl halides in the presence of pyridine or trimethylamine and, if necessary, by the addition of dry benzene or dichloromethane as diluent. Elimination of the acetyl group at the 2′-O-position was accomplished by refluxing in methanol or methanol - water[7]. As another useful route, direct and selective 4″-O-

Scheme 1.



acylation of **1** took place with acyl halides in dry pyridine or trimethylamine at temperatures below 5°C. The two synthetic routes for 4″-O-phenylacetylation are shown in Scheme 1.

Structure-antimicrobial Activity Relationships

In preliminary experiments, the 4″-O-phenylacetyl-derivative (**3**) was found to be more active than DLM A_4 (carbomycin A). To observe a correlation between the antibiotic activity and methylene chain length of phenylalkanoyl group in the PAD homologs, 4″-O-benzyl- (**13**), 4″-O-phenylacetyl-, 4″-O-hydrocinnamoyl- (**14**) and 4″-O-phenylbutyryl-derivative (**15**) were synthesized. The minimal

Table 1. Antibacterial spectra of PAD derivatives and others.

Compound	MIC (μg/ml)								
	S. aureus FDA 209P	*S. aureus* Smith	*S. aureus* Smith*	*S. aureus* (TC, EM, LM, CP)^r	*S. aureus* (PcG, TC, EM, LM)^r	*E. coli* K-12	*Streptococcus pneumoniae***	*Str. pyogenes***	*Sarcina lutea*
3	0.2	0.4	0.4	>100	100	100	0.2	0.4	<0.05
4	0.2	0.2	0.2	>100	100	50	<0.2	0.2	<0.025
5	0.4	0.8	0.8	>100	>100	50	<0.2	<0.2	0.05
6	0.8	3.2	1.6	>100	>100	>100	0.2	0.4	0.2
7	0.2	0.4	0.4	>100	>100	50	<0.2	<0.2	0.05
8	0.2	0.2	0.2	>100	100	50	0.2	0.2	0.1
9	0.4	0.8	0.8	>100	100	50	0.2	0.2	0.025
10	0.4	0.4	0.4	>100	>100	50	0.1	0.2	0.05
11	0.4	0.8	0.4	>100	>100	100	0.2	0.4	0.05
12	<0.2	0.4	0.4	>100	>100	100	0.2	0.2	0.05
13	0.8	1.6	1.6	>100	>100	>100	0.8	0.4	—
14	0.4	0.4	0.4	>100	>100	100	0.2	0.8	—
15	0.4	0.8	0.8	>100	>100	>100	0.4	0.8	—
DLM A_4	0.2	1.6	1.6	>100	>100	100	0.2	0.4	0.05

*: 25% horse serum, **: 10% horse blood.

inhibitory concentrations (MICs) of the derivatives, determined by the serial broth dilution method in brain heart infusion media, are shown in the lower columns of Table 1.

Since **3** (PAD) gave the lowest MIC value, a series of analogs having substituents in the benzene ring of **3** were synthesized. Various derivatives having *para*-substituents on the phenylacetyl function were selected on the basis of electronegativity[5]. The physicochemical properties and analytical data of the 12 analogs are summarized in Table 2.

MICs of **3** against a wide variety of bacteria were determined and the results are shown in Table 3. Josamycin and DLM A_4 were used as reference antibiotics. It is obvious that **3** is the most active

Table 2. Chemical properties of deltamycin derivatives.

No.	Compound	Yield (%)	Melting point (°C)	TLC (Rf)		$[\alpha]_D^{21}$ (c 0.5, $CHCl_3$)	MS (M^+) (m/e)
				Solvent a*	Solvent b*		
3	PAD	54.6	188~190 (dec.)	0.58	0.56	−60.9	875
4	NPAD	26.0	230~234 (dec.)	0.52	0.53	−59.2	920
5	OPAD	37.7	210~214 (dec.)	0.39	0.18	−53.8	891
6	DPAD	35.1	231~232 (dec.)	0.48	0.52	−60.8	935
7	CPAD	11.7	206~207 (dec.)	0.58	0.56	−60.7	909
8	SPAD	27.9	160~166 (dec.)	0.38	0.40	−50.2	953
9	FPAD	15.0	141~145 (dec.)	0.62	0.58	−51.7	943
10	LPAD	29.6	187~192 (dec.)	0.55	0.53	−39.3	893
11	MPAD	7.8	222~225 (dec.)	0.54	0.53	−45.6	905
12	YPAD	22.7	146~153 (dec.)	0.49	0.47	−38.7	900
13	BZD	30.2	179~183 (dec.)	0.66	0.58	−49.3	861
14	HCD	21.5	192~196 (dec.)	0.53	0.52	−54.9	889

No.	Compound	Molecular Formula	Elemental analysis					
			Calcd.			Found		
			C	H	N	C	H	N
3	PAD	$C_{45}H_{65}NO_{16}\cdot\frac{1}{2}C_6H_6$	63.00	7.49	1.53	62.83	7.54	1.41
4	NPAD	$C_{45}H_{64}N_2O_{18}\cdot\frac{1}{6}C_6H_6$	59.15	7.02	2.99	59.21	7.07	2.86
5	OPAD	$C_{45}H_{65}NO_{17}\cdot\frac{1}{2}H_2O$	59.99	7.38	1.55	59.88	7.16	1.31
6	DPAD	$C_{47}H_{69}NO_{18}\cdot\frac{1}{2}H_2O$	59.73	7.46	1.48	59.85	7.35	1.30
7	CPAD	$C_{45}H_{64}NO_{16}Cl$	59.37	7.09	1.54	59.38	7.13	1.35
8	SPAD	$C_{46}H_{67}NO_{18}S\cdot\frac{3}{4}H_2O$	57.14	7.06	1.45	57.03	7.06	1.40
9	FPAD	$C_{46}H_{64}NO_{16}F_3\cdot\frac{1}{2}H_2O$	57.97	6.87	1.47	57.98	6.68	1.36
10	LPAD	$C_{45}H_{64}NO_{16}F\cdot\frac{1}{2}H_2O$	59.85	7.25	1.55	59.73	7.15	1.78
11	MPAD	$C_{46}H_{67}NO_{17}$	60.98	7.45	1.55	60.89	7.44	1.30
12	YPAD	$C_{46}H_{64}N_2O_{16}\cdot H_2O$	60.11	7.23	3.05	59.92	6.89	3.35
13	BZD	$C_{44}H_{63}NO_{16}\cdot\frac{1}{2}H_2O$	60.68	7.41	1.61	60.64	7.58	1.36
14	HCD	$C_{46}H_{67}NO_{16}\cdot\frac{3}{2}H_2O$	60.25	7.69	1.53	60.28	7.28	1.70

Abbreviations: PAD, 4″-O-deacyl-4″-O-phenylacetyl-DLM; NPAD, 4″-O-(*p*-nitrophenylacetyl)-; OPAD, 4″O-(*p*-hydroxyphenylacetyl)-; DPAD, 4″-O-(3,4-dimethoxyphenylacetyl)-; CPAD, 4″-O-(*p*-chlorophenylacetyl)-;SPAD, 4″-O-(*p*-methysulfonylphenylacetyl)-; FPAD, 4″-O-(*p*-trifluorophenylacetyl)-; LPAD, 4″-O-(*p*-fluorophenylacetyl)-; MPAD, 4″-O-(*p*-methoxyphenylacetyl)-; YPAD, 4″-O-(*p*-cyanophenylacetyl)-; BZD, 4″-O-benzyl-; HCD, 4″-O-hydrocinnamoyl-.

* Solvent a = C_6H_6 - Me_2CO (1 : 1), Solvent b = $CHCl_3$ - MeOH (20 : 1).

Table 3. Antibacterial spectra of PAD.

Test organism	MIC (μg/ml)		
	PAD	DLM A_4	JM
Staphylococcus aureus FDA 209P	0.2	0.4	0.2
" " Smith	0.4	0.8	1.6
" " (STH)^r	0.2	0.2	0.2
" " (EM)^r	0.2	0.4	0.4
" " (SPM)^r	0.8	0.8	3.1
" " (SM, STM)^r	0.2	0.2	0.2
" " (PcG, KM, NM)^r	0.4	0.8	0.8
" " (PcG)^{r}B	0.2	0.4	0.8
" " (PcG)^{r}R	0.4	0.4	0.8
" " (SM, CP, TC, EM, LM)^r	>100	>100	>100
" " (EM, OM, PcG, TC)^r 1	0.2	0.4	0.8
" " (")^r 2	0.4	0.8	0.8
" " (EM, OM)^r	0.4	0.4	0.4
" " (EM, OM, CP, TC)^r	>100	>100	>100
" " (TC, CP, PcG)^r	0.4	0.4	0.4
Escherichia coli K-12	100	100	100
*Streptococcus pneumoniae**	0.2	0.4	0.4
*Streptococcus pyogenes**	0.4	0.4	0.4
Mycobacterium smegmatis ATCC 607**	12.5	12.5	12.5
Bacillus subtilis ATCC 6633	0.2	0.2	0.4
Sarcina lutea A9852	0.2	0.2	0.2
Klebsiella pneumoniae ATCC 10031	3.1	6.2	6.2
Salmonella gallinarum ATCC 9184	100	100	100
Shigella sonnei	100	100	100

Brain heart infusion broth was used as medium for all strains.

* Horse blood (25%) was added to the medium.

** Glycerin (10%) was added to the medium.

Abbreviations: josamycin, JM; erythromycin, EM; oleandomycin, OM; streptothricin, STH; tetracycline, TC; leucomycin, LM; streptomycin, SM; chloramphenicol, CP; spiramycin, SPM; penicillin G, PcG; neomycin, NM; kanamycin, KM; ( )^r, strain resistant to ( ).

of the three macrolides.

MICs of PAD derivatives having *para*-substituents on the phenylacetyl function are listed in the upper columns of Table 1. The derivatives having electron-withdrawing functionality at the *para*-position, NPAD (*p*-nitro-, **4**), SPAD (*p*-methylsulfonyl, **8**), FPAD (*p*-trifluoromethyl-, **9**) and CPAD (*p*-chloro-, **7**), demonstrated the best antibiotic activity.

In addition to the antibacterial activities of PAD analogs, the antimycoplasmal activity of **3** and **4** and MICs of **3** against various types of *M. gallisepticum* were determined. These results are shown in Tables 4 and 5 along with those of tylosin and erythromycin as reference antibiotics. Compound **3** and **4** were as active, or more active than tylosin.

In Vivo Activities

The concentration of **3** in plasma after oral administration was determined in mice and dogs. In the case of mice, the antibiotic was orally administered at the calculated dose of 200 mg per kg. Blood samples were withdrawn from the eye at 0.5, 1.0, 1.5, 2.0 and 2.5 hours after the administration.

Table 4. Antimycoplasmal spectra of PAD and NPAD in comparison with reference antibiotics.

	MIC (μg/ml)						
	Mycoplasma gallisepticum S6	*M. pneumoniae* Mac	*M. pulmonis* PG22	*M. fermentans*	*M. hominis* PG21	*M. salivarium* ATCC 14277	*M. agalactiae*
Medium	I	I	I	I	II	II	I
PAD	0.016	<0.05	2.5	0.1	1.25	25.0	10.0
NPAD	0.016	<0.05	1.25	0.1	1.25	12.5	10.0
DLM A_4	0.004	0.10	0.31	0.16	1.25	0.78	2.5
" A_3	0.020	<0.10	25.0	0.20	0.39	0.39	5.0
" A_2	0.039	0.10	>50.0	0.78	1.56	0.78	5.0
" A_1	0.039	<0.10	25.0	0.20	0.78	0.78	5.0
" X	0.310	0.31	>50.0	3.13	50.0	25.0	>100
EM	0.390	0.10	25.0	>25.0	>100	>100	>100
TYL	0.039	0.10	0.20	0.10	3.13	12.5	10.0

Medium I: Difco PPLO broth w/o CV+10% glucose.
Medium II: " +10% arginine.
TYL: tylosin

Table 5. MIC against various *Mycoplasma gallisepticum* of PAD.

Test organism	MIC (μg/ml)		
	TYL	EM	PAD
M. gallisepticum TS-18	0.020	0.020	0.039
" " SP-23	0.020	0.020	1.25
" " A-72	>50	>50	>50
" " E-5	3.13	>50	25
" " A-69	6.25	>50	6.25
" " A-68	3.13	>50	3.13
" " E-11	12.5	>50	>50
" " E-40	0.313	0.078	0.313
" " S-4A	0.313	5.0	0.313
" " Chiba-3T	0.020	0.039	<0.010
" " A-76	<0.010	<0.010	0.020

Medium: Difco PPLO w/o CV+1% glucose +10% yeast extract solution (25%)+20% hourse serum+penicillin G K-salt 500 u/ml+Tl acetate 500 μg/ml+0.005% PR indicator.

The plasma obtained by centrifugation was bioassayed on *Sarcina lutea*. The results are shown in Fig. 1 along with those of DLM A_4.

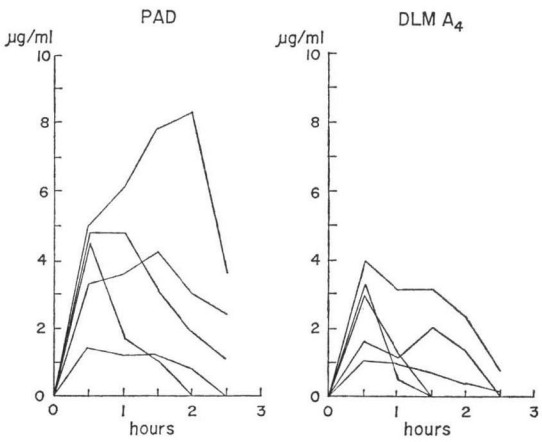
In dogs, the antibiotic was administered at the calculated dose of 25 mg per kg through a gastric tube inserted into the dog's stomach. Blood samples were taken at 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 hours after the administration. The plasma samples were bioassayed on the above-mentioned test organism. The results are shown in Fig. 2 along with those of josamycin.

Inactivation of **3** in rat liver homogenate was studied together with those of DLM A_1, A_2, A_3 and A_4. After being contacted with a fixed amount of the homogenate for 3 hours at 37°C, each antibiotic was extracted with ethylacetate. The organic layer was evaporated under reduced pressure, the residue dissolved in methanol and quantitatively spotted on silica gel TLC. From the densitogram,

after development and coloration with 10% H_2SO_4, it was found that the remaining % of each antibiotic was as follows: **3** 16.1%; A_1, 2.8%; A_2, 1.1%; A_3, 0.4%; A_4, 5.3%. The ester bond at 4″-O-position of **3** resists to hydrolyze *in vivo* better than those of DLMs. This finding is consistent with the blood level data of **3** and DLM A_4.

The chemotherapeutic activity of **3** was tested on infected mice with the pathogen *S. aureus* Smith being used intraperitoneally at a dose of $LD_{50} \times 100$. The antibiotic was subcutaneously or orally administered immediately after the bacterial challenge. As shown in Table 6, **3** showed activity equivalent to josamycin.

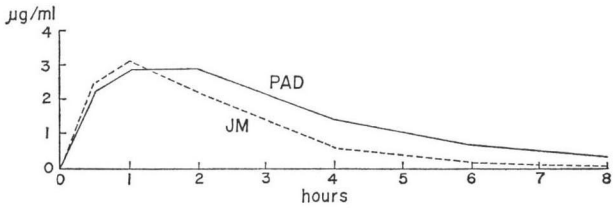
Fig. 1. Blood levels of PAD in mice.
Animal: mouse, ddy, male, Shizuoka
Dose: 200 mg/kg, P.O.
Assay: disc bioassay with *Sarcina lutea*.



Toxicity

The acute toxicity of **3** was tested in mice which had received intraperitoneally 1,000 mg per kg of **3** and all were surviving after 10 days. Therefore, the LD_{50} of **3** was greater than 1,000 mg per kg.

Fig. 2. Blood levels of PAD in dogs.
Animal: mongrel dogs.
Dose: 25 mg/kg, P.O.
Assay: disc bioassay with *Sarcina lutea*.
Data are average of 6 dogs.



Experimental

Melting points were measured by the KOFLER method and were uncorrected. IR absorption spectra were measured with a Hitachi EPI-G_2 spectrophotometer, UV absorption spectra with a Hitachi EPS-3T spectrophotometer, PMR spectra with a JEOL JNM PS-100 spectrometer, mass spectra with a Hitachi RMU-6M mass spectrometer and optical rotation values with a JASCO DIP-180 automatic polarimeter.

Table 6. CD_{50} of PAD in mice.

Compound	200 mg/kg (S.C.)	200 mg/kg×2 (P.O.)
PAD	22	150
JM	13	150
DLM A_4	60	150

Pathogen: *S. aureus* Smith
Animal: mouse, ddy, male, Shizuoka.

Minimal inhibitory concentrations against bacteria

The MIC values of the 4″-O-acyl derivatives were determined by two-fold tube dilution method in Brain heart infusion broth (BHI, Nissui Pharmaceuticals) of pH 7.5. The test organisms were grown in Trypto-soy broth (TSB, Eiken Chemicals) for 24 hours and diluted with saline to produce a final bacterial population in each tube of 10^6 colony forming unit per ml. The MIC was determined as the lowest concentration of drug that prevented macroscopic growth after incubation of 18 hours at 37°C. For *Streptococcus pyogenes* and *Streptococcus pneumoniae*, TSB and BHI were supplmented with 10% defibrinated horse blood.

Minimal inhibitory concentrations against mycoplasma

The liquid used for the growth and the MIC determination consisted of 7 parts of PPLO broth (Difco), 2 parts of unheated horse serum, 1 part of 25% yeast extract prepared from Nitten dry yeast (Nippon Beet Sugar Manufacturing) according to the method described by CHANOCK *et al.*[8] fortified with 500 units per ml of penicillin G and 500 μg/ml of thallium acetate and added with 1% of glucose and 0.002% of phenol red as the pH indicator by growth of mycoplasma. The glucose was replaced by L-arginine when culturing *Mycoplasma hominis* and *M. salivarium*. Each antibiotic was first dissolved in a small amount of MeOH and then sterile, distilled water was added. Serial two-fold dilutions of each drug were prepared by CHANOCK's liquid medium or PPLO enrichment broth. The test organisms were grown in the above-mentioned medium and diluted to produce a final mycoplasmal population in each tube of 10^6 colony forming unit per ml. Cultures were incubated at 37°C for 5 days. During the period of incubation, it was examined daily for change in pH by observing, for comparison, uninoculated and inoculated media containing no drugs. The lowest concentration of a drug inhibiting the color change completely was regarded as the MIC of the drug.

Deacylation test of PAD by rat liver homogenate

Fresh rat liver (9.2 g) was homogenized in 46 ml of sucrose-Tris buffer (pH 7.6) (0.25 M sucrose, 0.01 M tris-HCl buffer and 0.01 M Mg^{++} solution) and filtered through several sheets of gause. The liver homogenate (5.0 ml) was mixed with **3** in MeOH (1 mg/0.5 ml) and 0.1 M pH 7.0 phosphate buffer (10 ml) and shaken on a incubator at 37°C. The reaction mixture (5 ml) was taken out with a pipette, adjusted to pH 9.0 with a drop of aqueous 1 N NaOH and extracted with EtOAc (5 ml). The solvent layer was dried with anhydrous Na_2SO_4, evaporated to dryness and taken up in 0.2 ml of MeOH. The MeOH solution (5 μl) was spotted on a pre-coated TLC plate (Kiesel gel 60 F_{254}, E. Merck). The TLC plate was developed with a solvent system of $CHCl_3$ - MeOH (10: 1), impregnated in 10% aqueous H_2SO_4 and heated at 80°C uniformly. From the standard curve of **3** on the TLC plate, the remaining concentration of **3** by rat liver homogenate was determined. In a similar manner, the deacylation test of DLM A_1, A_2, A_3 and A_4 was carried out.

2′-O-Acetyl-4″-O-deacyl-DLM, (2′-O-acetyl-DLM X) (**2**)

1 (2.6 g) [DLM X: PMR ($CDCl_3$) δ1.18~1.38 (15H, $-CH_3$), 2.22 (3H, s, $-OCOCH_3$), 2.52 (6H, s, $-N(CH_3)_2$), 3.57 (3H, s, $-OCH_3$), 6.68~6.80 (2H, m, -CH=CH-), 9.63 (1H, s, -CHO); MS M^+, (*m/e*) 757; *Anal.* Calcd. for $C_{37}H_{59}NO_{15}\cdot\frac{5}{4}H_2O$: C, 56.94, H, 7.78, N, 1.79, Found: C, 56.97, H, 7.66 N, 1.50] was dissolved in 25 ml of dry acetone and 1.7 ml of acetic anhydride was added with cooling to 0~5°C and stirring. After reaction with stirring at room temperature for 10 hours was completed, the solvent was distilled off *in vacuo* at 35°C and 300 ml of ice-water was added to the residue. After the aqueous suspension was adjusted to pH 3.0 by the addition of 5% aqueous HCl, it was washed with 30 ml of benzene and the aqueous layer was adjusted to pH 9.0 by the addition of 5% aqueous $NaHCO_3$ and was extracted twice with 200 ml of benzene. The benzene layer was dried and evaporated to dryness *in vacuo*. Crystallization of the residue from benzene - *n*-hexane gave 1.7 g of crystalline **2**. Mp 188~189°C; $[\alpha]_D^{21}$ −77.9° (*c* 0.5, $CHCl_3$); UV (MeOH) nm (log ϵ) 240 (4.16); PMR ($CDCl_3$) δ 2.06 (3H, s, $-OCOCH_3$), 2.22 (3H, s, $-OCOCH_3$); IR (KBr) cm^{-1} 1240 ($-OCOCH_3$); MS M^+ (*m/e*) 799.

Anal. Calcd. for $C_{39}H_{61}NO_{16}\cdot\frac{1}{4}H_2O$: C, 58.23, H, 7.68, N, 1.74
Found: C, 58.20, H, 7.79, N, 1.35

4″-O-Phenylacetyl-4″-O-deacyl-DLM, (PAD) (**3**)

Compound **2** (1.7 g) was dissolved in 15 ml of dry pyridine and 1.5 g of phenylacetylchloride were added dropwise with stirring and ice-cooling. After stirring for 3 hours at room temperature, the reaction mixture was poured into 300 ml of ice-water. After sufficient mixing, the aqueous suspension was adjusted to pH 9.0 by the addition of aqueous 5% NaOH and was extracted twice with 250 ml of benzene. The benzene layer was washed twice with 50 ml of aqueous 2% $NaHCO_3$, followed by drying and evaporation to a solid. The residue obtained was dissolved in MeOH and chromatographed on a Sephadex LH-20 column (100~200 mesh, 6.5×33.0 cm). The eluate was fractionated and each fraction was monitored by TLC. The fractions containing the desired product were collected and

evaporated to dryness. The residue of crude 2'-O-acetyl-PAD was dissolved in 50 ml of MeOH and refluxed for 6 hours to complete the deacetylation reaction. After the solvent was distilled off, 100 ml of ice-water was added to the cooled residue. The aqueous suspension was adjusted to pH 2.5 by the addition of aqueous 5% HCl and was washed twice with 200 ml of benzene to remove impurities. The aqueous layer was adjusted to pH 9.0 by the addition of aqueous 5% NaOH and was extracted twice with 400 ml of benzene. The solvent layer was dried and evaporated to dryness. Crystallization of the residue from the mixed solvent of benzene - *n*-hexane gave 1.02 g of **3**. UV (MeOH) nm (log ϵ) 240 (4.19); IR (KBr) cm^{-1} 1605, 1500 (Ph); PMR ($CDCl_3$) δ 3.72 (2H, s, $PhCH_2$), 7.31 (5H, PhH).

PAD through selective 4''-O-phenylacetylation (**3**)

To a solution of 200 mg of **1** in 1.0 ml of anhydrous pyridine, 0.1 ml of phenylacetylchloride was added dropwise with stirring and ice-cooling. After stirring for 5 hours in a range of 0~5°C, the reaction mixture was poured into 30 ml of ice-water. By the same manner as described in the above experimental, 54 mg of crystalline **3** was obtained.

4''-O-(*p*-Substituted-phenylacetyl)-4''-O-deacyl-DLM (**4**~**12**)

General procedure: Compound **2** (1.0 g, 1.25 m mol.) was dissolved in 10 ml of dry pyridine and *p*-substituted-phenylacetylchloride (5.0 m mol.) was added dropwise with stirring and ice-cooling. After stirring for 3~16 hours at room temperature, the reaction mixture was poured into 200 ml of ice-water. The aqueous suspension was adjusted to pH 9.0 and was extracted with 300 ml of benzene. By the same manner as described in the **3** synthesis, *p*-substituted-PAD derivatives were obtained.

4''-O-(*p*-Nitrophenylacetyl)-4''-O-deacyl-DLM, (NPAD) (**4**)

UV (MeOH) nm (log ϵ), 242 (4.19); IR (KBr) cm^{-1}, 1530 ($-NO_2$), 1355 ($-NO_2$); PMR ($CDCl_3$) δ 3.80 (2H, s, $PhCH_2-$), 7.42, 8.10 (each 2H, each d, J=8, PhH).

4''-O-(*p*-Hydroxyphenylacetyl)-4''-O-deacyl-DLM, (OPAD) (**5**)

From **2** and *p*-methoxycarbonyloxy-phenylacetylchloride was obtained 4''-O-(*p*-methoxycarbonyloxyphenylacetyl)-2'-O-acetyl-DLM X. The intermediate was refluxed in MeOH and the solvent was evaporated to dryness. After treating the powder with 5 ml of 0.4% NaOH-MeOH solution at 20°C for 5 minutes, 0.42 g of **5** was obtained by crystallization from benzene - *n*-hexane. UV (MeOH) nm (log ϵ), 232 (4.26); IR (KBr) cm^{-1}, 3550 (–OH), 1600 (Ph), 1520 (Ph); PMR ($CDCl_3$) δ 3.56 (2H, s, $PhCH_2-$), 6.66, 7.06 (each 2H, each d, J=8, PhH).

4''-O-(3,4-Dimethoxyphenylacetyl)-4''-O-deacyl-DLM, (DPAD) (**6**)

UV (MeOH) nm (log ϵ), 236 (4.29); IR (KBr) cm^{-1}, 1600 (Ph), 1520 (Ph), 1085 (CH_3O-); PMR ($CDCl_3$) δ 3.59 (2H, s, $PhCH_2-$), 3.82 (6H, s, $(CH_3O)_2Ph-$), 6.73~6.79 (3H, m, PhH).

4''-O-(*p*-Chlorophenylacetyl)-4''-O-deacyl-DLM, (CPAD) (**7**)

UV (MeOH) nm (log ϵ), 226 (4.22), 240 (sh); IR (KBr) cm^{-1}, 1500 (Ph), 1095 (Ph–Cl); PMR ($CDCl_3$) δ 3.64 (2H, s, $PhCH_2-$), 7.22 (4H, d, PhH).

4''-O-(*p*-Methylsulfonylphenylacetyl)-4''-O-deacyl-DLM, (SPAD) (**8**)

UV (MeOH) nm (log ϵ), 227 (4.31), 244 (sh); IR (KBr) cm^{-1}, 1600 (Ph), 1305 ($-SO_2-$), 1150 ($-SO_2-$); PMR ($CDCl_3$) δ 3.82 (2H, s, $PhCH_2-$), 7.52, 7.80 (each 2H, each d, J=8, PhH).

4''-O-(*p*-Trifluoromethylphenylacetyl)-4''-O-deacyl-DLM, (FPAD) (**9**)

UV (MeOH) nm (log ϵ), 241 (4.18); IR (KBr) cm^{-1}, 1325 ($-CF_3$); PMR ($CDCl_3$) δ 3.77 (2H, s, $PhCH_2-$), 7.42, 7.58 (each 2H, each d, J=8, PhH).

4''-O-(*p*-Fluorophenylacetyl)-4''-O-deacyl-DLM, (LPAD) (**10**)

UV (MeOH) nm (log ϵ), 240 (4.15); IR (KBr) cm^{-1}, 1605 (Ph), 1510 (Ph), 695 (Ph-F); PMR ($CDCl_3$) δ 3.68 (2H, s, $PhCH_2-$), 6.95, 7.12 (each 2H, each d, J=8, PhH).

4''-O-(*p*-Methoxyphenylacetyl)-4''-O-deacyl-DLM, (MPAD) (**11**)

UV (MeOH) nm (log ϵ), 231 (4.36); IR (KBr) cm^{-1}, 1580 (Ph), 1510 (Ph), 1080 (CH_3O-); PMR ($CDCl_3$) δ 3.63 (2H, s, $PhCH_2-$), 3.77 (3H, s, CH_3O-), 6.84, 7.22 (each 2H, each d, J=8, PhH).

4''-O-(*p*-Cyanophenylacetyl)-4''-O-deacyl-DLM, (YPAD) (**12**)

UV (MeOH) nm (log ϵ), 233 (4.47), 237 (4.46); IR (KBr) cm^{-1}, 2230 (-CN), 1505 (Ph); PMR ($CDCl_3$) δ 3.78 (2H, s, $PhCH_2$-), 7.43, 7.62 (each 2H, each d, J=8, PhH).

4''-O-Benzyl-4''-O-deacyl-DLM, (BZD) (**13**)

UV (MeOH) nm (log ϵ), 233 (4.32); IR (KBr) cm^{-1}, 1600 (Ph), 1490 (Ph).

4''-O-Hydrocinnamoyl-4''-O-deacyl-DLM, (HCD) (**14**)

UV (MeOH) nm (log ϵ), 241 (4.15); IR (KBr) cm^{-1}, 1600 (Ph), 1495 (Ph).

Acknowledgement

The authors wish to express their thanks to Prof. Y. YAMADA of Tokyo College of Pharmacy, Tokyo, for his helpful advice and to Miss Y. ISOBE of our laboratories, for her technical assistance.

References

1) SHIMAUCHI, Y.; K. OKAMURA, A. KOKI, M. HASEGAWA, M. DATE, Y. FUKAGAWA, K. KOUNO, T. ISHIKURA & J. LEIN: Deltamycins, new macrolide antibiotics. I. Producing organism and fermentation. J. Antibiotics 31: 261~269, 1978
2) SHIMAUCHI, Y.; K. KUBO, K. ŌSUMI, K. OKAMURA, Y. FUKAGAWA, T. ISHIKURA & J. LEIN: Deltamycins, new macrolide antibiotics. II. Isolation and physicochemical properties. J. Antibiotics 31: 270~275, 1978
3) SHIMAUCHI, Y.; K. KUBO, K. ŌSUMI, K. OKAMURA, Y. FUKAGAWA, T. ISHIKURA & J. LEIN: Deltamycins, new macrolide antibiotics. III. Chemical structures. J. Antibiotics 32: 878~883, 1979
4) TACHIBANA, A.; M. SHIBATA, F. KUMAGAI, K. MORIYAMA, K. YANO & T. OSONO: Studies on josamycin propionate. II. Distribution and metabolism. Jap. J. Antibiotics 26: 122~129, 1973
5) HAHN, F. E.; J. E. HAYES, C. L. WISSEMAN, Jr., H. E. HOPPS & J. E. SMADEL: Mode of action of chloramphenicol. VI. Relation between structure and activity in the chloramphenicol series. Antibiot. & Chemoth. 6: 531~543, 1956
6) MUTOH, Y. *et al.*: Manufacture of 4''-deacyldeltamycin. Japan Patent, Showa 53-15,159, 1978
7) UZU, K.; H. TAKAHIRA, H. KATO, K. SUGIURA & S. ISHII: The studies on spiramycin derivatives. New spiramycin ester, acetylspiramycin. *in* "Progress in Antimicrobial and Anticancer Chemotherapy (Proc. 6th Internat. Congr. Chemother.) Vol. 1, pp. 49~55, 1970
8) CHANOCK, R. H.; L. HAYFLICK & M. F. BARILE: Growth on artificial medium of an agent associated with a typical pneumonia and its identification. Proc. Nat. Acad. Sci. 48: 41~49, 1962