STUDIES ON LANKACIDIN-GROUP (T-2636) ANTIBIOTICS

VII. STRUCTURE-ACTIVITY RELATIONSHIPS OF LANKACIDIN-GROUP ANTIBIOTICS

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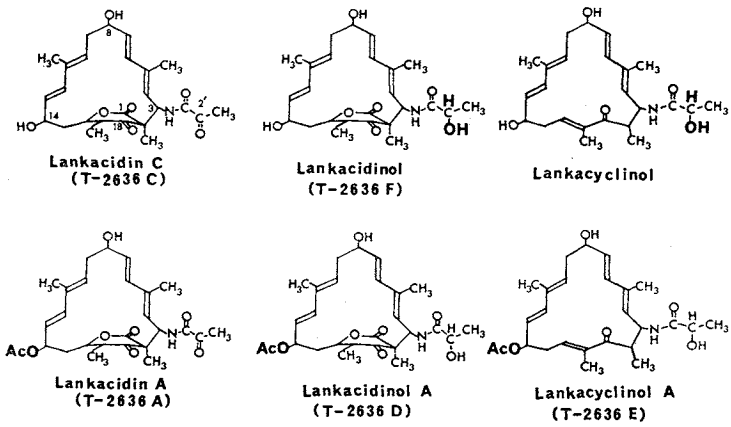
The lankacidin-group (T-2636) antibiotics, neutral seventeen-membered macrocyclic antibiotics, are mainly active against Gram-positive bacteria. Chemical modifications of certain functions of lankacidin C has provided a group of lankacidin C analogs and derivatives. These new antibiotics were examined for their *in vitro* antimicrobial activities, their therapeutic effects against experimental infections in mice, and for their acute toxicities in mice and subacute toxicities in rats.

Some of the lankacidin C mono-esters were found to be superior in their biological properties to the parent antibiotic.

The antibiotics, lankacidin A, lankacidin C, lankacidinol A, lankacyclinol A, lankacidinol* and lankacyclinol** have been isolated from *Streptomyces rochei* var. *volubilis* and their chemical structures have been determined (see Chart 1.[1~6]).

Chart 1. Chemical structures of lankacidin-group antibiotics isolated from the culture filtrate of *Streptomyces rochei* var. *volubilis*.



Among these antibiotics, lankacidin C shows the strongest antimicrobial activities *in vitro* against Gram-positive bacteria, mycoplasma, *Neisseria gonorrhoeae, Vibrio cholerae, Xanthomonas oryzae* and clinically isolated staphylococci. Lankacidin A and lankacidinol are less active than lankacidin C.[2,4,7] The remaining members have very poor activity. Lankacidins A and C administered orally

* The names of these antibiotics have been tentatively designated as T-2636 A, C, D, E and F, respectively.

** The metabolite described in the following paper.

show strong protective effect in mice infected with *Staphylococcus aureus* 308 A-1 (intraperitoneal infection). Lankacidinol A and lankacidinol have low effectivity when administered intraperitoneally.[7] Lankacidins A and C are low toxic substances in acute toxicity but show a slight depression of body weight gain in the experiments of rat subacute toxicity.

This paper deals with the synthesis and biological evaluation of lankacidin C derivatives. These derivatives were prepared with the aim of obtaining more effective derivatives with decreased toxicity.

Materials

The physico-chemical constants of the derivatives are shown in Table 1.

1. Chemical Procedure

Lankacidin C 8,14-diacetate: A solution of lankacidin A (100 mg) in Ac_2O (0.5 ml) and pyridine (1 ml) was kept at 25°C overnight and then poured into ice water. The filtered mass was crystallized from ether to give colorless prisms (80 mg).

The diesters shown below were synthesized using this procedure with minor modifications.

Starting material	(g)	Product	(g)
Lankacidin C	0.23	C 8,14-dipropionate	0.23
〃	0.23	C 8,14-dicrotonate	0.13
Lankacidin A	0.25	C 8-propionyl-14-acetate	0.245
〃	0.25	C 8-crotonyl-14-acetate	0.173
〃	0.50	C 8-(m-bromo)-benzoyl-14-acetate	0.30
Lankacidin C 8-acetate	0.10	C 8-acetyl-14-propionate	0.08

Lankacidin C 8,14-disuccinate: To a solution of lankacidin C (230 mg) in pyridine (4 ml), succinic anhydride (500 mg) was added. The mixture was allowed to stand at 0~5°C for 15 hours and extracted with AcOEt and then added to ice cold 2% $NaHCO_3$. The basic solution was adjusted with N HCl to pH 3 and reextracted with AcOEt. The organic layer was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. Addition of AcOEt and ether to the concentrate gave colorless needles (50 mg).

Lankacidin C 8,14-di-(trifluoro)-acetate: To a solution of lankacidin C (920 mg) in THF (10 ml), trifluoroacetic anhydride (1.5 ml) was added at 0~5°C and the mixture was stirred at 25°C for 2 hours. After addition of H_2O, the reaction mixture was immediately extracted with ether. The extract was washed with H_2O, concentrated to small volume and allowed to stand at 0~5°C. Crude crystals were recrystallized from ether to yield colorless needles (750 mg).

Lankacidin C 8-trifluoroacetate: Lankacidin C 8,14-di-(trifluoro)-acetate (860 mg) was applied to chromatography on silica gel (30 g). The eluate of benzene—AcOEt (from 7: 3 to 6: 4) was concentrated under reduced pressure and crystallized from ether to give colorless prisms (270 mg).

Lankacidin C 8-propionate and C 14-propionate: A solution of lankacidin C (920 mg) in pyridine (10 ml) and propionic anhydride (0.25 ml) was allowed to stand at 25°C for 7 hours. The solution was poured into iced water and extracted with AcOEt. The concentrate of the extract was separated by preparative thin-layer chromatography (prep. TLC) on silica gel HF_{254} (Merck Co.) with the solvent system of AcOEt—benzene (1: 2) to obtain lankacidin C 8-propionate (131 mg) and 14-propionate (138 mg) (recrystallized from ether and ether-AcOEt, respectively).

Lankacidin C 8,14-dibutyrate, C 8-butyrate and C 14-butyrate: To a solution of lankacidin C (20 g) in pyridine (200 ml), butyryl chloride (6 ml) was added at 0~5°C. The reaction mixture was allowed to stand at room temperature overnight. The mixture was poured into iced water (6 liters) and extracted twice with AcOEt (2 liters). The extract was washed with N/10 HCl (1 liter), H_2O (1 liter), 2% $NaHCO_3$ (1 liter) and H_2O (1 liter ×2). The organic layer was dried and the residue was purified by chromatography on silica gel (300 g, 0.05~0.20 mm, Merck Co.). Lankacidin C 8,14-dibutyrate (3.6 g), 14-butyrate (3.9 g) and 8-butyrate (2.8 g) were obtained from each eluate of benzene—AcOEt (8: 2), (7: 3) and (6: 4), respectively.

Table 1. Physico-chemical constants of the derivatives of lankacidin-group antibiotics

Compound	m.p. (decomp.) (°C)	$[\alpha]_D^{22\sim25°}$ $c=0.5$ EtOH	Elemental analysis						Formula	UV; λ_{max}^{EtOH} nm (ε)
			Calcd.			Found				
			C	H	N	C	H	N		
Lankacidin C										
〃 8-propionate	203~204	−206	65.23	7.23	2.72	64.95	7.36	2.64	$C_{28}H_{37}NO_8$	226 (57200)
〃 8-butyrate	203	−210	65.76	7.42	2.64	65.76	7.47	2.74	$C_{29}H_{39}NO_8$	225 (47200)
〃 8-iso-butyrate	191	−190	〃	〃	〃	66.21	7.47	2.65	〃	225 (41200)
〃 8-valerate	180	−196	66.28	7.60	2.58	66.02	7.69	2.65	$C_{30}H_{41}NO_8$	225 (47500)
〃 8-iso-valerate	194	−201	〃	〃	〃	65.49	7.30	2.43	〃	225 (43800)
〃 8-caprate	169	−193	68.49	8.38	2.09	68.43	8.51	2.25	$C_{35}H_{51}NO_8$	227 (51900)
〃 8-palmitate	140	−141	70.56	9.00	2.01	70.01	9.09	2.16	$C_{41}H_{63}NO_8$	226 (45500)
〃 8-crotonate	198~200	−160	66.02	7.07	2.65	66.16	6.40	2.63	$C_{29}H_{37}NO_8$	226 (58000)
〃 8-trifluoroacetate	150~153	−217	58.37	5.80	2.52	58.53	5.69	2.89	$C_{27}H_{32}NO_8F_3$	226 (45000)
〃 8-benzoate	206	− 99.5	68.16	6.62	2.49	67.85	6.63	2.58	$C_{32}H_{37}NO_8$	230 (55400)
〃 8-phenylpropionate		−140	69.01	6.98	2.37	68.86	6.84	2.37	$C_{34}H_{41}NO_8$	226 (44600)
〃 8-nicotinate	186	−103	65.94	6.43	4.96	65.90	6.29	4.70	$C_{31}H_{36}N_2O_8$	227 (54800)
〃 14-propionate	195~196	−218	65.23	7.23	2.72	65.00	7.27	2.76	$C_{28}H_{37}NO_8$	227 (53600)
〃 14-butyrate	173	−204	65.76	7.42	2.64	65.68	7.34	2.57	$C_{29}H_{39}NO_8$	227 (42600)
〃 14-iso-butyrate	181	−180	〃	〃	〃	65.86	7.63	2.23	〃	227 (40400)
〃 14-valerate	173	−188	66.28	7.60	2.58	66.02	7.58	2.63	$C_{30}H_{41}NO_8$	227 (41100)
〃 14-iso-valerate	182	−211	〃	〃	〃	66.31	7.43	2.49	〃	226 (41300)
〃 14-caprate	125	−202	68.49	8.38	2.09	68.06	8.50	2.17	$C_{35}H_{51}NO_8$	227 (46400)
〃 14-palmitate		−123	70.56	9.00	2.01	70.84	9.43	1.75	$C_{41}H_{63}NO_8$	227 (35100)
〃 14-crotonate	196	−167	66.02	7.07	2.65	65.77	7.30	2.69	$C_{29}H_{37}NO_8$	226 (58500)
〃 14-benzoate	186~188	− 90	68.16	6.62	2.49	67.92	6.51	2.60	$C_{32}H_{37}NO_8$	228 (52500)
〃 14-phenylpropionate		−157	69.01	6.98	2.37	68.69	6.99	2.31	$C_{34}H_{41}NO_8$	227 (43500)
〃 14-nicotinate	185~187	− 94	65.94	6.43	4.96	65.51	6.51	4.55	$C_{31}H_{36}N_2O_8$	226 (50000)
〃 8,14-dipropionate	185~186	−206	65.13	7.23	2.45	64.98	7.20	2.38	$C_{31}H_{41}NO_9$	226 (50400)
〃 8,14-dibutyrate	172	−184	66.09	7.56	2.34	65.83	7.44	2.23	$C_{33}H_{45}NO_9$	226 (48200)
〃 8,14-di-iso-butyrate	189	−171	〃	〃	〃	66.37	7.84	2.19	〃	226 (42300)
〃 8,14-divalerate	155	−171	66.96	7.87	2.23	66.69	7.84	2.15	$C_{35}H_{49}NO_9$	226 (47900)
〃 8,14-di-iso-valerate	199	−194	〃	〃	〃	67.06	7.57	2.26	〃	226 (45000)
〃 8,14-dicrotonate	193~195	−120	66.54	6.94	2.35	66.21	7.02	2.31	$C_{33}H_{41}NO_9$	226 (63100)
〃 8,14-di-(trifluoro)-acetate	152~154	−235	53.46	4.80	2.15	53.49	4.81	2.17	$C_{29}H_{31}NO_9F_6$	227 (45600)
〃 8,14-disulcinate	148~152	−210	60.08	6.26	2.12	59.99	6.61	2.51	$C_{33}H_{41}NO_{13}$	226 (59600)
〃 8-acetyl-14-propionate	190~192	−176	64.62	7.05	2.51	64.33	7.10	2.78	$C_{30}H_{37}NO_9$	227 (54600)
Lankacidin A										
〃 8-propionate	174~178	−217	64.62	7.05	2.51	64.24	6.98	2.58	$C_{30}H_{39}NO_9$	226 (52500)
〃 8-crotonate	115~124	−170	65.36	6.90	2.46	65.55	7.24	2.41	$C_{31}H_{39}NO_9$	227 (59200)
Lankacidinol										
〃 8-acetate	183	−218	64.40	7.41	2.78	63.98	7.35	2.73	$C_{27}H_{37}NO_8$	228 (48800)
〃 8-acetate (2′-D type)	173	−196	〃	〃	〃	64.24	7.33	2.84	〃	228 (46800)
〃 14-propionate	140	−221	64.97	7.59	2.71	64.88	7.84	2.42	$C_{28}H_{39}NO_8$	229 (46000)
〃 2′,14-diacetate		−139	63.83	7.21	2.56	63.88	7.89	2.66	$C_{30}H_{39}NO_9$	227 (41500)

In almost the same method, the following esters were prepared from lankacidin C in pyridine by using acid chlorides.

Starting material Lankacidin C (g)	Di- (g)	8- (g)	14- (g)	Ester
20	0.215	1.35	1.2	Isobutyrate
20	2.1	2.8	3.3	Valerate
20	1.5	2.7	2.8	Isovalerate
20	—	2.1	2.2	Caprate
20	—	1.1	2.3	Palmitate
0.92	—	0.075	0.124	Crotonate
0.92	—	0.052	0.079	Benzoate
0.92	—	0.085	0.097	Phenyl propionate

Lankacidin C 14-nicotinate: A solution of lankacidin C (920 mg) in THF (10 ml) and pyridine (2 ml) was added to nicotinyl chloride hydrochloride (500 mg) and kept at room temperature for 15 hours. The reaction mixture was poured into a mixture of AcOEt and H_2O. The aqueous layer after neutralization was again extracted with AcOEt. Combined organic extracts were washed with 2% $NaHCO_3$, dried and concentrated *in vacuo*. The main product was purified by column chromatography and followed by preparative TLC plates on silica gel. Recrystallization from AcOEt gave colorless crystals (94 mg).

2. Enzymatic Deacylation (Method I)[8]

(1) A solution of lankacidin C 8,14-diacetate (10.8 g) in MeOH (2.5 liters) was stirred at 37°C for 3 hours with the broth filtrate of *Streptomyces rochei* var. *volubilis* which had been preextracted with AcOEt. After evaporation of MeOH, the reaction solution was extracted with AcOEt (3 liters) and the extract was dried, concentrated and crystallized from AcOEt to yield lankacidin C 8-acetate (7.9 g).

The lankacidin C 8-monoesters prepared by the above-described method are as follows:

Starting material	(g)	Product	(g)
Lankacidin C 8,14-dipropionate	0.1	C 8-propionate	0.075
〃 C 8-propionyl-14-acetate	10.0	〃	7.0
〃 C 8-butyryl-14-acetate	0.1	C 8-butyrate	0.078
〃 C 8-valeryl-14-acetate	0.1	C 8-valerate	0.065
〃 C 8-crotonyl-14-acetate	0.1	C 8-crotonate	0.075
〃 C 8-benzoyl-14-acetate	0.1	C 8-benzoate	0.01
〃 C 8-nicotinyl-14-acetate	0.15	C 8-nicotinate	0.09

(2) To a solution of lankacidinol 2′,8,14-triacetate[4] (1.5 g) in MeOH (150 ml) was added the crude enzyme (450 mg)/H_2O (450 ml) prepared from the broth filtrate of *Streptomyces rochei* var. *volubilis* and the mixture was stirred at 25°C for 3 hours. After addition of H_2O, the reaction solution was extracted with methyl isobutyl ketone (MIBK) and the extract was evaporated *in vacuo*. The residue was chromatographed on a column of silica gel (30 g) and eluted with benzene - AcOEt (6 : 4), (4: 6), and (2: 8) to give lankacidin C 8-acetate (14 mg), lankacidinol 2′,8-diacetate (22 mg) and lankacidinol 8-acetate (168 mg), respectively. By the same method, lankacidinol 2′,14-di-acetate (1.5 g) gave lankacidin C (54 mg), lankacidinol 2′-acetate (53 mg) and lankacidinol (34 mg).

3. Enzymatic Acylation (Method II)[8]

A solution of lankacidinol (1.5 g) in AcOEt (680 ml) was added to above-mentioned enzyme solution (2.7 liters). The reaction mixture was stirred at room temperature for 4 hours. The MIBK extract was dried and concentrated *in vacuo*. The residual oil was purified by chromatography on silica gel (30 g). Each eluate, obtained using benzene—AcOEt (6: 4), (4: 6), and (2: 8) gave lankacidin A (58 mg), lankacidinol 2′,14-diacetate (70 mg, precipitated with AcOEt-hexane), and lankacidinol A (770 mg), respectively. Similarly, using PrOEt, lankacidinol 14-propionate (180 mg) was obtained from lankacidinol (0.5 g).

Methods

1. Antimicrobial Activity

Antimicrobial activities of lankacidin C derivatives were examined by the paper disc technique or by serial agar dilution method against *Staphylococcus aureus* 209 P, oleandomycin-erythromycin resistant *S. aureus* 209 P (OE-R), and *Sarcina lutea* PCI 1001.[2]

2. Protecting Effect

The *in vivo* activities of lankacidin C derivatives were assessed against mice (CF 1/H) infected with *S. aureus* 308 A-1 or *Stretptococcus pyogenes* E-14.[7] Single doses of the antibiotic were administered orally or intraperitoneally immediately after challenge. The 50% effective dose (ED_{50}) was determined on the basis of 7-day observation by the method of REED and MUENCH.[9]

3. Acute Toxicity

Male ICR-JCL mice weighing 18~24 g were used. Various doses of lankacidin C derivatives suspended in 5% gum arabic solution were administered orally or intraperitoneally. Three animals were used in each group.

The 50% lethal doses (LD_{50}) were calculated from the mortality for 7 days after administration according to the method of LITCHFIELD and WILCOXON.[10]

4. Subacute Toxicity in Rats

Female rats (Donryu strain) weighing 100~155 g at the start of the experiment were divided into 5 groups consisting of 5 animals each. Five rats kept in a cage were allowed free access to diet prepared at the Hikari factory of Takeda and to drinking water. Several derivatives of lankacidin C were suspended at the concentration of 100 mg/ml in 0.2% sodium carboxymethyl cellulose (CMC) solution. These suspensions in daily dose of 1,000 mg/kg were administered by intragastric caterization (6 times a week for one month). Control groups were given only 0.2% CMC solution. Behavioral changes were observed daily and body weights measured two times a week. Blood samples obtained from the rats at the termination of the administration period were subjected to routine hematological and biochemical examinations.[12]

Results and Discussion

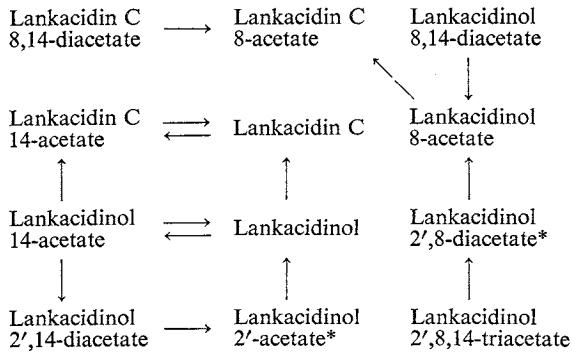
Synthesis and Structure of the Esters

Various kinds of acyl derivative of lankacidins C, A or C 8-acetate[1] were obtained by acylation with acid chlorides or acid anhydrides in anhydrous pyridine, tetrahydrofuran, acetone, acetic acid or their mixtures at 0~25°C for 1~48 hours. When lankacidin C was treated under these conditions, 8,14-diester, 8- or 14-monoester and starting material were obtained in almost equal amount. However, in the case of aliphatic esters, the yield of diester became progressively lower with increasing chain length of the acid chloride. Lankacidin C 8-trifluoroacetate and 8-chloro lankacidin A were rapidly hydrolized in aqueous solution to give lankacidins C and A, respectively. Moreover, the mesylate or tosylate of lankacidin A were rapidly hydrolized to starting material.

Specific enzymatic reactions readily assisted in the preparation of monoesters. Monoesters of lankacidin C at position 8 or 14 were also prepared by using the crude enzyme obtained from the filtered broth of *S. rochei* var. *volubilis*. The former was obtained enzymatically from lankacidin A 8-ester in methanolic aqueous solution (method I) while the latter was obtained from lankacidin C and an acyl donor $C_nH_{2n+1}CO$ (n=0~2)) (method II).[8] Using these methods, the yields of esters were about 70~80%.

Furthermore, various acetates of lankacidinol were prepared by using the crude enzyme. When lankacidinol was treated according to method II, lankacidinol A, lankacidin A and lankacidinol 2′,14-diacetate were obtained. Using method I lankacidinol 2′,8,14-triacetate gave lankacidin C 8-acetate,

Chart II. Enzymic pathway of lankacidin-group antibiotics



* Minor product.

lankacidinol 2′,8-diacetate and 8-acetate. Lankacidinol 8-acetate was identical with the sample prepared from lankacidinol 8,14-diacetate by method I. Treatment of lankacidinol 2′,14-diacetate according to method I afforded lankacidin C, lankacidinol 2′-acetate and lankacidinol. From these results, it is concluded that the rate of enzymatic reaction at 2′-position is slower than that at 14-position. Finally, lankacidinol 8-acetate and lankacidinol were dehydrogenated at 2′-position to yield lankacidin C 8-acetate and lankacidin C, respectively.

The above-mentioned enzymatic pathways are shown schematically in Chart 2.

The structures of the esters were suggested by Rf values of TLC (Table 2) and confirmed by the absorption peaks at 3450~3650 cm^{-1} (hydroxyl region) and 1700~1740, 1200~1300 cm^{-1} (ester region) in the infrared spectra, by the parent peaks or degradative patterns in mass spectra and especially, by chemical shifts of the hydroxyl methine proton in nuclear magnetic resonance spectra (the signals of $\delta^{CDCl_3}_{ppm}$ 4.06 (1H, m, H_8) and 4.26 (1H, m, H_{14}) shifted to 5.0~5.3 ppm, Figs. 1 and 2).

Table 2. Rf values of lankacidin-group antibiotics and related compounds on TLC

Antibiotic	Solvent system			
	(1)*	(2)*	(3)*	(4)*
Lankacidin C 8,14-diacetate			0.66	0.53
〃 A (14-acetate)	0.79	0.69	0.61	
〃 C 8-acetate	0.62	0.54	0.37	
〃 C	0.57	0.54	0.25	
Lankacidinol 2′,8,14-triacetate			0.63	0.30
〃 2′-14-diacetate	0.74	0.66	0.45	
〃 8,14-diacetate	0.74	0.62	0.39	
〃 2′,8-diacetate	0.67	0.56		
〃 A (14-acetate)	0.50	0.45	0.18	
〃 2′-acetate	0.52	0.44		
〃 8-acetate	0.52	0.44	0.12	
〃	0.28	0.26		
Lankacyclinol 2′,8,14-triacetate			0.57	0.16
〃 A (14-acetate)	0.42	0.42		
〃	0.21	0.20		

* (1) Benzene—Me_2CO (1: 1), SiO_2 f_{254} (Tokyokasei Co.)
(2) Methyl ethyl ketone—AcOEt (2: 8), Kieselgel F_{254} (Merck Co.)
(3) AcOEt—Benzene (2: 1), SiO_2 f_{254}
(4) AcOEt—Benzene (1: 2), SiO_2 f_{254}

Fig. 1. NMR spectrum of lankacidin C 8-propionate (100 MHz, $CDCl_3$)

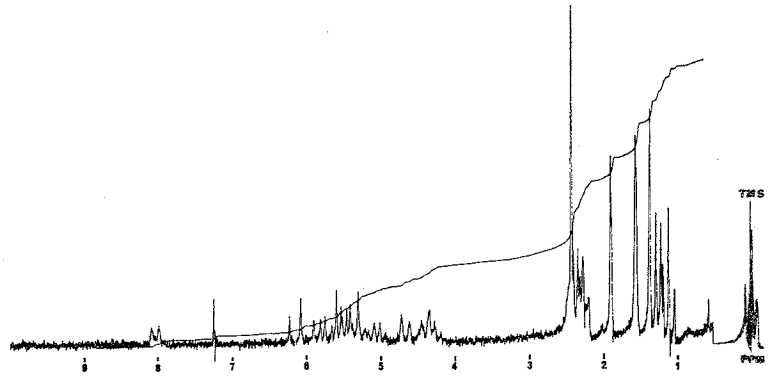
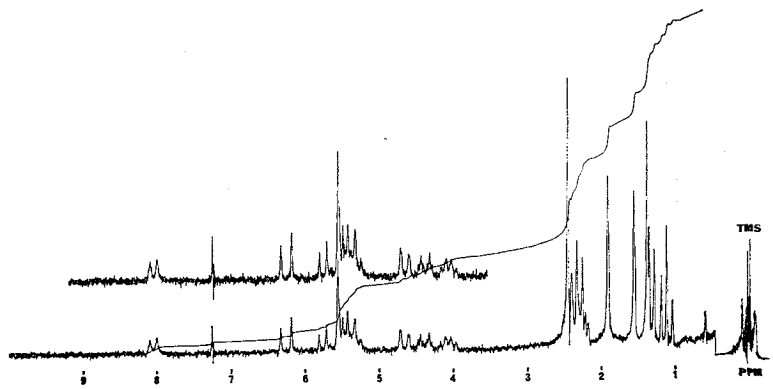


Fig. 2. NMR spectrum of lankacidin C 14-propionate (100 MHz, $CDCl_3$)



Biological Activities

In vitro antimicrobial activities of lankacidin C derivatives[1,5,6] obtained in the course of structure elucidation studies have been determined. The hydrogenated derivatives of known macrolides are ordinarily active against several bacteria,[11] however, 6,7-dihydrolankacidin C[1,5] and 6,7,12,13-tetrahydro lankacidin C[1,5] were inactive. The 2′-epimer of lankacidinol,[5] obtained by reduction of lankacidin C with sodium borohydride, has equal antimicrobial activity as lankacidinol. In contrast, lankacidinol 18-ol[5] and lankacidinol A 18-ol[1,5] did not show any antimicrobial activity. 8-Oxo lankacidinol A,[5] 8-oxo lankacidin A,[1,5] and 14-oxo lankacidin C 8-acetate[1,5] synthesized by oxidation were inactive, but 14-oxo lankacidin C[1,5] was as active as lankacidinol A. Lankacyclinol A[6] and lankacyclinol[6] obtained from lankacidinol A or lankacidinol by basic decomposition, slightly inhibited *S. aureus*. 8,14-Dimethyl lankacidin C[6] did not exhibit any antimicrobial activity.

The relationship between chemical structure and biological activities of lankacidin C esters is shown in Table 3. The diesters possess a weak antibacterial activity and a low protective effect. However, lankacidin C 8-monoesters were relatively more active than lankacidin C and displayed no cross-resistance against macrolide-resistant strains of *S. aureus*. In the *in vivo* studies, some of the lankacidin C 8-monoesters were almost as effective as lankacidin A when administered orally to infected mice. However, *in vitro* the antibacterial activity of lankacidin C 14-monoesters was decreased to about one-tenth of lankacidin C and in contrast with lankacidin C 8-monoesters, showed a partial cross-resistance against macrolide-resistant strains of *S. aureus*. However, some of the lankacidin

Table 3. Biological activities of lankacidin-group antibiotics and related compounds

Compound	MIC (μg/ml) *St. aureus* 209P	ED_{50} (mg/kg)*1 PO	LD_{50} (g/kg)*2 IP	PO	IP
Lankacidin C[1,3]	1.0	87	2.0	>10	4.5
8-acetate[1]	0.5~1.0	40 (A 57)*3		>10	5~10
8-propionate	1.0	30 (A 40)		>10	>10
8-butyrate	0.5	72 (C 28)*3			> 5
8-iso-butyrate	0.5	90 (C >80)			
8-valerate	0.5	26 (C 28)			> 5
8-iso-valerate	0.5	128 (C 60)			
8-caprate	100	>320 (C 120)			
8-palmitate	>100	>160 (C 28)			
8-crotonate	0.5~1.0	50*4			
8-trifluoroacetate	0.5	*ca.* 90 (C <60)			
8-benzoate	0.5	*ca.* 100 (C <60)			
8-phenylpropionate	0.5~1.0	*ca.* 120 (C <60)			
8-nicotinate	0.5				
14-acetate (=A)[1,3]	10~20	78	6.0	>10	8~10
14-propionate	20	39 (A 33)	1.5	>10	>10
14-butyrate	50	29 (C 45)			> 5
14-iso-butyrate	50	107 (C >80)			
14-valerate	100	69 (C 28)			> 5
14-iso-valerate	50	128 (C 60)			
14-caprate	>100	237 (C 120)			
14-palmitate	>100	>160 (C 28)			
14-crotonate	20~50	< 50*4			
14-benzoate	10	120 (C <60)			
14-phenylpropionate	10	*ca.* 100 (C <60)			
14-nicotinate	10	*ca.* 150 (A <150)			
8,14-diacetate[1,3]	10~20	≧100			> 5
8,14-dipropionate	50	>100			
8,14-dibutyrate	>100	50~100			> 5
8,14-di-iso-butyrate	>100				
8,14-divalerate	>100	>100			> 5
8,14-di-iso-valerate	>100				
8,14-dicrotonate	50~100				
8,14-dibenzoate	100				
8,14-disuccinate	50	>100 (C <60)			2.5~5.0
8,14-di-(trifluoro)-acetate	10~20				
8-propionyl-14-acetate	20				
8-acetyl-14-propionate	50				
8-crotonyl-14-acetate	20~50				
8-nicotinyl-14-acetate	10				
8-chloro-14-acetate	>100				
Lankacidinol[1,4]	50~100	>400	36	>10	*ca.* 5
8-acetate	10		9.4		
14-acetate (=A)[1,3]	100	>200	10~35	>10	>10

Table 3. (Continued)

Compound	MIC (μg/ml) *St. aureus* 209P	ED_{50} (mg/kg)*1 PO	LD_{50} (g/kg)*2 IP	PO	IP
2′-acetate	50				
14-propionate	100		>80		
8,14-diacetate	50		15		
2′,8-diacetate	50				
2′,14-diacetate	20		>80		
2′,8,14-triacetate[1,3,4]	>100				> 5
18-ol[5]	>100		>100		1~2.5
18-ol 14-acetate[1,5]	>1,000				
Lankacidinol (2′-D type)[5]	50~100				
6-Dihydrolankacidin C[1,5]	>100				
6,12-Tetrahydro lankacidin C[1,5]	>1,000		>100		> 5
8-Oxo lankacidin A[1,5]	>1,000				
8-Oxo lankacidinol A[5]	>1,000				
14-Oxo lankacidin C[1,5]	50~100				
14-Oxo lankacidin C 8-acetate[1,5]	>100				
8,14-Dimethyl lankacidin C[6]	>100				
Iso-lankacidinol[8]	>100				
Iso-lankacidinol O[6]	>100				
Lankacyclinol[6]	>100		>80		> 5
Lankacyclinol A[3,6]	100				

*1 Mice (CF-1) infection caused by *Staph. aureus* 308A-1.
*2 Mice (ICR-JCL).
*3 Control sample, C: lankacidin C, A: lankacidin A.
*4 *Str. pyogenes* E-14.

C 14-esters were as effective by oral administration as lankacidin C or C 8-esters *in vivo*. Mono- or di-acetates of lankacidinol showed a similar trend in antimicrobial activity *in vitro*. Lankacidinol and its 8,14-diacetate, 8-acetate and 14-acetate possessing a 2′-hydroxyl group showed protective effect by intraperitoneal administration.

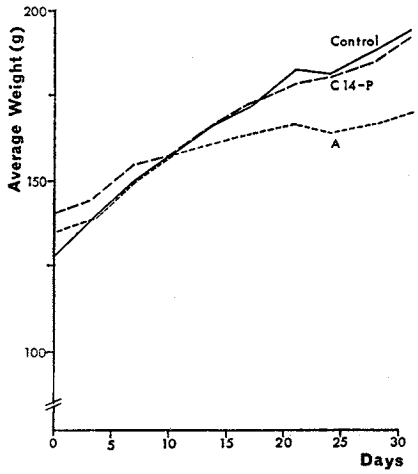
The above-mentioned results may be explained in terms of the stereo-chemical requirements for the lankacidin-group antibiotics to show *in vitro* and *in vivo* antimicrobial activities. The total loss of activity in the hydrogenated derivatives having a flexible ring structure and in the oxygenated derivatives having a strained ring structure may be attributed to steric changes of the seventeen-membered ring.

Products resulting from reduction of the 2′-carbonyl function have weak *in vitro* antimicrobial activities. These procucts are effective when administered intraperitoneally to infected mice, but they are without effect when administered orally. This difference in activity may be accounted by the lack of absorption from the intestinal tract. The carbonyl function at the 2′-position is not essential for antibiotic activity in lankacidin-group but the lactone group is essential.

The functional group at 8-position appears not to be an active site of the antibiotic.

The finding that 14-ester has little activity *in vitro*, but has a considerable activity *in vivo* may be

Fig. 3. Growth curves for rats given orally lankacidin A and C 14-propionate (1 g/kg/day)
The data are expressed in mean of 5 animals



explained as follows. The hydroxyl group at 14-position is essential for activity, it is inactivated by acylation and reactivated by enzymatic deacylation in the body fluid of the experimental animals.

When the antibiotics were administered orally in mice, the respective LD_{50} values were more than 10 g/kg. The intraperitoneal LD_{50} values of the monoesters were higher than those of lankacidin C or lankacidinol, these latter compounds being insoluble in the aqueous phase.

In order to determine the optimum compound subacute toxicity studies were performed with the biologically active derivatives. Fig. 3 shows the growth curves for rats given lankacidin A and C 14-propionate.

The administration of lankacidin C 14-propionate in rats did not exhibit any effect in regard to body weight gain, while a small reduction in body weight was recorded for the rats given lankacidin A. Also, no significant abnormalities were observed on macroscopical examination of the essential organs likewise, hematological and biochemical studies of blood, except that a hypertrophy of the caecum (approximately two-fold) and slight anemia was observed in both groups (H. YOKOTANI *et al.*, private communication).

On the basis of these results, lankacidin C 14-propionate was selected as the most favorable candidate for further biological evaluation.

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