STUDIES ON LANKACIDIN-GROUP (T-2636) ANTIBIOTICS

VIII. METABOLISM OF LANKACIDIN C 14-PROPIONATE IN RATS AND MICE

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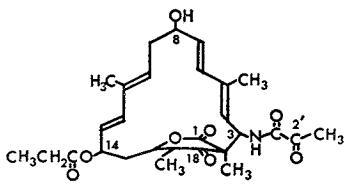
The metabolic fate of lankacidin C 14-propionate-^{14}C was studied in rats and mice. In these animals, the antibiotic administered orally was absorbed slowly, distributed widely in tissues and metabolized to be excreted mainly in bile. The metabolites in bile were then excreted into feces together with the unabsorbed radioactivity. The elimination of the antibiotic from the body was completed within 72 hours in both species. In rats, 77 and 6.5% of the radioactivity was excreted in feces and urine, respectively. In mice, 88 and 5.5% was eliminated into feces and urine, respectively. No significant amount of radioactivity was excreted in the expiratory air of either animal. After oral administration, the blood level of radioactivity reached a peak after 2 hours in rats and after 15 minutes in mice. Of the tissues tested, relatively higher concentrations were noted in liver, kidney, lung and spleen in rats and mice. At any time after the administration to mice, the levels of antibiotic activity in the infected region of liver, kidney and abdominal ascites were much higher than the blood levels. The antimicrobial activities in these tissues were mainly derived from the metabolites, lankacidin C and lankacidinol. The biliary metabolites identified were lankacidin C, lankacidinol, lankacyclinol, lankacidinol 14-P and T-2636 H in rats, and lankacidin C, lankacidinol and lankacyclinol in mice. The results obtained are discussed in relation to therapeutic activities of these antibiotics.

Lankacidin C 14-propionate whose structure is shown in Chart 1, is a propionyl derivative of lankacidin C, one of the lankacidin-group antibiotics isolated from the culture broth of *Streptomyces rochei* var. *volubilis*.[1~6]

Chart 1. Chemical structure of lankacidin C 14-P-^{14}C



This compound has been selected as the most favourable derivative since it is active against Gram-positive bacteria and clinically isolated resistant strains of staphylococci. It is effective by oral administration against infections in the mouse due to *Staphylococcus aureus* 308 A–1 and shows low toxicity.[7,8]

The present paper describes the metabolic fate of ^{14}C-labeled lankacidin C 14-propionate (lankacidin C 14-P-^{14}C) and reports observations concerning its absorption, distribution, excretion and metabolic routes in rats and mice.

Materials and Methods

Materials

Lankacidin C-^{14}C was prepared by using *Streptomyces rochei* var. *volubilis* and L-methionine-CH_3-^{14}C. Fermentation conditions were essentially the same as those reported by HIGASHIDE *et al.*[1] The radioactive methyl moiety of L-methionine was incorporated into the skeleton of lankacidin C as

a methyl-^{14}C group. Labeled lankacidin C was then propionated by the crude enzyme(s) obtained from the fermentation broth of this organism.[8,9] Lankacidin C 14-P-^{14}C was purified by thin-layer chromatography (TLC) using solvent systems 3 to give a final product of better than 97% purity with a specific radioactivity of 50 μCi/mg.

Animals

Animals used were male Sprague-Dawley rats (SD-JCL) weighing 200~300 g and male CH_1 mice weighing 25~35 g, and were purchased from CLEA Japan, Inc., Tokyo. They were maintained on a usual chow diet (CE-2, CLEA Japan, Inc., Tokyo) for more than 2 weeks before use.

Collection of Body Fluids and Excreta

Lankacidin C 14-P-^{14}C was dissolved in glycofurol (Hoffmann-La Roche Inc., U.S.A.)[10] for oral or intravenous administration. The dose selected were 50 and 200 mg/kg of body weight, respectively, corresponding to the ED_{50} and the ED_{100} values for oral administration of the antibiotic in mice infected with *Staphylococcus aureus* 308 A-1.[7] Blood was obtained from the tail vein or inferior cava vein in rats and from the inferior cava vein in mice. Urine, feces and expired air were collected by using the usual metabolic cages. For the measurement of the radioactivity in the expired air, air was introduced into the cage and the issuing air was passed through a trap containing 5 N KOH solution. Bile was collected from the biliary-cannulated rats and mice.

Measurement of Radioactivity

The radioactivity was measured by a liquid scintillation counter, Aloka model LSC-502 (Nihon musen Co., Tokyo) with an automatic quenching monitor. Radioactivity in urine and bile was counted directly in a dioxane-phosphor mixture.[11] Radioactivity in blood, tissues, feces or gastrointestinal contents was determined in a toluene-phosphor mixture[11] after combustion by the method of DAVIDSON and OLIVERIO.[12] In some experiments, the radioactivity was extracted with 10 ml of the dioxane-phosphor mixture from appropriate amounts of blood, tissue homogenates (1: 1, tissue/water, v/v) or suspension of feces (1: 9, feces/water, w/v) and counted as described above. Recovery of radioactivity was found to be quantitative. The radioactive carbon dioxide in the expired air trapped in 5 N KOH solution was counted in the toluene-phosphor mixture after regeneration and reabsorption into Hyamine 10X solution (Packard Instrumental Co. Inc., U.S.A.).

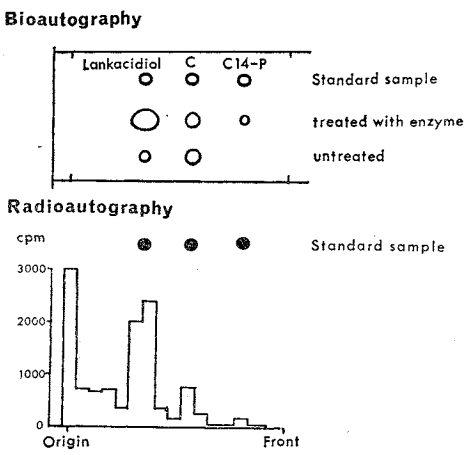
Measurement of Antimicrobial Activity

Antimicrobial activity in blood, bile or urine was determined by the cup method after appropriate dilution with water and by the paper disc method after extraction of tissue homogenates (1: 1, tissue/water, v/v) with 4 volumes of acetone. The test organism used was *Sarcina lutea* PCI 1001. As described in the "Results", antimicrobial activities in blood, tissues of mice given lankacidin C 14-P-^{14}C were derived mainly from two metabolites, lankacidin C and lankacidinol. Antimicrobial activity of lankacidin C is about 10 times that of lankacidinol and therefore the antimicrobial activity in the samples for all practical purpose indicates the amount of compound C. The amount of lankacidinol in the samples were estimated after being converted to lankacidin C by the crude enzyme prepared from the culture filtrate of *S. rochei* var. *volubilis*.[8,9]

Estimation of Lankacidin C 14-P-^{14}C and Metabolites by TLC

For the estimation of lankacidin C 14-P-^{14}C

Fig. 1. Bioautogram and radioautogram of the sample by TLC



Sample; Acetone ext. of mouse liver(15 min. after dose)
Adsorbent; SiO_2 HF_{254}, Merck
Solvent system; Benzene: Acetone(1:1)

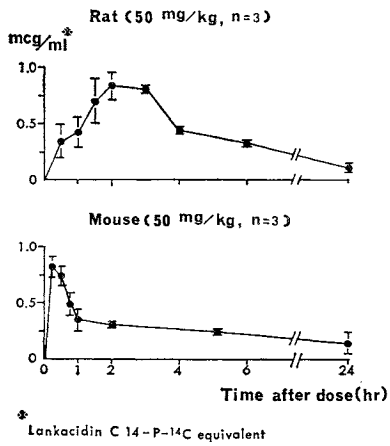
and its metabolites, aliquots of the acetone extracts of tissues or blood, bile and urine were subjected to TLC. Thin-layer plates of silica gel HF_{254} or F_{254} (E. Merck AG, Darmstadt) were used with the following solvent systems: system 1, methyl ethyl ketone - ethyl acetate (2: 8); system 2, benzene - acetone (1: 1); and system 3, benzene - ethyl acetate (1: 1). Radioactive spots on TLC-plates were scraped off from the plate for counting the radioactivity in the dioxane-phosphor mixture. The antimicrobially active components were examined by bioautographic technique using *Sarcina lutea* PCI 1001 as the test organism. A typical radioautogram and bioautogram are shown in Fig. 1.

Results

Blood Levels

After oral administration of 50 mg/kg of lankacidin C 14-P-^{14}C, the blood level of radioactivity reached a peak after 2 hours in rats (0.83 μg/ml, expressed as lankacidin C 14-P) and after 15 minutes in mice (0.83 μg/ml) (Fig. 2). The blood level of radioactivity decreased more rapidly in mice than in rats. These results indicate that the gastrointestinal absorption and subsequent tissue distribution of lankacidin C 14-P is more rapid in mice than in rats. At any given time after the administration, no significant amount of antimicrobial activity was detected in the blood of both animals. Protein binding was not observed after one hour incubation of lankacidin C 14-P, lankacidin C and lankacidinol (10, 100 μg/ml, pH 7.4) at 37°C in the plasma and whole blood of the mouse and rat.

Fig. 2. Blood levels of radioactivity after oral administration of lankacidin C 14-P-^{14}C
The data are expressed in mean ± S.D. (n=3)



Tissue Distribution

Table 1 shows results for tissue distribution of radioactivity in rats and mice after oral administration of 50 mg/kg of the labeled antibiotic. At 2 hours after the administration to rats, when the blood level of radioactivity was at its maximum (Fig. 2), the radioactivity was found to be widely distributed in tissues. The highest concentration was observed in stomach and intestine, followed by liver, adrenal and kidney, while the lowest was found in brain and testis. At 4 hours, the levels in brain, lung, spleen, adrenal, adipose tissue and testis further increased, while those in other tissues were unchanged or considerably decreased. The radioactivity decreased to very low levels at 24 hours, except for the liver and stomach in which relatively high concentrations were still detected.

In mice, the levels in liver, kidney, lung, spleen and blood were determined at 15 minutes, 2 and 24 hours after the oral administration. The highest concentration was noted in liver, followed by kidney, the levels in these two tissues being remarkably higher than the blood levels. Further, the radioactivity levels in the tissues of mice were significantly higher than those in the corresponding tissues of rats. This finding might be an indication of more rapid absorption of the orally administered antibiotic in mice than in rats.

Fig. 3 represents the levels of radioactivity and antibiotic activity in blood, liver, kidney and abdominal ascites of mice orally given 200 mg/kg of the labeled antibiotic. At any given time after

Table 1. Tissue levels of radioactivity after oral administration of lankacidin C 14-P-^{14}C (50 mg/kg)

Rats

Tissues	mcg/g or ml*		
	2 hours	4 hours	24 hours
Brain	0.210± 0.0563	0.552± 0.449	0.0328±0.0036
Lung	0.883± 0.473	5.41 ± 3.88	0.0155±0.0034
Heart	0.906± 0.570	0.765± 0.373	0.0345±0.0303
Liver	8.48 ± 3.17	7.98 ± 0.676	1.27 ±0.66
Stomach	243 ±75.1	618 ±166	1.15 ±0.56
Spleen	0.560± 0.270	9.33 ± 7.66	0.0708±0
Intestine	71.5 ±14.1	47.4 ± 18.6	0.440 ±0.132
Adrenals	3.25 ± 1.83	15.6 ± 11.2	0.434 ±0.247
Kidney	2.66 ± 1.25	2.79 ± 0.878	0.459 ±0.027
Muscle	0.588± 0.317	0.242± 0.178	0.0522±0.0115
Adipose	0.400± 0.0407	2.19 ± 1.57	0.239 ±0.047
Testis	0.186± 0.0735	0.449± 0.256	0.0368±0.0011
Blood	0.930± 0.539	0.787± 0.176	0.0395±0.036

Mice

Tissue	mcg/g or ml*		
	1/4 hour	2 hours	24 hours
Liver	15.2 ±2.75	15.7 ±2.70	2.70 ±0.45
Kidney	4.32±1.03	1.98 ±0.254	0.400 ±0.113
Lung	1.11±0.296	0.739±0.334	0.0496±0.0459
Spleen	1.57±1.49	0.389±0.057	0.129 ±0.222
Blood	2.16±0.596	0.798±0.277	0.144 ±0.109

The data are expressed in mean±S.D. (n=3).
* Lankacidin C 14-P-^{14}C equivalent.

Fig. 3. Antibiotic concentration of infected region after oral administration of lankacidin C 14-P-^{14}C in mice treated with mucin (200 mg/kg)
Each value of the metabolites was estimated by the techniques of radioautography and bioautography on thin-layer chromatogram

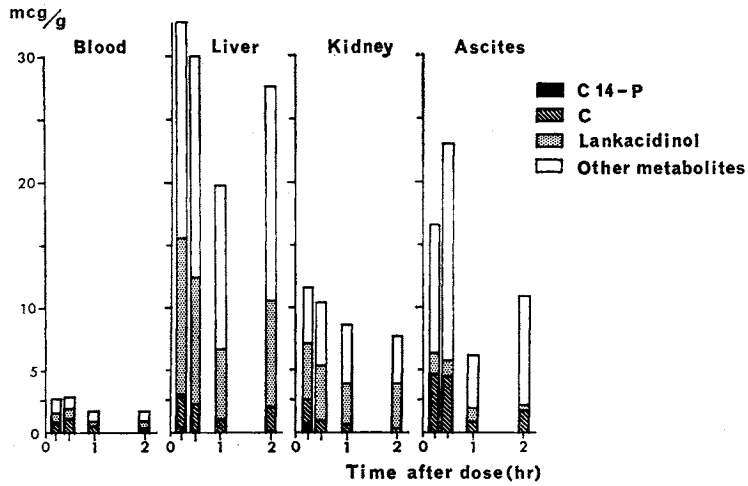


Table 2. Cumulative excretions of radioactivity after oral administration of lankacidin C 14-P-^{14}C in rats and mice (50 mg/kg)

Animal	Sample	Time after dose (hour)	Percentage of dose
Rats	Urine	0~5	1.53± 0.759
		5~24	4.40± 0.462
		24~48	6.00± 1.28
		48~72	6.54± 1.54
	Feces	0~24	6.45± 5.94
		24~48	62.9 ±21.3
		48~72	77.3 ±14.6
Mice	Urine	0~5	1.64± 0.30
		5~24	4.42± 1.63
		24~48	5.33± 0.634
		48~72	5.49± 1.55
	Feces	0~24	59.5 ±32.3
		24~48	83.2 ±16.3
		48~72	87.9 ±17.7

The data are expressed in mean±S.D. (n=3).

the administration, more than half of the radioactivity in the blood could be attributed to the antimicrobially active metabolites. Analogous results were also noted in the liver, kidney and peritoneal fluids. In these tissues the levels of radioactivity and antimicrobial activity were much higher than those in the blood. Active metabolites found in these tissues were lankacidin C and lankacidinol, the small amounts of lankacidin C 14-P being detected only in liver and kidney at early period after dosing.

Urinary and Fecal Excretions

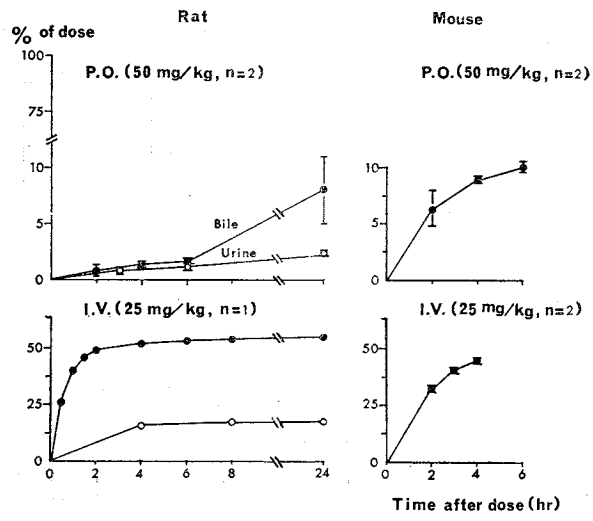
Both in rats and mice, excretion of radioactivity in urine and feces was complete within 72 hours after oral administration of lankacidin C 14-P-^{14}C, more than 70% of the dose being excreted in the first 48 hours (Table 2). Thus, 77.3 and 87.9% of the dosed radioactivity was recovered from the 72-hour feces of rats and mice, respectively, the remaining activity appearing in urine. No significant amount of radioactivity was detected in the expired air of both species.

Biliary Excretions

Excretion of the radioactive antibiotic in bile was studied in the biliary-cannulated rat and mouse (Fig. 4). The intravenous injection of the antibiotic in rats and mice resulted in a prompt excretion of the radioactivity in the bile, about half of the dose being recovered from 4-hour bile of both animals. On the other hand, the biliary excretion of radioactivity after the oral administration was found to be more rapid in mice than in rats. During the first 6 hours after the administration, mice excreted 10.1% of the dose in bile, while rats excreted only 1.58%. This might be an indication that the orally administered antibiotic was absorbed more rapidly in mice than in rats.

Urinary excretion of radioactivity was not significantly different for the intact and the cannulated rats, indicating that in this species the biliary

Fig. 4. Excretion of radioactivity after administration of lankacidin C 14-P-^{14}C in bile-duct cannulated rats and mice

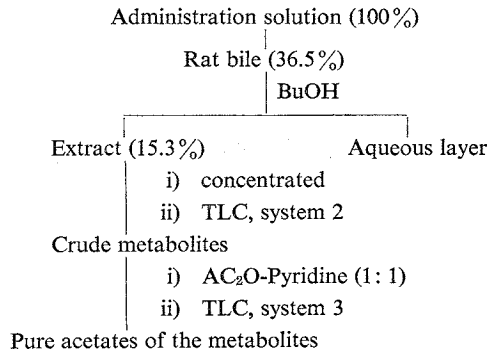


radioactivity is not efficiently reabsorbed from the intestinal tract.

Biliary Metabolites

As described above (Fig. 4), in rats as well as in mice the absorbed fraction of the antibiotic was excreted mainly in the bile. To clarify the chemical nature of the biliary metabolites, the bile was collected for 2 hours from 20 biliary-cannulated rats after intravenous injection of lankacidin C 14-P-^{14}C (25 mg/kg body weight), and was found to contain 36.5% of the injected radioactivity. Five metabolites were isolated from this sample as acetyl derivatives according to the method presented in Chart 2. An extract of the radioactive bile with butanol was concentrated *in vacuo* to yield an oily residue. The radioactivity in the residue was separated into 5 fractions by TLC with solvent system 2. Each fraction was eluted from the plate and acetylated with pyridine-acetic anhydride. The acetyl derivatives of the metabolites were further purified by TLC with solvent system 3.

Chart 2. Isolation procedure of biliary metabolites



	(%)
Lankacidin C	0.19
Lankacidinol 14-propionate	0.90
T-2636 H	0.87
Lankacidinol	4.90
Lankacyclinol	0.95
Total	7.81

Figs. 5 and 6 show the IR and MS spectra of the acetates obtained. The acetates of lankacidin C, lankacidinol 14-propionate and lankacidinol were identical with authentic samples as judged by the Rf values, IR and MS spectra.[2,3,8] Chemical structures of the other metabolites, lankacyclinol and T-2636 H, will be described elsewhere in detail.[13]

TLC analysis indicated that 0.26% and 1.7% of the radioactivity in the 6-hour bile of mice orally given lankacidin C 14-P-^{14}C could be attributed to lankacidin C and lankacidinol, respectively. From the same sample lankacyclinol was also detected.

Fig. 5-(a) IR spectrum of lankacidin C 8,14-diacetate (KBr)

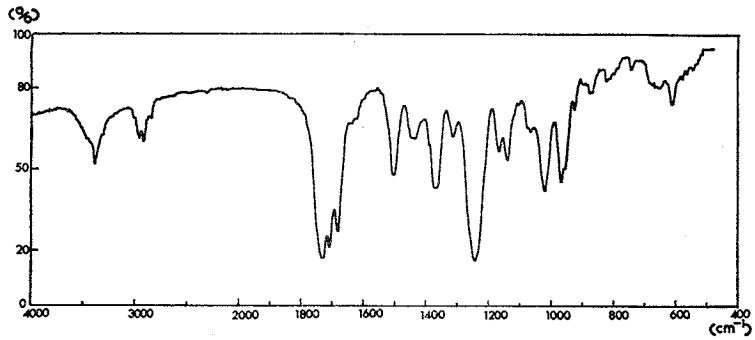


Fig. 5-(b) IR spectrum of lankacidinol 2′,8-acetyl-14-propionate (KBr)

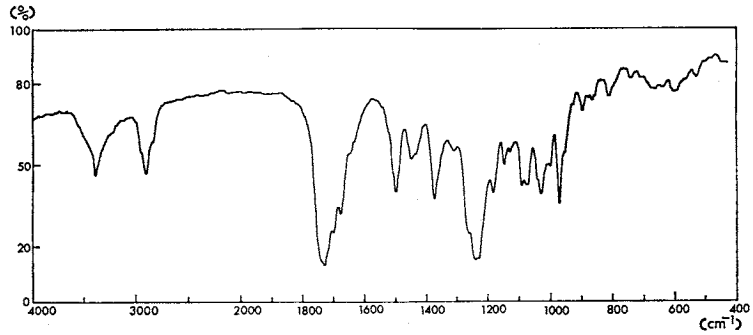


Fig. 5-(c) IR spectrum of T-2636 H triacetate (KBr)

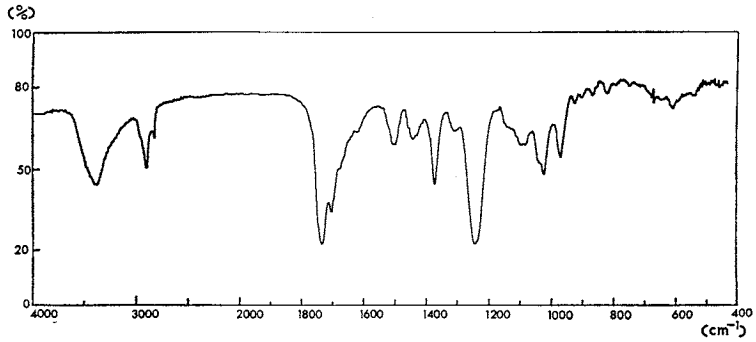


Fig. 5-(d) IR spectrum of lankacidinol 2′,8,14-triacetate (KBr)

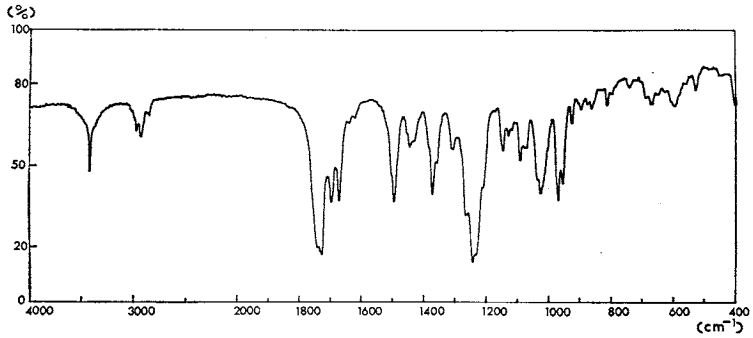


Fig. 5-(e) IR spectrum of lankacyclinol 2′,8,14-triacetate (KBr)

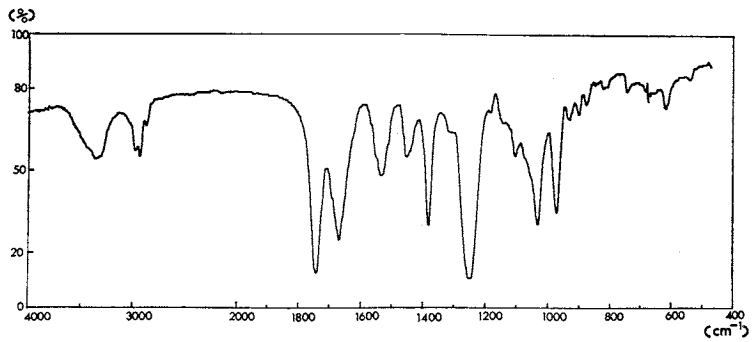


Fig. 6–(a) MS spectrum of lankacidin C 8,14-diacetate

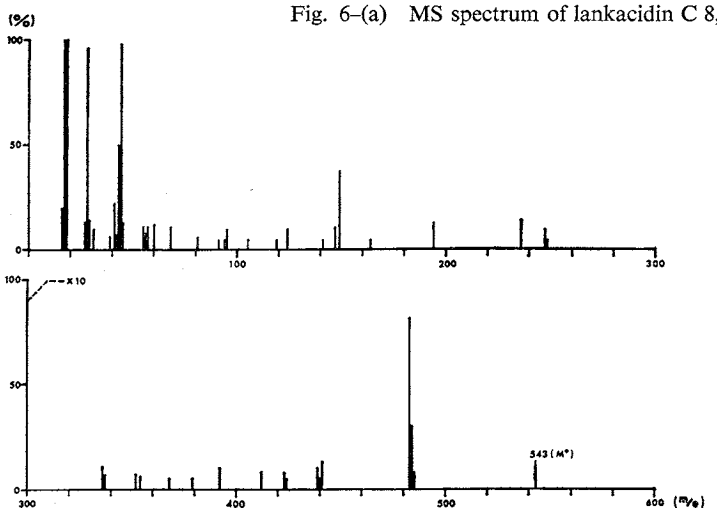


Fig. 6–(b) MS spectrum of lankacidinol 2′,8-diacetyl-14-propionate

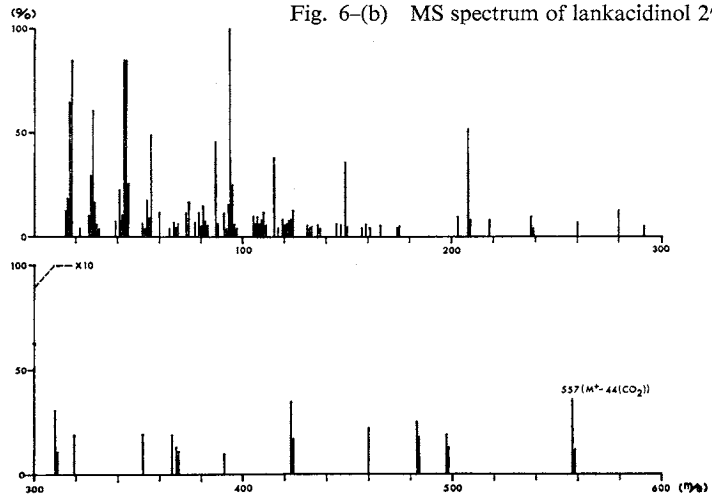


Fig. 6–(c) MS spectrum of T-2636 H triacetate

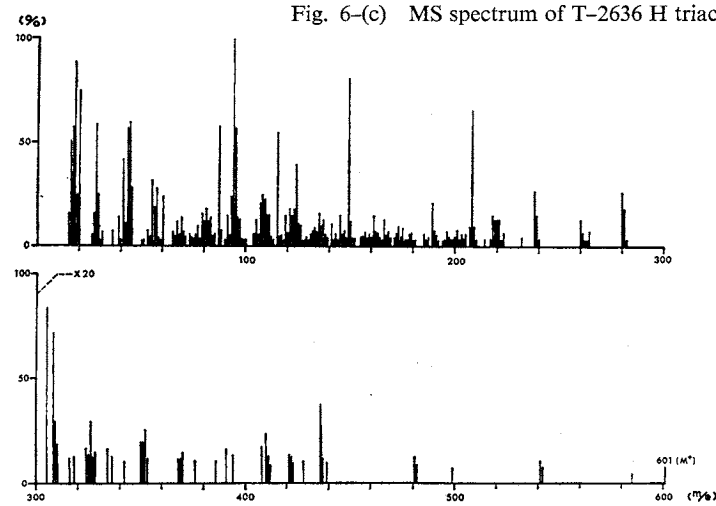


Fig. 6-(d) MS spectrum of lankacidinol 2′,8,14-triacetate

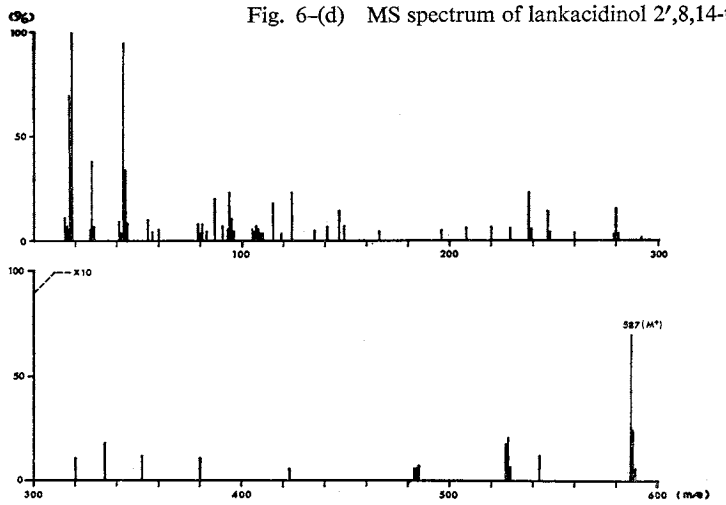
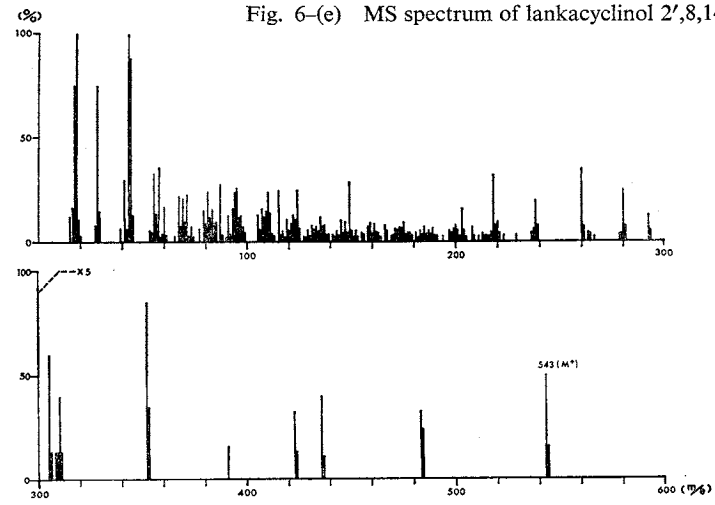


Fig. 6-(e) MS spectrum of lankacyclinol 2′,8,14-triacetate



Discussion

The metabolic fate of lankacidin C 14-P has been studied in rats and mice. Species differences have been noted in the rate of absorption and in the metabolic route. Both in rats and mice, the orally administered antibiotic was slowly absorbed, widely distributed in tissues and metabolized to be excreted into bile. The biliary metabolites were excreted into feces, together with the unabsorbed antibiotic.

It seems, however, that lankacidin C 14-P is absorbed from the alimentary tract more rapidly in mice than in rats. This conclusion is supported by the following findings: (1) after the oral administration, the blood level of radioactivity reached the maximum more rapidly in mice than in rats (Fig. 2); (2) tissue levels of radioactivity after oral dosing were significantly higher in mice than in rats (Table 1), and (3) biliary excretion of ^{14}C proceeded at a faster rate in mice than in rats (Fig. 4).

Some species differences were also noted in the metabolic route. The rat appears to metabolize lankacidin C 14-P primarily by hydrolytic and reductive processes, producing lankacidin C and lankacidinol 14-P, respectively. Lankacidin C was in part oxidized to yield T-2636 H and in part reduced to lankacidinol, and the latter was further decarboxylated to lankacyclinol (Chart 3). On the other

hand, the mouse seems to metabolize the antibiotic exclusively by the hydrolytic process followed by reduction and decarboxylation, producing lankacidin C, lankacidinol and lankacyclinol, respectively (Chart 4).

It has been established that in the rabbit the antibiotic is metabolized by the same route as in the mouse. The metabolites are identified as lankacidin C, lankacidinol and lankacyclinol whose antimicrobial activity were described by HARADA *et al.*[8].

In general, the blood level of an antibiotic after administration of an effective dose is usually higher than its minimum inhibitory concentration *in vitro*. This seems to be not the case for lankacidin C 14-P, since the blood levels of antimicrobial activity were extremely low in mice orally given the effective dose (50~200 mg/kg) of the antibiotic (Figs. 2 and 3).

Appreciable reservoirs of antimicrobial activity were, however, detected in liver, kidney and abdominal ascites of mice (Fig. 3).

In this connection, reference is made to the findings of BONVENTRE and IMHOFF[14] who showed that the proliferating regions of *Staphylococcus aureus* following intraperitoneal infection in mice were the peritoneal cavity, liver, kidney and spleen. Thus, it might be reasonable to consider that the therapeutic effect of lankacidin C 14-P against *S. aureus* infections in the mouse is largely derived from the active metabolites, lankacidin C and lankacidinol present in the areas of bacterial infection (peritoneal cavity) and proliferation (liver and kidney).

Chart 3. Metabolic pathway of lankacidin C 14-P-^{14}C in the rat

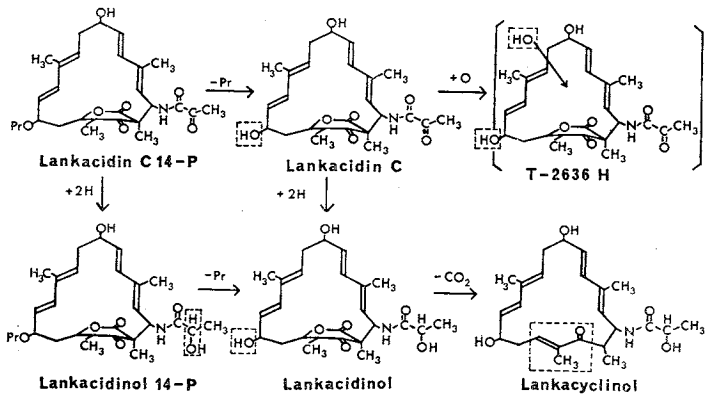
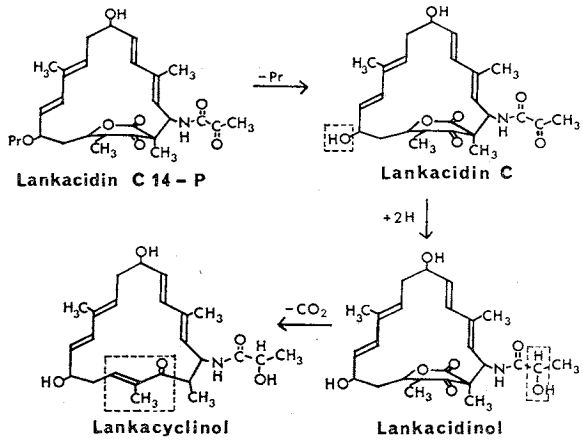


Chart 4. Metabolic pathway of lankacidin C 14-P-^{14}C in the mouse



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