STUDIES ON LANKACIDIN-GROUP (T-2636) ANTIBIOTICS. X

MICROBIAL CONVERSION OF LANKACIDIN-GROUP ANTIBIOTICS*

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Lankacidin C, a component of lankacidin-group (T-2636) antibiotics, was esterified to lankacidin C 8-butyrate in the presence of methyl butyrate by culture broth and by cell-free extract of *Bacillus megaterium* IFO 12108. In addition, methyl isobutyrate, methyl valerate and methyl isovalerate served as acyl donors for the esterification, and lankacidin C 8-isobutyrate, lankacidin C 8-valerate and lankacidin C 8-isovalerate were formed respectively. Lankacidin C 8,14-dibutyrate was hydrolyzed to lankacidin C 14-butyrate by the same organism.

Lankacidin-group (T-2636) antibiotics produced by *Streptomyces rochei* var. *volubilis* have been determined to be neutral seventeen-membered macrocyclic antibiotics[1,2,3,4]. Lankacidin C, a component of the antibiotics, showed strong antimicrobial activity mainly against Gram-positive bacteria.[5] Some lankacidin C mono-esters were found to be superior to lankacidin C in antimicrobial activity, protecting effects and toxicities.[6] Recently, they were also found to show antitumor effects.[7,8] Especially, lankacidin C 14-butyrate showed preferable biological properties in protecting and antitumor effects by oral administration in mice.[6,8]

It is very difficult to prepare lankacidin C mono-esters in high yield by chemical synthesis because lankacidin C has two hydroxyl groups (C-8 and C-14) that may be esterified. Thus, it is desirable to obtain microorganisms which esterify lankacidin C to lankacidin C mono-esters or which hydrolyze lankacidin C di-esters to lankacidin C mono-esters. It was reported that lankacidin C was esterified to lankacidin C 14-acetate (lankacidin A) in the presence of ethyl acetate by *S. rochei* var. *volubilis*[9] and that lankacidin C 8,14-diacetate was hydrolyzed to lankacidin C 8-acetate by the same organism.[6] This organism also catalyzes the esterification and the hydrolysis of formyl, acetyl and propionyl groups at the C-14 position. Subsequently, we found that lankacidin C 8,14-dibutyrate was hyrolyzed to lankacidin C 14-butyrate by *Bacillus megaterium* IFO 12108 and that lankacidin C was esterified to lankacidin C 8-butyrate in the presence of methyl butyrate by the same organism.

This paper describes the hydrolysis and the esterification of these lankacidin-group antibiotics by *B. megaterium* IFO 12108.

Materials and Methods

Antibiotics

Preparation methods of lankacidin C, lankacidin C mono-esters and lankacidin C di-esters were reported in the previous papers.[3,6]

* Microbial conversion of antibiotics. V. The preceding paper IV in this series corresponds to J. Antibiotics 27: 605~609, 1974.

Microorganism and culture conditions

B. megaterium IFO 12108 was obtained from the Institute for Fermentation, Osaka. The organism was grown in a medium at 28°C for 72 hours on a rotary shaker. The medium (pH 7.2) contained 2 % glucose, 1 % glycerol, 2 % soluble starch, 1 % soy bean flour, 1 % corn steep liquor (Corn Product Co., New York, U.S.A.), 1 % cottonseed meal (Okamura Seiyu Co., Osaka, Japan), 0.5 % Polypepton (Daigo Nutritive Chemicals, Osaka, Japan), 0.3 % NaCl and 0.5 % $CaCO_3$ in deionized water.

Preparation of cell-free extract

B. megaterium IFO 12108 was grown in a medium at 28°C for 48 hours on a rotary shaker. The medium (pH 7.2) contained 2 % dextrin, 0.5 % Polypepton, 0.5 % yeast extract (Difco Laboratories, Detroit, Michigan, U.S.A.) and 0.5 % meat extract (Wako Pure Chemicals, Osaka, Japan) in deionized water. Cells were harvested by centrifugation, washed twice with 0.02 M phosphate buffer (pH 7.2), suspended in the same buffer and disrupted with a sonic oscillator (Kubota Model 200 M) for 10 minutes. The disrupted cell suspension was centrifuged at 30,000 $\times g$ for 20 minutes to give the cell-free extract.

Assay of esterifying activity

Esterifying activity was assayed by measuring the amount of lankacidin C 8-butyrate formed from lankacidin C. To a mixture containing 400μmoles of phosphate buffer (pH 8.0), 8.0 mg (protein) of the cell-free extract and 2 mg of lankacidin C in a total volume of 4 ml, 0.5 ml of methyl butyrate was added. Since methyl butyrate was only slightly soluble in the mixture, the incubation was carried out with shaking at 37°C for 90 minutes. After the incubation, the reaction mixture was extracted with ethyl acetate. The amount of lankacidin C 8-butyrate in the extract was determined by TLC-bioautography.[10] The protein concentration was determined by the method of LOWRY *et al.*[11]

Results

Esterification of Lankacidin C to Lankacidin C 8-butyrate

Lankacidin C (360 mg) dissolved in methanol (18 ml) and methyl butyrate (90 ml) were added to the culture broth of *B. megaterium* IFO 12108 (720 ml). The reaction mixture was incubated at 28°C for 2 hours with shaking. Thin-layer chromatogram of the reaction mixture indicated that about 60 % of lankacidin C was converted to lankacidin C 8-butyrate.

The reaction mixture was then extracted with methyl isobutyl ketone (MIBK). The extract was washed with water and concentrated *in vacuo*. The concentrate was applied to a silica gel column and eluted with benzene-ethyl acetate (7~6 : 3~4). The eluate containing lankacidin C 8-butyrate was concentrated *in vacuo*. Lankacidin C 8-butyrate was crystallized from ether as colorless needles (144 mg); m.p. 201°C (decomp.). The UV, IR and NMR spectra and the elemental analysis of lankacidin C 8-butyrate thus prepared were identical with those of the authentic sample.[6]

Esterification of Lankacidin C by Cell-free Extract

Lankacidin C was esterified to lankacidin C 8-butyrate in the presence of methyl butyrate by the cell-free extract of *B. megaterium* IFO 12108.

The amount of lankacidin C 8-butyrate formed was proportional to the amount of added protein of the cell-free extract (Fig. 1) and to the incubation time (Fig. 2). The esterification required methyl butyrate as butyryl donor (Fig. 3), and showed the pH optimum at 8.0 (Fig. 4).

The esterifying activity with various butyryl donors is shown in Table 1. Methyl butyrate was more effective than ethyl butyrate. Propyl butyrate and butyl butyrate were less effective.

Fig. 1. Effect of cell-free extract on the esterification.

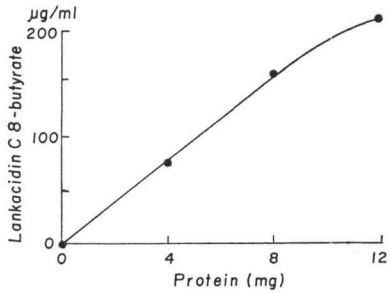


Fig. 2. Effect of incubation time on the esterification.

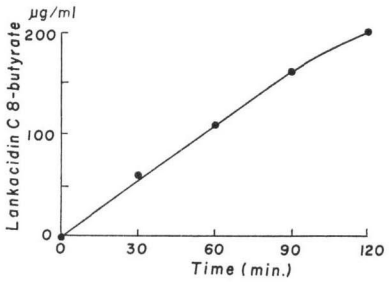


Fig. 3. Effect of methyl butyrate on the esterification.

To a mixture containing 400μmoles of phosphate buffer (pH 8.0), 8.0 mg (protein) of the cell-free extract and 2 mg of lankacidin C in a total volume of 4 ml, the indicated volume of methyl butyrate was added.

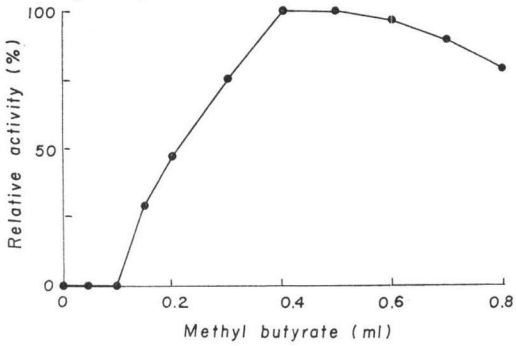


Fig. 4. Effect of pH on the esterification.

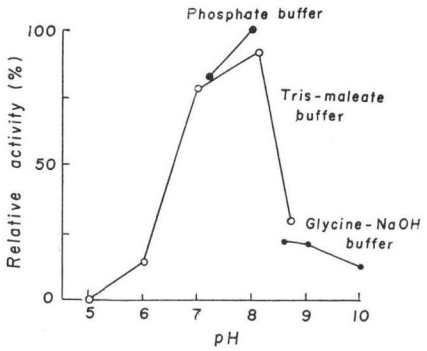


Table 1. Esterification with various butyryl donors.

Butyryl donor	Relative activity (%)
Methyl butyrate	100
Ethyl butyrate	65
Propyl butyrate	24
Butyl butyrate	24

The reaction mixture contained 0.5 ml of butyryl donor.

Table 2. Esterification of lankacidin C to lankacidin C 8-esters with various methyl esters.

Methyl ester	Lankacidin C 8-ester (μg/ml)
Methyl acetate	0
Methyl propionate	5
Methyl butyrate	160
Methyl isobutyrate	50
Methyl valerate	330
Methyl isovalerate	125
Methyl caprate	0

The reaction mixture contained 0.5 ml of methyl ester.

The esterification of lankacidin C to lankacidin C 8-esters was examined using various methyl esters as acyl donors. In addition to methyl butyrate, methyl isobutyrate, methyl valerate and methyl isovalerate served as acyl donors, and lankacidin C 8-isobutyrate, lankacidin C 8-valerate and lankacidin C 8-isovalerate were formed respectively. Methyl acetate, methyl propionate and methyl caprate did not serve as acyl group donor (Table 2).

Hydrolysis of Lankacidin C 8,14-dibutyrate to Lankacidin C 14-butyrate

Lankacidin C 8,14-dibutyrate (400 mg) dissolved in methanol (200 ml) was added to the culture broth of *B. megaterium* IFO 12108 (800 ml). The reaction mixture was incubated at 28°C for 1 hour with shaking. Thin-layer chromatogram of the reaction mixture indicated

that lankacidin C 8,14-dibutyrate disappeared and was converted mainly to lankacidin C 14-butyrate and partially to lankacidin C.

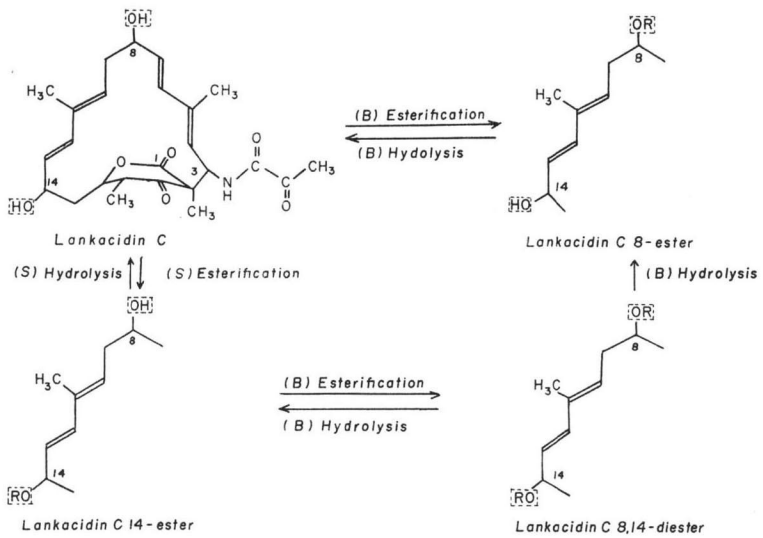
The reaction mixture was then extracted with MIBK. The extract was washed with water and concentrated *in vacuo*. The concentrate was applied to a silica gel column and eluted with benzene - ethyl acetate (7 : 3). The eluate containing lankacidin C 14-butyrate was concentrated *in vacuo*. Lankacidin C 14-butyrate was crystallized from ether as colorless needles (54 mg); m.p. 173°C (decomp.). The UV, IR and NMR spectra and the elemental analysis of lankacidin C 14-butyrate thus prepared were identical with those of the authentic sample.[6]

Discussion

It was reported that lankacidin C was esterified to lankacidin C 14-acetate (lankacidin A) in the presence of ethyl acetate by *S. rochei* var. *volubilis*.[9] In addition, lankacidin C 8,14-diacetate and lankacidin C 14-acetate were hydrolyzed to lankacidin C 8-acetate and lankacidin C, respectively, by the same organism.[8,9] As described in this paper, lankacidin C was esterified to lankacidin C 8-butyrate in the presence of methyl butyrate, and lankacidin C 8,14-dibutyrate was hydrolyzed to lankacidin C 14-butyrate by *B. megaterium* IFO 12108. Furthermore, lankacidin C 14-butyrate was esterified to lankacidin C 8,14-dibutyrate in the presence of methyl butyrate, and lankacidin C 8-butyrate was hydrolyzed to lankacidin C by the same organism (data not shown). The pathway of the esterification and the hydrolysis of these lankacidin-group antibiotics by *S. rochei* var. *volubilis* and *B. megaterium* IFO 12108 is schematically shown in Chart 1.

The esterase(s) of *B. megaterium* IFO 12108 that catalyzes the esterification and the hydrolysis is different from that of *S. rochei* var. *volubilis*. The esterase of *S. rochei* var. *volubilis* introduces and releases acyl groups at C-14 of lankacidin-group antibiotics. These acyl groups are either formyl, acetyl or propionyl groups. In contrast, the esterase(s) of *B. megaterium* IFO 12108 introduces and releases acyl groups at C-8 position, and the acyl groups are mainly butyryl and valeryl, but not formyl, acetyl and propionyl groups.

Chart 1. Pathway of the esterification and the hydrolysis of lankacidin-group antibiotics by *S. rochei* var. *volubilis* and *B. megaterium* IFO 12108.



R (acyl group)

(*S*): *S. rochei* var. *volubilis* formyl, acetyl, propionyl
(*B*): *B. megaterium* IFO 12108 butyryl, isobutyryl, valeryl, isovaleryl

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