LUMINAMICIN, A NEW ANTIBIOTIC

PRODUCTION, ISOLATION AND PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES

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(Received for publication May 22, 1985)

A new antibiotic, luminamicin, was isolated from the culture broth of an actinomycete strain OMR-59. It exhibits antibacterial activity against anaerobic bacteria, especially against *Clostridium* sp. The molecular formula of the antibiotic was determined as $C_{32}H_{38}O_{12}$ on the basis of high resolution mass spectrum, elemental analysis and NMR spectrum.

In the course of screening for antianaerobic antibiotics of actinomycete origin, we have found thiotetromycin[1] and clostomicin[2]. The continuing search led to the discovery of a new antibiotic, luminamicin, which showed antibacterial activity against anaerobic bacteria, especially against *Clostridium* sp. It was produced by strain OMR-59 isolated from a soil sample collected at Nerima-ku, Tokyo. Strain OMR-59 appears to belong to the genus *Nocardioides*[3] and further taxonomical studies are in progress.

The present paper deals with the production, isolation, physico-chemical and biological properties of luminamicin.

Producing Organism

The vegetative mycelium of strain OMR-59 grows abundantly and shows fragmentation on agar media, though sporophores have more than twenty spores per chain. Strain OMR-59 contains LL-diaminopimeric acid in the cell wall. From these results, strain OMR-59 appears to belong to the genus *Nocardioides*. Further taxonomical studies are in progress.

Fermentation

A stock culture of strain OMR-59 was inoculated into a 500-ml flask containing 100 ml of a sterile seed medium. The flask was shaken on a rotary shaker for 60~75 hours at 27°C. The seed medium was composed of glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5% and $CaCO_3$ 0.4%. Three hundred milliliters of the seed culture were transferred to 30 liters of a production medium consisting of glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, $CaCO_3$ 0.4%, $CoCl_2 \cdot 6H_2O$ 0.002% and agar 0.1% in a 50-liter jar fermentor. The fermentation was carried out at 27°C with aeration of 12.5 liters/minute and agitation of 300 rpm. The amount of the antibiotic produced was determined by a paper disk agar diffusion method using *Clostridium perfringens* as a test organism.

A typical time course for the fermentation is shown in Fig. 1. The antibiotic production started 17~25 hours after inoculation, then gradually increased and reached a maximum (26 μg/ml) at 46

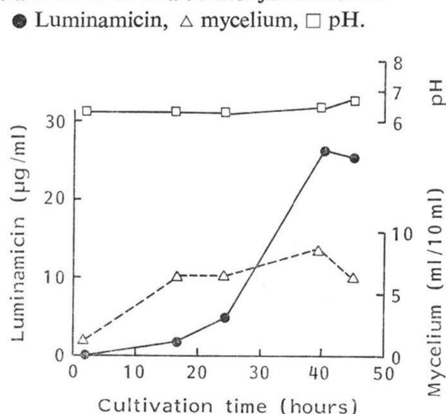

Fig. 1. Time course of luminamicin production by strain OMR-59 in a 50-liter jar fermentor.
● Luminamicin, △ mycelium, □ pH.

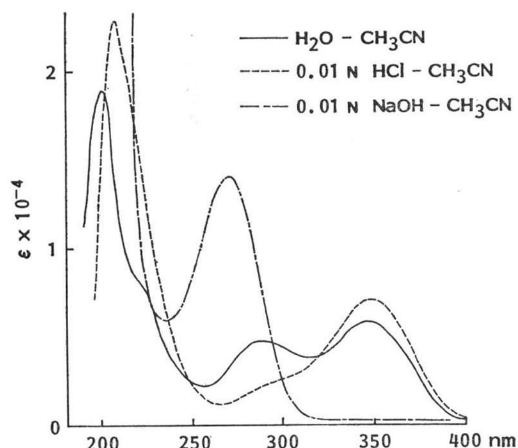

Fig. 2. UV spectrum of luminamicin.

Table 1. Physico-chemical properties of luminamicin.

Nature	Acidic, colorless needles
MP	245°C
Anal	Found: C 59.82, H 5.85
EI-MS	Calcd for $C_{32}H_{38}O_{12}$: *m/z* 614.236
	Found: *m/z* 614.236
Formula	$C_{32}H_{38}O_{12}$
UV λ_{max} nm (ε)	230 (sh), 291 (4,770), 347 (5,760) in H_2O - CH_3CN, 300 (sh), 348 (7,280) in 0.01 N HCl - CH_3CN, 271 (14,200) in 0.01 N NaOH - CH_3CN
IR cm^{-1}	1760, 1730, 1640, 1600

sh: Shoulder.

hours.

Isolation

The culture broth (60 liters), adjusted to pH 4.0 with 12 N HCl, was centrifuged to obtain about 40 liters of a supernatant fluid and 1.5 kg of wet mycelial cake. The mycelial cake was extracted with 7 liters of 85% aqueous acetone. After the removal of acetone by evaporation under reduced pressure, the aqueous solution was combined with the supernatant fluid. The mixture was passed through a column of non-ionic porous resin, Diaion HP-20 (2 liters). After washing the column with 4 liters of 50% aqueous acetone, the active principle was eluted with 5 liters of 80% aqueous acetone. The active fractions (3 liters) were collected and concentrated *in vacuo* to 360 ml. The aqueous solution was adjusted to pH 3.0 with 6 N HCl and extracted twice with 300 ml of ethyl acetate. The extracts were pooled and concentrated *in vacuo* to dryness to yield a brown paste (1.5 g). The paste, dissolved in 3 ml of benzene, was applied to a silica gel column (Merck, Kiesel gel 60, 30 g) packed in benzene; then the active principle was eluted sequentially with 200 ml of benzene containing 10% acetone; 150 ml of benzene containing 20% acetone; and finally with 200 ml of benzene containing 30% acetone. A head pressure of 1~2 kg/cm^2 was used to maintain a flow rate of approximately 2.0 ml/minute. The active fractions were concentrated *in vacuo* to give of a yellowish powder (280 mg). The powder was finally purified by HPLC apparatus (Jasco Tri Rotar V, column: YMC-Pack A-324 ODS 10×300 mm, solvent: 65% aqueous CH_3CN, flow rate: 3.0 ml/minute,

Fig. 3. IR spectrum of luminamicin (KBr).

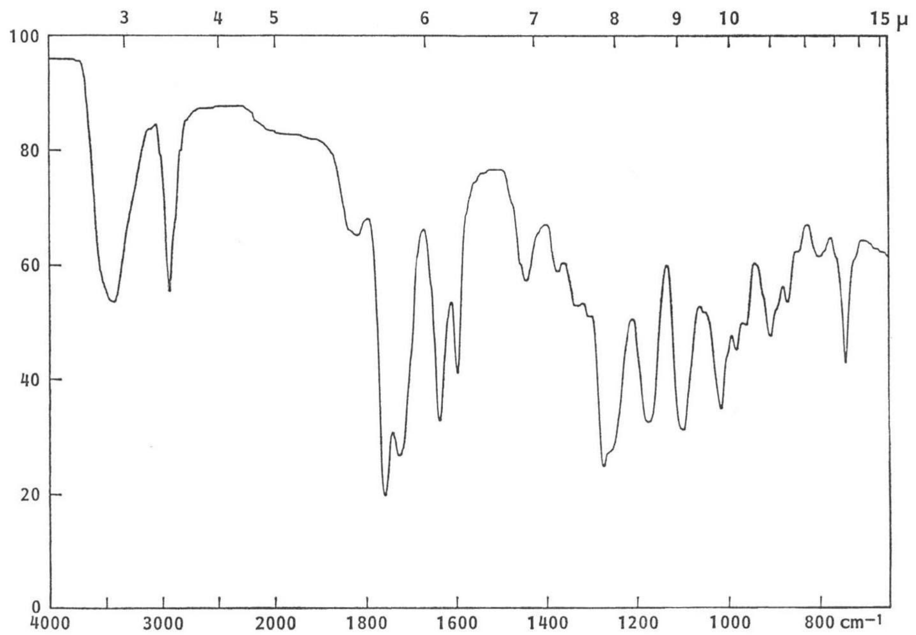

Fig. 4. ^{1}H NMR spectrum of luminamicin (400 MHz, acetone-d_6).

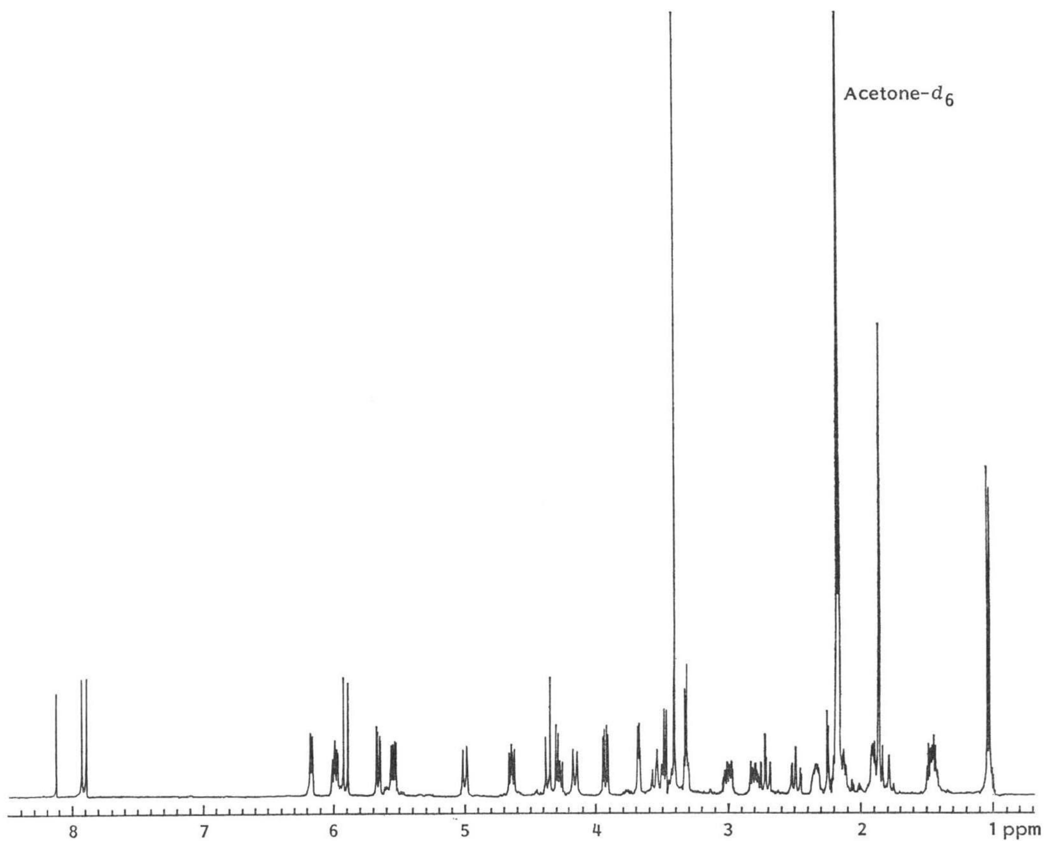

Table 2. Antimicrobial activity of luminamicin.

Microorganism	MIC (μg/ml)	
	Luminamicin	Vancomycin
Staphylococcus aureus FDA 209P	>100	NT
Bacillus subtilis ATCC 6633	100	NT
Micrococcus luteus ATCC 9341	25	NT
Escherichia coli NIHJ	>100	NT
Salmonella typhimurium KB 20	>100	NT
Klebsiella pneumoniae KB 214	>100	NT
Proteus vulgaris IFO 3167	>100	NT
Pseudomonas aeruginosa IFO 3080	>100	NT
Clostridium perfringens ATCC 3624	3.12	1.56
C. kainantoi IFO 3353	6.25	1.56
C. difficile ATCC 9689	6.25	1.56
C. kluyveri IFO 12016	12.5	1.56
Bacteroides fragilis ATCC 23745	12.5	50
Fusobaterium varium ATCC 8501	>100	>100

Anaerobic organisms were grown under anaerobic conditions.
NT: Not tested.

detection: UV 210 nm). Active fractions (retention time, 14.1 minutes) were combined and concentrated *in vacuo* to give a white powder. Colorless needles (160 mg) were obtained by crystallization from chloroform.

Physico-chemical Properties

Table 1 shows the physico-chemical properties of luminamicin. It is soluble in methanol, acetone and ethyl acetate, slightly soluble in chloroform, diethyl ether and benzene, and insoluble in water and *n*-hexane. The antibiotic gives a Rf value of 0.44 (benzene - acetone, 3: 1) on a precoated Merck 60 F_{254} silica gel plate.

The molecular formula was determined as $C_{32}H_{38}O_{12}$ by elemental analysis (the compound contained no nitrogen atom, Table 1), high resolution mass spectrometry (found m/z 614.236, calcd 614.236, Table 1) and ^{13}C NMR spectrum.

The UV, IR and ^{1}H NMR spectra of luminamicin are shown in Figs. 2, 3 and 4, respectively.

Biological Properties

Antimicrobial activities were assayed by a conventional agar dilution method using heart infusion agar for aerobic, and GAM agar for anaerobic bacteria with Gas Pak systems. The *in vitro* antibacterial spectrum of luminamicin is shown in Table 2. Luminamicin showed no activity against aerobic bacteria, except *Micrococcus luteus*. On the contrary, luminamicin showed selective activity against anaerobic bacteria, especially against *Clostridium* sp.

Intraperitoneal injection to mice at 100 mg/kg did not show any toxic effect.

Discussion

From the above physico-chemical properties luminamicin was differentiated from any other known antibiotics. Consequently, it was concluded that luminamicin is a new antibiotic.

Luminamicin was found to be selectively active against *Clostridium* strains including *C. difficile* which is known to cause pseudomembranous colitis[4]. Luminamicin was less active against some

anaerobes than vancomycin which is used clinically in therapy of pseudomembranous colitis (Table 2).

References

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