ACULEXIMYCIN, A NEW ANTIBIOTIC FROM *STREPTOSPORANGIUM ALBIDUM*

II. ISOLATION, PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES

TAKAYA IKEMOTO

Teikyo University School of Medicine
11-1, Kaga, 2-chome, Itabashi-ku, Tokyo 173, Japan

TOSHIAKI KATAYAMA, AKIO SHIRAISHI and TATSUO HANEISHI*

Fermentation Research Laboratories, Sankyo Co., Ltd.
2-58, 1-chome, Hiromachi, Shinagawa-ku, Tokyo 140, Japan

(Received for publication June 9, 1983)

A new larvicidal antibiotic, aculeximycin, was found in the culture broth of an actinomycete identified as *Streptosporangium albidum*. Aculeximycin was isolated from the culture filtrate by adsorption on a Diaion HP-20 column and successive elution with acidic aqueous acetone. It was extracted from the concentrated active fraction with 1-butanol and subjected to column chromatography on a Sephadex LH-20 column.

Aculeximycin exhibited strong larvicidal activity against mosquito larvae as well as antimicrobial activities against Gram-positive and Gram-negative bacteria, yeasts and molds.

As described in the preceding paper, a new larvicidal antibiotic, aculeximycin was produced by an actinomycete identified as *Streptosporangium albidum*.

Physicochemical properties and biological properties of aculeximycin differ from those of known antibiotics produced by Streptosporangium such as sporaviridin as well as those of antibiotics from streptomycetes.

In the present paper, isolation and physicochemical and biological properties of aculeximycin will be described.

Isolation

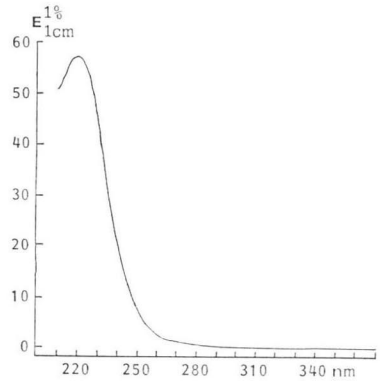
Fermentation broth (2.5 liters) containing the antibiotic was adjusted to pH 6.5 with 6 N NaOH and filtered with the aid of infusorial earth (Celite 545 from Johns-Manville Products Corp., Calif., U.S.A.). The filtrate (2.1 liters) was adsorbed on 300 ml of Diaion HP-20 (Mitsubishi Chemical Ind. Ltd., Japan) column. The column was washed with 600 ml of deionized water followed by washing with 50% aqueous acetone and the antibiotic was eluted with 50% acidic aqueous acetone (pH 2.0). The active fraction (1.5 liters) was neutralized to pH 7.0 and concentrated to remove acetone under reduced pressure. The concentrate was extracted twice with 100 ml of 1-butanol each time. The extracts were pooled and concentrated to 20 ml under reduced pressure. The concentrate was dropped into one liter of acetone under continuous stirring to form a light white precipitate. It was redissolved in 30 ml of deionized water and lyophilized to give 580 mg of white powder. One hundred and sixty mg of the lyophilized preparation dissolved in 5 ml or 50% aqueous methanol was applied on a Sephadex LH-20 column (300 ml) equilibrated with 50% aqueous methanol and developed with the same solvent mixture. The con-

* To whom all correspondence should be addressed.

Table 1. Physicochemical properties of aculeximycin in comparison with sporaviridin.

	Aculeximycin	Sporaviridin[2]
Nature	Basic, water soluble, amorphous powder. Extracted with 1-butanol	Basic, water soluble, amorphous powder. Extracted with 1-butanol
Mp	>200°C	>200°C
$[\alpha]_D^{25}$	+2.6° (*c* 0.76, H_2O)	+9.0° (*c* 1, H_2O)
Elementary analysis (%)	C 51.08 H 7.96 N 1.82	C 55.41 H 8.47 N 2.26
UV max. ($E_{1cm}^{1\%}$)	221 nm (55.5) in MeOH	227 (sh. 174), 233 (178), 242 nm (sh. 114) in H_2O
IR (KBr)	1680, 1640, 1120~1000 cm^{-1}	

Fig. 1. Ultraviolet absorption spectrum of aculeximycin in methanol.



centrated active fraction was lyophilized to yield 105 mg of white amorphous powder, which showed a single spot (I_2 vapor) on silica gel TLC plates (Merck Co. Ltd., Silica gel 60 F_{254}, Art 5715) developed with a solvent mixture (1-BuOH - AcOH - H_2O, 3: 1: 1).

Physicochemical Properties

The antibiotic was obtained as a white amorphous powder, soluble in water and methanol, but only sparingly soluble in acetone. The antibiotic reacted positively on TLC plates to sulfuric acid, iodine and potassium permanganate. It behaved as a basic substance on high voltage

Fig. 2. Infrared absorption spectrum of aculeximycin in KBr disc.

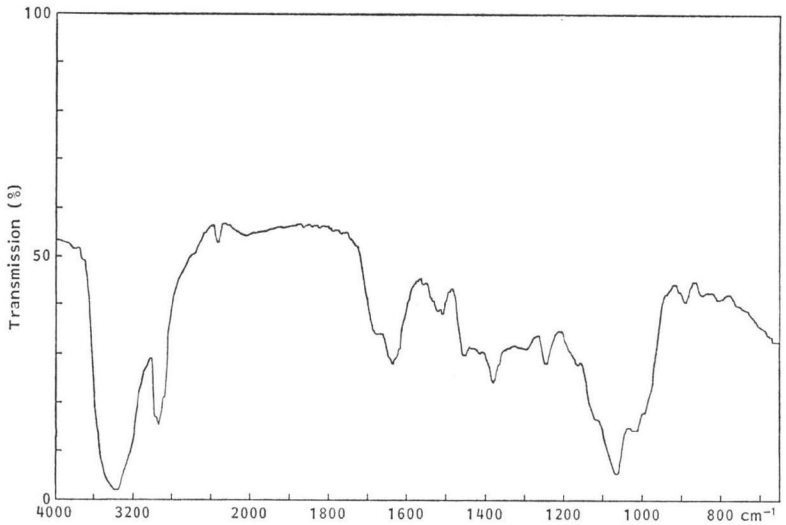


Fig. 3. ^1H NMR spectrum of aculeximycin in D_2O.

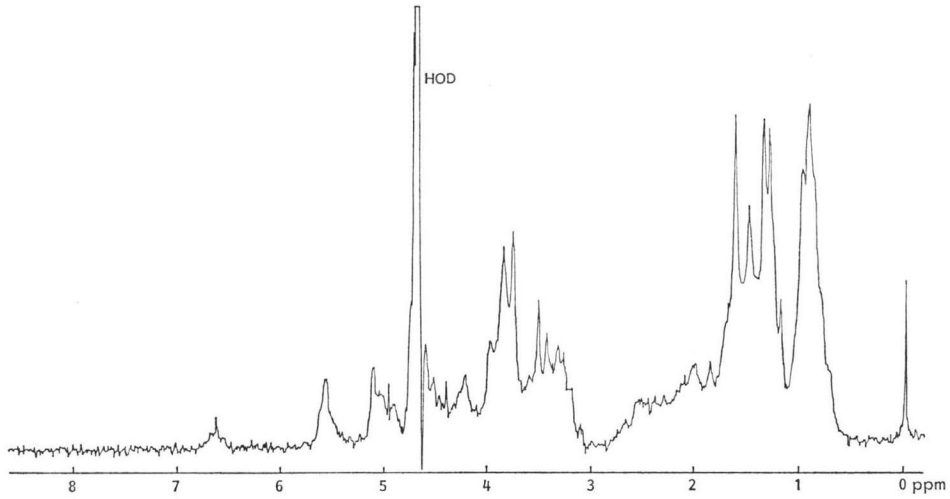


Table 2. Antimicrobial spectrum of aculeximycin.

Test organism	MIC (μg/ml)	Test organism	MIC (μg/mJ)
Staphylococcus aureus FDA 209P JC-1	0.39	*Serratia marcescens* SANK 73060	>100
S. aureus 56	0.78	*Pseudomonas acidovorans* SANK 72782	50
S. aureus 337	1.56	*P. aeruginosa* SANK 73575	50
S. aureus 42	1.56	*P. aeruginosa* NCTC 10490	>100
S. epidermidis SANK 71575	1.56	*Proteus vulgaris* OX 19	25
Streptococcus faecalis SANK 71778	>100	*P. mirabilis* SANK 70461	>100
Bacillus subtilis PCI 219	0.78	*P. mirabilis* SANK 71873	12.5
B. cereus SANK 70176	0.78	*Candida albicans* YU 1200	3.13
B. megaterium SANK 79959	0.78	*Saccharomyces cerevisiae* SANK 50170	1.56
Escherichia coli NIHJ JC-2	>100	*Pyricularia oryzae* SANK 16975	3.13
E. coli B IAM 1268	>100	*Pellicularia filamentosa* f. *sasakii* SANK 16376	1.56
E. coli 665	>100		
Klebsiella pneumoniae PCI 602	100	*Penicillium chrysogenum* SANK 12768	3.13
K. pneumoniae 846	>100	*Aspergillus niger* SANK	3.13

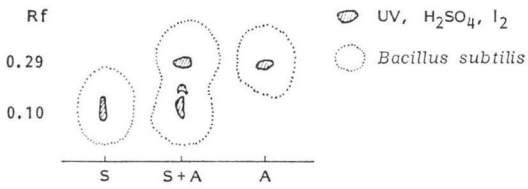
paper electrophoresis (55 Volts/cm, 0.6 mA/cm) in buffer at pH 1.8 (formic acid - acetic acid - water, 25: 75: 900) for 30 minutes. The relative mobility of the antibiotic was 0.5 when the mobility of alanine was defined as 1.0.

It showed no parent peak in EI or FD mass spectrometric analysis. These results, as well as other physical and chemical properties, are summarized in Table 1. The UV, IR and ^1H NMR spectra of the antibiotic are shown in Figs. 1, 2 and 3, respectively.

Biological Activity

The minimal inhibitory concentration (MIC) of aculeximycin against bacteria, yeasts and molds were determined by a serial two-fold agar dilution method. The results are shown in Table 2. The medium used for bacteria was Muller-Hinton agar (Difco Lab., U.S.A.); the medium for yeasts and molds was Sabouraud-dextrose agar. The MICs were read after one day at 37°C for bacteria, and 3 days at 27°C for yeasts and molds.

Fig. 4. Silica gel TLC of aculeximycin and sporaviridin.



S: Sporaviridin, A: Aculeximycin.
Silica gel 60 F_{254} (Merck Co. Ltd., Art 5715).
Solvent; 1-BuOH - AcOH - H_2O, 3: 1: 1.

Aculeximycin was active against Gram-positive bacteria, especially *Staphylococcus aureus, Staphylococcus epidermidis* and some species of Bacillus and also active against fungi such as *Candida albicans* and *Pyricularia oryzae*.

The larvicidal activity was expressed in term of IC_{50} determined by the mortality of first instar mosquito larvae in aqueous solutions containing different concentrations of the antibiotic at room temperature for 24 hours and the antibiotic was found to have potent activity against mosquito larvae with IC_{50} of 0.66 ppm.

The toxic effect of the antibiotic on killifish (ten fishes per group) was determined after incubation in one liter of a serial two-fold diluted antibiotic at room temperature for seven days. The mortalities of the fishes treated with the antibiotic at doses of 0.5 and 0.25 ppm were 100% and 30%, respectively.

The acute toxicity (LD_{50}) of the antibiotic in mice by intravenous injection is about 0.6 mg/kg.

Among known antibiotics, the one most related to aculeximycin in physicochemical properties and antimicrobial spectrum is sporaviridin, which is produced by *Streptosporangium viridogriseum*. These two antibiotics are similar with respect to their physicochemical properties as summarized in Table 1 as well as their antimicrobial spectra and toxicities in mice[2]. Nevertheless they were clearly differentiated from each other by their ultraviolet absorption spectra and direct comparison on silica gel TLC plate as shown in Fig. 4. The differences suggest that aculeximycin is a new antibiotic.

A second antibiotic, a sporaviridin-like substance produced by *S. albidum* could not be compared with aculeximycin, because of the lack of a detailed description of the physicochemical and biological properties[3].

Acknowledgment

We wish to express our gratitude to Prof. M. SASA of Teikyo University School of Medicine for his encouragement during the course of these studies. We are also grateful to Dr. T. FURUMAI of Nippon Roche Research Center for his providing sporaviridin.

References

1) IKEMOTO, T.; H. MATSUNAGA, K. KONISHI, T. OKAZAKI, R. ENOKITA & A. TORIKATA: Aculeximycin, a new antibiotic from *Streptosporangium albidum*. I. Taxonomy of producing organism and fermentation. J. Antibiotics 36: 1093~1096, 1983
2) OKUDA, T.; Y. ITO, T. YAMAGUCHI, T. FURUMAI, M. SUZUKI & M. TSURUOKA: Sporaviridin, a new antibiotic produced by *Streptosporangium viridogriseum* nov. sp. J. Antibiotics, Ser. A 19: 85~87, 1966
3) FURUMAI, T.; H. OGAWA & T. OKUDA: Taxonomic study on *Streptosporangium albidum* nov. sp. J. Antibiotics 21: 179~181, 1968