AN ANTIMYCOPLASMA ANTIBIOTIC ASTEROMYCIN: ITS IDENTITY WITH GOUGEROTIN

Sir:

Because of the high correlation between antitumor activity and antimycoplasma activity, we have been using a *Mycoplasma* species as a "detector organism" in our screening program for antitumor antibiotics. As a result, a water-soluble pyrimidine antibiotic of basic nature was found in a ferment of a *Streptomyces* species indexed as S-514 in our culture collection. *Streptomyces* S-514 was isolated from a soil sample of Tsukushino, Fukuoka Prefecture, Japan.

The antibiotic is active primarily against various *Mycoplasma* species and is cytotoxic to several tissue culture cell-lines. We compared the antibiotic with known antibiotics having antimycoplasma activity, and believing it to be a new one, named it asteromycin. Subsequently, we concluded that asteromycin is identical with the antibacterial antibiotic gougerotin[1,2,3], when we compared the physicochemical properties of the two antibiotics. It is of interest that no mention of either antimycoplasma or antitumor activity has been reported for gougerotin.

We report herein the results of the isolation and characterization of asteromycin and its identity with gougerotin.

The producing strain, S-514, was maintained on a KRAINSKY's agar slant. The organism was propagated in a modified WAKSMAN's medium consisting of 2% starch, 0.5% meat extract, 1% peptone, 0.5% NaCl and 0.2% $CaCO_3$ (pH 7.2) for 20 hours at 27°C. Three ml of this seed culture was inoculated into 100 ml of the same medium in a 500-ml flask and the fermentation conducted at 27°C on a reciprocating shaker. The titer, assayed by the pulp disc diffusion method against *Mycoplasma gallisepticum*, reached a maximum of 150 mcg/ml at 96 hours. The pH of the medium was 8.2~8.4 at the time of maximum potency.

In order to isolate the antibiotic 10 liters of the clarified culture filtrate was applied to a column (6×90 cm) of Amberlite IRC-50 (H^+). The column was washed extensively with water and the antibiotic was eluted with 0.1 N HCl containing 80% acetone (1,000 ml). Active fractions (350 ml) were combined and concentrated *in vacuo*. The active eluate was then passed through an Amberlite IR-45 (OH^-) column to remove impurities. After lyophilization, 9 g of a brown powder with a potency of 32~62 mcg/ml was obtained in 60~65% overall yield from the filtrate. This crude powder was further purified by alumina column (3×70 cm) chromatography. The antibiotic, dissolved in 90% methanol, was placed onto the column which was developed stepwise with 90%, 70% and 50% methanol solutions (250 ml each step). The active fractions, eluted with 50% methanol, were combined

Table 1. Physicochemical properties of asteromycin and gougerotin

Property	Asteromycin	Gougerotin
Nature	basic, colorless needles	basic, colorless needles
M. P.	188~200°C (dec.)	200~215°C (dec.)
Optical rotation	$[\alpha]_D^{25}$ +57° (*c* 1, H_2O)	$[\alpha]_D^{25}$ +45° (*c* 1, H_2O)
Analysis (%)	C 38.11, H 6.15, N 18.59	C 41.23~41.57, H 6.03~6.14, N 20.94~20.03
Formula	$C_{16}H_{25}O_8N_7 \cdot 3H_2O$	$C_{16}H_{25}O_8N_7 \cdot H_2O$
U.V. λ_{max} nm ($E_{1cm}^{1\%}$)		
in H_2O	234 (89.1), 266 (89.1)	228 (96), 269 (96)
in 0.1 N NaOH	266 (89.1)	228 (S), 269 (96)
in 0.1 N HCl	274 (129.6)	276 (141)
Color reaction		
positive	ninhydrin, ELSON-MORGAN, biuret, SAKAGUCHI, EHRLICH	ninhydrin, biuret, SAKAGUCHI
negative	MOLISCH, anthrone, $FeCl_3$	MOLISCH, $FeCl_3$

Fig. 1. Ultraviolet absorption spectra of asteromycin and gougerotin.

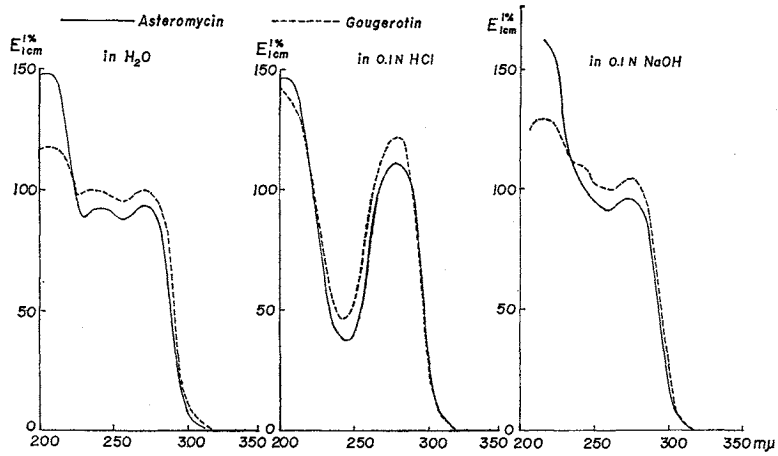
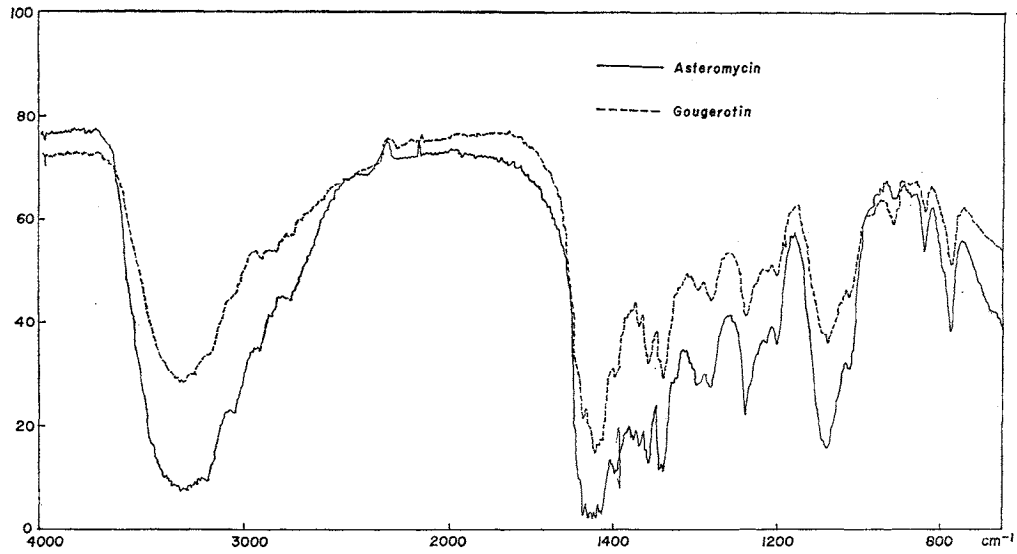


Fig. 2. Infrared absorption spectra of asteromycin and gougerotin (KBr).



and concentrated. Lyophilization yielded 1.5 g of amorphous white powder. This powder was further purified by recrystallization from methanol-water-ethylether (4 : 1 : 8), giving 300 mg colorless needle crystals.

The physicochemical properties of asteromycin and gougerotin are very similar as summarized in Table 1 and Fig. 1. The difference in the elemental analysis is explained by crystalline asteromycin containing two more moles of hydration water.

The identification of asteromycin with an authentic sample of gougerotin was confirmed by conducting a mixed melting point determination and by comparing their infrared spectra (Fig. 2).

Asteromycin inhibits the growth of several *Mycoplasma* species at concentrations of 1.0~5.0 mcg/ml by the broth dilution method

Table 2. Antimycoplasmal activity of asteromycin by broth dilution method

Organism	MIC* (mcg/ml)
Mycoplasma salivarium	6.0
M. hominis	6.0
M. orale	1.5
M. fermentans	3.0
M. pneumoniae Mac	3.0
M. gallisepticum	1.5
M. pulmonis mA	6.0
Acholeplasma laidlawii	3.0

* MIC=minimum inhibitory concentration

(Table 2). It has a weak but broad antibacterial spectrum, with minimum inhibitory concentrations of the order of 500~2,000 mcg/ml. Determination of the acute toxicity of asteromycin in mice by the intraperitoneal route gave an LD_{50} of 250 mg/kg. In a subacute toxicity test, lethal effects (LD_{50}) were observed when doses of 10 mg/kg/day were given intraperitoneally for one week. Asteromycin is active against Sarcoma-180 ascites tumor in mice at a dose of 1.5 mg/kg/day. The minimum amount needed to cause 50 % inhibition of the growth of HeLa cells is 1~2 mcg/ml.

From the results described above the identity of gougerotin and asteromycin is confirmed. We should like to emphasize that *Mycoplasma* species are the most susceptible organisms to this antibiotic.

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