CAPSIMYCIN, A NEW ANTIBIOTIC. I

PRODUCTION, ISOLATION AND PROPERTIES

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(Received for publication October 9, 1978)

Capsimycin is a new antifungal antibiotic produced by a strain of *Streptomyces* sp. C 49–87. The active substance in the fermented broth was isolated by solvent extraction followed by silica gel column chromatography. The antibiotic melts at 186°C (decomp.) and has a molecular formula $C_{30}H_{40}N_2O_6$. It exhibits marked inhibitory activity against *Phytophthora capsici* (Leaf blight disease of cucumber) and *Pythium debaryanum* (Damping-off disease of cucumber).

In our laboratories we have devised a pot test with cucumber seedlings to test for organisms active against *Phytophthora capsici*. As a result of this screening program, *Streptomyces* No. C 49–87 was found to produce a new antibiotic with anti-*Phytophthora capsici* activity. It was designated capsimycin.

Capsimycin is considered to be new because it differs from other antibiotics in certain of its physico-chemical properties.

This paper describes its production, isolation and characterization.

Materials and Methods

Production of the antibiotic

Streptomyces sp. strain C 49–87 was used to inoculate 100 liters of medium in a fermentor. The culture was incubated at 27°C for 90 hours with aeration and agitation. The medium used was as follows: 1.0% glucose, 3.5% sucrose, 3.5% wheat embryo, 0.5% yeast, 0.3% $(NH_4)_2SO_4$, 0.2% NH_4Cl, 0.9% $CaCO_3$, 0.004% $FeSO_4$, 0.2% $MgSO_4 \cdot 7H_2O$, 0.005% $MnSO_4$ and 0.01% $ZnSO_4$.

The antibiotic activity was determined by the pot test method with cucumber seedlings using *Phytophthora capsici* as the test organism. The broth was filtered using diatomaceous earth as a filter aid. The filtrate was adjusted to pH 4.0 and extracted with ethyl acetate (50 liters). The mycelial cake was extracted with acetone. The aqueous solution obtained by evaporating the acetone was extracted with ethyl acetate. The ethyl acetate extracts were combined and extracted with alkaline water (pH 8.9). After being adjusted to pH 4.0, the aqueous phase was again extracted with ethyl acetate and the extract was concentrated to a small volume under reduced pressure. The addition of *n*-hexane to the concentrated solution precipitated about 15 g of crude antibiotic. This was dissolved in a small amount of chloroform and passed through a silica gel column (Wako-gel C-200, 150 g). The column was washed successively with portions of chloro form (2 liters total). To elute the antibiotic a mixture of chloroform - methanol (20: 1) was used. Fractions active against *Phytophthora capsici* were combined, evaporated to dryness *in vacuo* and crystallized from ethanol to give capsimycin as colorless needles (5 g).

Results

Physico-chemical Properties

Capsimycin is a weakly acidic substance with pKa′ 5.5 in 50% aqueous tetrahydrofuran and the following physical and chemical properties:

Solubility: soluble in ethyl acetate, acetone, methanol and ethanol; insoluble in ether, benzene and *n*-hexane.

Melting point: 186°C (decomp).

Optical rotation: $[\alpha]_D^{20}$ +196° (*c* 1, chloroform).

Color reactions: positive to LEMIEUX, 2,4-dinitrophenylhydrazine and $FeCl_3$ reactions; negative to ninhydrin, MOLISCH and anthrone reactions.

Thermostability: when capsimycin was dissolved in methanol-water and kept at 100°C for 5 minutes, it is stable in the pH range from pH 4~11, but unstable at more acidic pH values. In crystalline form it was stable for 2 weeks at 70°C, 4 weeks at 60°C and 2 months at 45°C.

Table 1. Thin-layer chromatography of capsimycin (silica gel GF_{254}).

Solvents	Rf value
$CHCl_3$	0.00
$CHCl_3$ - MeOH (1:1)	0.75
$CHCl_3$ - MeOH (3:1)	0.39
$CHCl_3$ - MeOH (5:1)	0.30
EtOAc	0.02
EtOAc - MeOH (1:1)	0.45
EtOAc - MeOH (3:1)	0.12

The Rf values of capsimycin on silica gel GF_{254} TLC plates using various solvents are shown in Table 1. Its position on the plates was detected by its ultraviolet absorption and by charring after spraying with 10% H_2SO_4.

Elemental analysis

Found: C, 68.66; H, 7.66; O, 18.34; N, 5.30.

Calcd. for $C_{30}H_{40}N_2O_6$ (MW 524.64): C, 68.68; H, 7.69; O, 18.30; N, 5.34.

The molecular weight was confirmed from the mass spectrum which showed a molecular ion peak at *m/e* 524.

The ultraviolet absorption spectrum (Fig. 1) showed λ_{max} at 220 nm ($E_{1cm}^{1\%}$ 918) and 325 nm

Fig. 1. Ultraviolet absorption spectra of capsimycin.

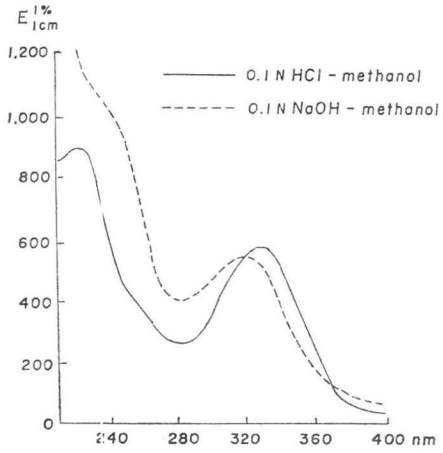

Fig. 2. I.R. absorption spectrum of capsimycin (in KBr).

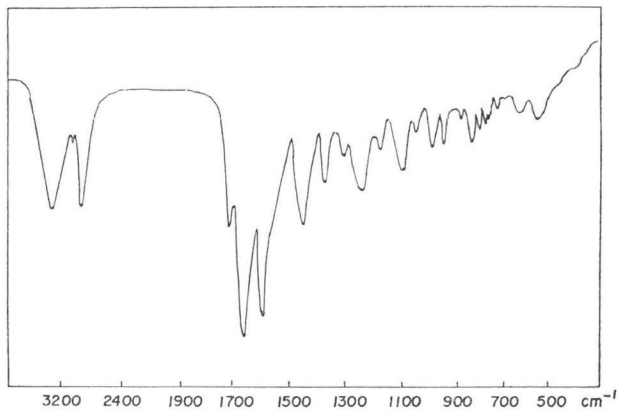

Fig. 3. ^{1}H-NMR spectrum of capsimycin.

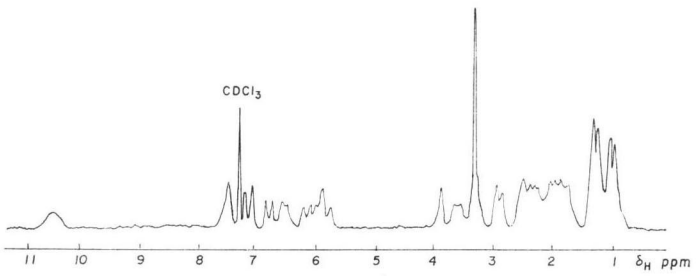

Fig. 4. ^{13}C-NMR spectrum of capsimycin.
100 MHz, $CDCl_3$=76.9 ppm.

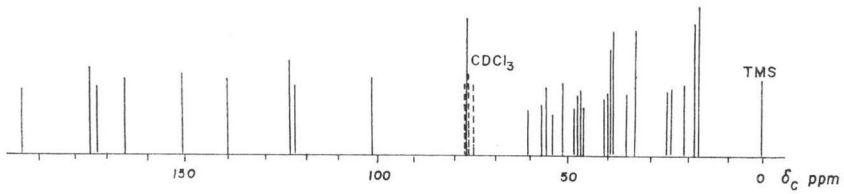

Table 2. ^{13}C-NMR resonances of capsimycin.

No.	δC ppm	Multiplicities*	No.	δC ppm	Multiplicities*
1	17.34	q	16	50.16	d
2	17.67	q	17	53.70	d
3	20.94	t	18	55.05	q
4	25.53	t	19	58.07	d
5	27.35	t	20	61.54	d
6	33.75	d	21	77.31	d
7	36.97	t	22	100.65	s
8	38.91	t	23	122.79	d
9	38.96	t	24	124.28	d
10	40.54	d	25	139.85	d
11	41.09	d	26	150.87	d
12	45.84	d	27	166.23	s
13	47.12	d	28	173.57	s
14	47.59	d	29	175.74	s
15	48.79	d	30	196.16	s

* Multiplicities in the off-resonance spectrum. s: singlet, d: doublet, t: triplet, q: quartet.

Table 3. Protection of cucumbers in pot tests with capsimycin.**

Test product	ppm	Protective value (%)*	
		Phytophtora capsici	*Pythium debaryanum*
Capsimycin	100	100	100
	50	100	100
	25	100	83
Captafol	400	66	66

* Protective value $= \left[1 - \frac{\text{fungus titer (antibiotic-treated leaf)}}{\text{fungus titer (untreated leaf)}}\right] \times 100.$

** No phytotoxicity was seen.

($E^{1\%}_{1cm}$ 594) in a 0.1 N hydrochloric acid methanol solution and λ_{max} 320 nm ($E^{1\%}_{1cm}$ 567) in a 0.1 N sodium hydroxide methanol solution.

The infrared spectrum (Fig. 2) showed the presence of NH or OH functions at 3380 cm^{-1} and an amide function at 1580 cm^{-1}.

The ^{1}H-NMR spectrum taken at 100 MHz in $CDCl_3$ (Fig. 3), showed the presence of two C-methyl, one O-methyl, one epoxide and one *trans* double bond functions. The ^{13}C-NMR spectrum of capsimycin in $CDCl_3$ is shown in Fig. 4, chemical shifts and multiplicities in the off-resonance spectrum are summarized in Table 2.

Biological Properties

Capsimycin was active against several fungi (Table 3), but inactive against most bacteria.

The LD_{50} (*per os*) in mice was 600~700 mg/kg.

Discussion

Capsimycin can be differentiated from known antibiotics. Those with absorption maxima within the same wavelength range as capsimycin are ikarugamycin[1] ($C_{29}H_{38}N_2O_4$), althiomycin[2] ($C_{27}H_{28}N_8O_{10}S_3$), antimycin[3] ($C_{28}H_{40}N_2O_9$), blastmycin[4] ($C_{26}H_{36}N_2O_9$), methymycin[5] ($C_{25}H_{43}NO_7$), vulgarin[6], streptolydigin[7] ($C_{32}H_{44}N_2O_9$), equisetin[8] ($C_{22}H_{31}NO_4$) and tirandamycin[9] ($C_{22}H_{27}NO_7$).

Since they are different from capsimycin in elementary analysis, optical rotation, melting point, infrared spectrum and NMR spectrum, we believe capsimycin to be a new antibiotic.

Acknowledgement

The authors are indebted to Dr. S. KAWABATA for the ^{13}C-NMR spectrum.

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