A NEW ANTIHERPETIC AGENT, AH-135Y, PRODUCED BY *Streptomyces albovinaceus* STRAIN NO. AH-135

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As the most prominent acyclic derivative of guanine, 9-[(2-hydroxyethyl)methyl]guanine (acyclovir, ACV)[1] is now in clinical use for the treatment of deseases caused by herpes simplex viruses (HSV). However, ACV-resistant mutants of HSV are increasing as a result of loss or alteration of thymidine kinase (TK) activity or alternation in the virus DNA polymerase[2]. We started the screening in order to search for novel antiherpetic agent in place of ACV. Recently, we isolated a new antiherpetic agent, AH-135Y (Fig. 1) from the culture filtrate of a streptomycete, *Streptomyces albovinaceus* strain No. AH-135. This compound turned out to belong to the glutarimide antibiotics and has antiviral activity against herpes simplex virus (HSV)-I.

Among 520 *Streptomyces* strains tested, a potent strain for the antiherpes agent, strain No. AH-135, was selected. This strain was isolated from a soil sample obtained from Yaku-shima, Kagoshima Prefecture. From the key characters based on (W; RF; C^-; SM), that is, white series of aerial mycelium; *Rectus-Flexibiles*; chromogenicity negative; smooth spore wall, this strain was classified as a strain belonging to *Streptomyces albovinaceus* Kudrina with minor exception on carbon sources utilization. Therefore, it was called *Streptomyces albovinaceus* No. AH-135, hereafter.

A slant culture of *Streptomyces albovinaceus* strain No. AH-135 was maintained on a MGA agar slant at 4°C. The slant culture was inoculated into 200-ml Erlenmeyer flasks containing 50 ml of a seed medium which consisted of 2% glucose, 3% soluble starch, 1% soybean flour, 1% corn steep liquor, 0.5% Polypepton, 0.3% NaCl, 0.5% $CaCO_3$ (pH 7.0). The flasks were incubated on a rotary shaker (180 rpm, 5-cm radius) at 28°C for 2 days. A 200-ml portion of the seed culture was transferred into a 10-liter jar fermenter containing 5 liters of a production medium having the same composition as the seed medium. The fermentation was carried out at 28°C for 3 days under agitation of 400 rpm and aeration of 4 liters per minute. The activity reached the maximum in culture after 96-hour fermentation.

Culture broth (7.2 liters) was centrifuged and the supernatant fluid was extracted with ethyl acetate at pH 2. AH-135Y was isolated from the concentrated organic layer by column chromatography on Diaion HP-10 (Mitsubishi Chemical Industries, Ltd.), followed by that on Diaion HP-20SS (the same company) using methanol as the eluent. The fractions of AH-135Y were concentrated. An excess of ether was added to precipitate white powder. Purified AH-135Y was obtained with yield of 10 mg.

Physico-chemical properties are shown in Table 1. AH-135Y was obtained as white powder with the mp at 220°C. It was optically inactive when measured in methanol (*c* 0.1). The compound was soluble in methanol, ethanol, acetone, ethyl acetate or dimethyl sulfoxide, and slightly soluble in chloroform, and insoluble in water, ether and *n*-hexane. The UV absorption maxima of AH-135Y

Fig. 1. Structure of AH-135Y.

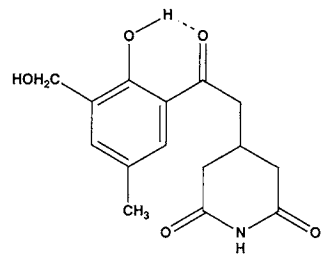


Table 1. Physico-chemical properties of AH-135Y.

Property		
Nature	White powder	
MP (°C)	220°C	
$[\alpha]_D^{25}$ (MeOH)	Inactive	
EI-MS (*m/z*)	291	
Molecular formula	$C_{15}H_{17}NO_5$	
UV λ_{max} nm (ε)	MeOH	Acidic MeOH
	219 (18,472)	219 (14,821)
	260 (9,066)	260 (7,066)
	343 (4,026)	342 (3,257)
IR ν_{max} (KBr) cm^{-1}	3510, 1715, 1660, 1270	

Table 2. NMR data of AH-135Y.

Position	δ_C (ppm)	δ_H (ppm)
1	156.2	—
2	130.6	—
3	134.8	7.49 (1H, s)
4	127.2	—
5	128.3	7.65 (1H, s)
6	118.1	—
7	205.3	—
8	42.3	3.19 (2H, d, J=6 Hz)
9	25.9	2.57 (1H, m)
10	36.9	2.39, 2.62 (2H, m, dd, J=10, 17 Hz)
11	172.8	—
12	—	10.77 (1H, s)
13	172.8	—
14	36.9	2.39, 2.62 (2H, m, dd, J=10, 17 Hz)
1-OH	—	12.20 (1H, s)
2-CH_2	57.1	4.51 (2H, d, J=6 Hz)
2-CH_2OH	—	5.17 (1H, s)
4-CH_3	20.1	2.29 (3H, s)

in methanol were observed at 219 (shoulder; ε 18,472), 260 (ε 9,066) and 343 nm (ε 4,026). This absorption was virtually unchanged in acidic methanol. In alkaline methanol, the solution became turbid to form an inactive compound. It showed absorptions at 3510, 1715 and 1660 cm^{-1} due to the hydroxyl, imide and carbonyl group, respectively, in the IR spectrum. The MW of 291 was obtained from EI-MS. The elementary analysis of AH-135Y afforded a molecular formula $C_{15}H_{17}NO_5$, which agreed with the M^+ (m/z 291) by EI-MS. The compound gave a positive color reaction to the ferric chloride. These results indicated the presence of a phenol, hydroxyl or imide group. The UV spectrum of AH-135Y was similar to that of actiphenol[3], actiketal[4] and non-kang 101 G[5] which belong to the glutarimide antibiotics.

The structure of AH-135Y was elucidated from the ^1H NMR, ^{13}C NMR (Table 2), ^1H-^1H COSY and ^{13}C-^1H COSY spectral data and the results of the HMBC (Fig. 2) experiment. The ^1H NMR spectrum showed seventeen signals due to one methyl, four methylene, one NH and two OH protons. The ^{13}C NMR spectrum exhibited thirteen signals consisting of one methyl, four methylene, one methine, six aromatic and three carbonyl carbons. The methylene signal at δ 36.9 showed higher intensity than that of δ 42.3, and the carbonyl signal at δ 172.8 showed higher intensity than that of δ 205.3. These may be explained by the presence of two carbons having same structure. In the ^1H NMR spectrum, the reason for the low field shift of the hydroxyl signal at δ 12.20 may be explained by the intramolecular hydrogen bonding with the carbonyl group. The correlation between protons and carbon was elucidated from the ^1H-^1H COSY and the ^{13}C-^1H COSY spectra. The HMBC experiment showed the presence of an aromatic ring and a glutarimide ring (Fig. 2). From these results, we proposed the structure of AH-135Y as shown in Fig. 1. It is closely related to actiphenol, having a methyl group at C-3 in place of the hydroxymethyl group.

Fig. 2. Long range coupling observed by HMBC experiment (in DMSO-d_6).

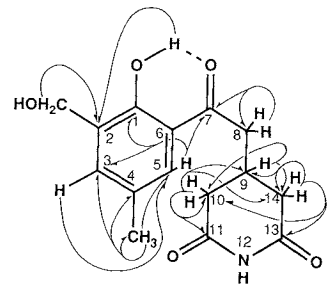
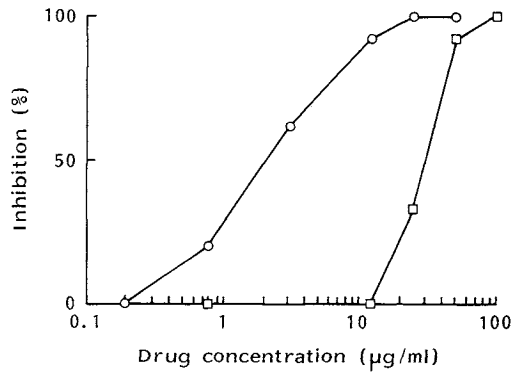


Fig. 3. Antiherpetic activity (○) and cytotoxicity (□) of AH-135Y.

○ Inhibition of plaque formation by HSV-I infection, □ inhibition of growth of Vero cells.



The antiviral and anticellular activity of AH-135Y was measured by the plaque reduction assay and cell growth inhibition test using Vero cells and HSV-I (KOS). AH-135Y showed the antiherpetic activity of 2.1 μg/ml as EC_{50} against HSV-I, and cytotoxicity of 30.0 μg/ml as IC_{50} against Vero cells (Fig. 3) and therefore selectivity (the ratio of IC_{50} to EC_{50}) was 14.3. AH-135Y showed similar antiherpetic activity *in vitro* to that of idoxuridine (IDU) reported by SUZUTANI *et al.*[6]. For the cytotoxicity *in vitro*, however, AH-135Y showed

Table 3. Antimicrobial spectrum of AH-135Y.

Strain used	MIC (μg/ml)
Bacillus subtilis	>100
Staphylococcus aureus	>100
Micrococcus luteus	>100
Escherichia coli	>100
Proteus vulgaris	>100
Pseudomonas aeruginosa	>100
Saccharomyces cerevisiae	1
Candida albicans	>100
Aspergillus niger	>100
A. oryzae	>100

weaker activity than IDU.

AH-135Y showed antifungal activity exclusively against *Saccharomyces cerevisiae* (MIC=1μg/ml), but did not show antibacterial activity (Table 3). Actiphenol on the other hand did not inhibit the growth of bacteria and *Saccharomyces cerevisiae*[7]. Glutarimide antibiotics are reported to have antifungal[8], antimitogenic[9] or antiviral activities[10]. Glutarimide antibiotics which have an aromatic or unsaturated ring in their structures are also reported to show weaker activity against fungi and bacteria than those with a saturated ring. Though AH-135Y has an aromatic ring in its structure, it showed strong activity against HSV-I. Details on the mechanism of action of AH-135Y remain to be examined.

References

1) ELION, G. B.; P. A. FURMAN, J. A. FYFE, P. DE MIRANDA, L. BEUCHAMP & H. J. SCHAEFFER: Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. Proc. Natl. Acad. Sci. U.S.A. 54: 5716~5720, 1977
2) SCHNIPPER, L. E. & C. S. CREEMPACKER: Resistance of herpes simplex virus to acycloguanosine; the role of the viral thymidine kinase and DNA polymerase loci. Proc. Natl. Acad. Sci. U.S.A. 77: 2270~2273, 1980
3) HIGHET, R. J. & V. PRELOG: Sttoffwechselprodukte von Actinomyceten, Actiphenol. Helv. Chim. Acta 42: 1523~1526, 1956
4) SONODA, T.; H. OSADA, J. UZAWA & K. ISONO: Actiketal, a new member of the glutarimide antibiotics. J. Antibiotics 44: 160~163, 1991
5) HUA, J.-C. & Y.-Y. XIE: Isolation and structure of antibiotics non-kang 101 F and 101 G. Hua Hsueh Hsueh Pao. 38: 275~282, 1980
6) SUZUTANI, T.; H. MACHIDA & T. SAKUMA: Efficacies of antiherpes-virus nucleosides against two strains of herpes simplex virus type I in Vero and human embryo lung fibroblast cells. Antimicrob. Agents. Chemother. 32: 1046~1052, 1988
7) ASZALOS, A.; H. HOBERECHT & A. I. COHEN: Structure of antibiotic C-73X. J. Med. Chem. 10: 281~284, 1967
8) UBRIZSY, G. & J. VOROS: The inhibiting effect of antibiotics on wood-destroying fungi. Acta Agron. Acad. Sci. Hung. 12: 167~172, 1965
9) SONODA, T.; H. OSADA, J. MAGAE & K. ISONO: Epiderstatin and its related glutarimide antibiotics inhibit the cell growth induced by mitogen stimulation. Agric. Biol. Chem. 54: 1259~1263, 1990
10) HAFF, R. H.: Inhibition of the multiplication of pseudorabies virus by cycloheximide. Virology 22: 430~431, 1964