HERBIMYCIN B, A NEW BENZOQUINONOID ANSAMYCIN WITH ANTI-TMV AND HERBICIDAL ACTIVITIES

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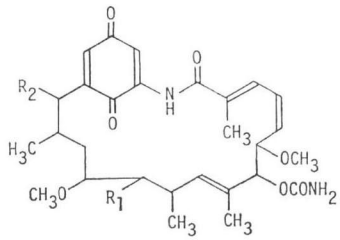
A new benzoquinonoid ansamycin, herbimycin B was isolated from the culture broth of *Streptomyces hygroscopicus* No. AM-3672, a herbimycin A-producing strain. Herbimycin B showed potent anti-TMV activity. Herbicidal effect of herbimycin B was less than that of herbimycin A.

In the previous papers[1,2], we reported that *Streptomyces hygroscopicus* strain No. AM-3672 produced herbimycin A**, a new benzoquinonoid ansamycin which had potent herbicidal activity, and its structure (Fig. 1) was determined by ^{1}H- and ^{13}C-NMR spectral analyses and the biosynthetic method of feeding experiments with ^{13}C-labeled precursors followed by ^{13}C-NMR analysis of the labeled product. Further investigation led to the discovery of a new component, herbimycin B from the culture filtrate of the same organism.

The present paper deals with the isolation, characterization and biological properties of herbimycin B. In addition, biological comparisons of the two antibiotics, herbimycins A and B as regards herbicidal and anti-tobacco mosaic virus (TMV) activities were studied.

Fig. 1. Structures of herbimycins A and B.



Herbimycin		R_1	R_2
	A	OCH_3	OCH_3
	B	OH	H

Production of Herbimycin B

Streptomyces hygroscopicus No. AM-3672 (FERM-P 4335) was transferred into a 500-ml flask containing 100 ml of seed medium and cultivated at 30°C for 72 hours on a rotary shaker (240 rpm). The seed culture (300 ml each) was transferred into three 30-liter jar fermentors containing 20 liters of production medium and the fermentation was carried out at 30°C for 43 hours

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** Herbimycin[1,2] is now re-named herbimycin A.

with agitation of 350 rpm and with aeration of 20 liters/min. The composition of seed and production medium was 2.5% glucose, 1.0% soybean meal, 0.4% KCl, 0.25% yeast extract, 0.1% meat extract, 0.5% $(NH_4)_2SO_4$, 0.02% K_2HPO_4 and 0.3% $CaCO_3$ (pH 7.2 before sterilization), and Disfoam BC-51Y (Nihon Yushi-Chemical Co., Ltd.) was used as an antifoam agent.

Isolation of Herbimycin B

Herbimycins were monitored by thin-layer chromatography (TLC) on silica gel (Merck, GF_{254}), eluted with ethyl acetate - *n*-hexane - chloroform - methanol (9: 6: 1: 1, v/v) after visualization by irradiation with UV light. The Rf values of herbimycins A and B were 0.65 and 0.47, respectively.

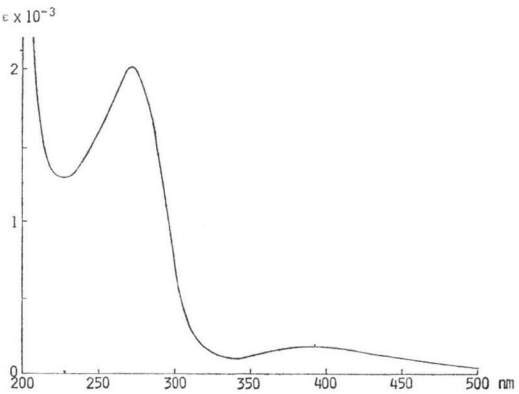
After the culture supernatant obtained from a 72-hour culture (60 liters) was concentrated under reduced pressure to 15 liters, the antibiotic was extracted with 10 liters of ethyl acetate. The solvent layer was washed successively with 5 and 2 liters of 5% sodium bicarbonate solution, 3 liters of saturated sodium carbonate solution and 7 liters of water. The washed extract was concentrated *in vacuo* to give a yellowish brown paste. A 2-liter methanolic solution of the paste was mixed with 60 g of activated carbon. After the carbon cake was washed with methanol, the antibiotic was eluted with 2.5 liters of ethyl acetate. The eluate was concentrated under reduced pressure to about 150 ml and left overnight at 5°C to give crude crystals. The crude crystals were recrystallized three times from ethyl acetate to afford 600 mg of herbimycin B as yellow needles, which gave a single spot on silica gel TLC. The mother liquor was subjected to the purification process described for herbimycin A[1].

Physicochemical Properties of Herbimycin B

The physical and chemical properties of herbimycin B are summarized as follows.

1) Melting point: 229°C (decomposition)
2) Optical rotation: $[\alpha]_D^{20}+109°$ (*c* 1.0, chloroform)
3) Elemental analysis: Found; C 62.41; H 7.28; N 5.27%
   Calculated for $C_{28}H_{38}N_2O_8$; C 63.40; H 7.17; N 5.28%
4) Molecular weight (by mass spectrometry): 530
5) Molecular formula: $C_{28}H_{38}N_2O_8$
6) UV absorption: λ_{max}^{MeOH} nm (ε), 272 (20,000); 395 (1,900)
7) Solubility: Soluble in acetone, methanol, ethanol, dimethylsulfoxide and N, N-dimethylformamide; to a lesser extent in chloroform, ethyl acetate and benzene; but soluble with difficulty or insoluble in water, ethyl ether and *n*-hexane.

Fig. 2. UV spectrum of herbimycin B (MeOH).



The mass spectrum of herbimycin B showed a molecular ion at *m/z* 530 and a fragment peak at *m/z* 487. The accurate mass measurement of the ion at 487 (Found, 487.256; Calcd. for $C_{27}H_{37}NO_7$, 487.257) indicated that it corresponds to $C_{27}H_{37}NO_7$ [M^+-43 (HCON)]. The

Fig. 3. IR spectrum of herbimycin B (KBr).

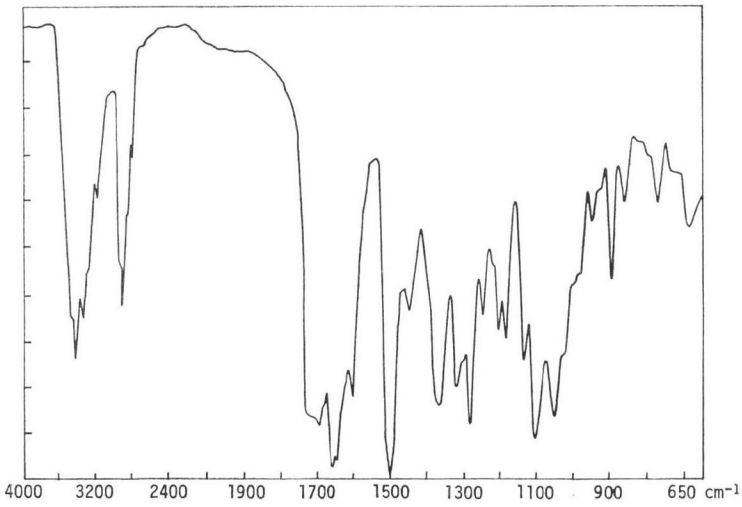
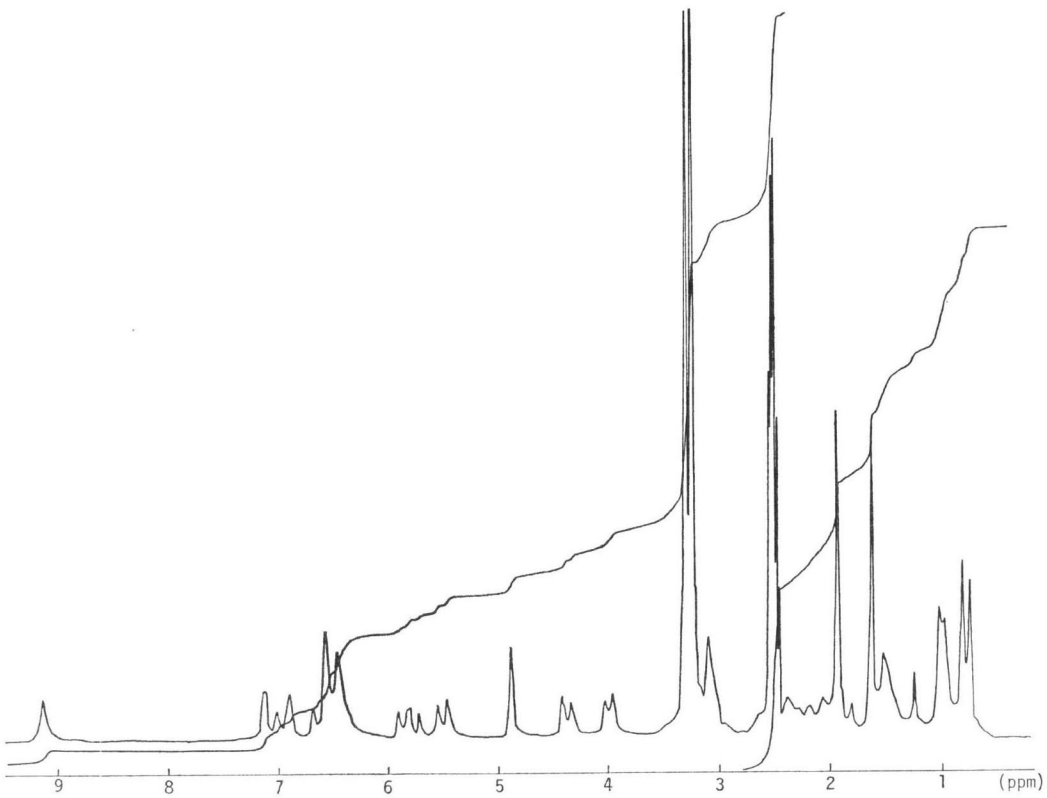


Fig. 4. ^{1}H-NMR spectrum of herbimycin B (100 MHz, DMSO-d_6).



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

The physicochemical properties described above suggest that herbimycin B is a new benzoquinonoid ansamycin which is closely similar to herbimycin A ($C_{30}H_{42}N_2O_9$). Recently, we determined the structure of herbimycin B (Fig. 1) based on the proton spin decoupling and ^{13}C-NMR comparison of

herbimycin B with A (manuscript in preparation).

Biological Properties of Herbimycin B

The antimicrobial activity of herbimycin B was determined by the conventional agar dilution method using Heart infusion agar for bacteria (37°C, 24 hours) and potato dextrose agar for yeasts and fungi (27°C, 72 hours). Herbimycin B was weakly active against *Piricularia oryzae* and *Trichophyton rubrum* (Table 1), but inactive against bacteria as are other benzoquinonoid ansamycins.

The herbicidal and anti-TMV activities of herbimycin B were compared with those of herbimycin A as shown in Tables 2 and 3. The herbicidal activity of both antibiotics was assayed by the following two methods: a pre-emergence system conducted before germination of the seed and a post-emergence system conducted two weeks after germination were used and the activity was evaluated on a 0~5 scale. It was found that the herbicidal activity of herbimycin B is more effective in the pre-emergence system than post-emergence. However, with most plants the herbicidal effect of herbimycin B was less than that of herbimycin A (Table 2).

Table 1. Antimicrobial activity of herbimycin B.

Test organism	MIC μg/ml
Staphylococcus aureus FDA 209 P	>100
Bacillus subtilis PCI 219	>100
Sarcina lutea PCI 1001	>100
Mycobacterium smegmatis ATCC 607	>100
Escherichia coli NIHJ	>100
Salmonella typhimurium	>100
Pseudomonas aeruginosa P-3	>100
Erwinia aroideae J-29	>100
Xanthomonas oryzae	>100
Candida albicans	>100
Saccharomyces sake	>100
Piricularia oryzae	100
Aspergillus niger	>100
Microsporum gypseum	>100
Trichophyton interdigitale	>100
Trichophyton rubrum	100

Table 2. Herbicidal activity of herbimycins A and B.

	Test plant	Pre-emergence system							Post-emergence system					
		Herbimycin A (g/are)				Herbimycin B (g/are)			Herbimycin A			Herbimycin B		
		100	50	25	12.5	100	50	25	100	50	25	100	50	25
Monocotyledon	*Oryza sativa*	2	1	1	0	0	0	0	0	0	0	0	0	0
	Echinochloa crus-galli	5	5	5	4	2	1	0						
	Digitaria adscendens	5	5	5	4	2	1	0	5	4	4	0	0	0
	Cyperus microiria	5	5	5	5	5	5	5	5	5	5	2	2	2
Dicotyledon	*Chenopodium ficifolium*	5	5	5	4	5	5	5	5	5	5	5	5	5
	Portulaca oleracea	5	5	5	4	5	5	4	4	4	3			
	Galinsoga ciliata	5	5	5	5	5	5	3	4	3	2	0	0	0
	Rorippa atrovirens	5	5	4	4	4	4	3	5	4	3			

The score of mortality (0~5): 0, no activity; 1, less than 20 %; 2, 20~40 %; 3, 40~70 %; 4, 70~90 %; 5, more than 90 %.

Anti-TMV activity of the herbimycins, geldanamycin[3,4] and macbecin I[5,6,7] was tested by a local lesion method. Herbimycin B, as well as herbimycin A and geldanamycin, were found to have potent anti-TMV activity as shown in Table 3.

Herbimycin B is less toxic than herbimycin A whose LD_{50} is 19 mg/kg (i.p., mice).

Discussion

Herbimycin B is a new component isolated from the culture broth of *Streptomyces hygroscopicus* No. AM-3672, a herbimycin A producing strain. The physicochemical and biological properties indicated it to be an analog of herbimycin A. Geldanamycin[3], maytansine[9], maytanprine[10], maytanbutine[10], ansamitocins[11,12], colubrinol[13], macbecin I[6] and herbimycin A[1] are known to belong to the benzoquinonoid ansamycin group[8]. However, none of their physicochemical properties are identical to those of herbimycin B.

Herbicidal activity of herbimycin B was weaker than that of herbimycin A (Table 2). The herbicidal activities of herbimycin A and macbecin I are shown in Table 4. Macbecin I possesses potent herbicidal activity, but its effect was slightly less than that of herbimycin A.

As shown in Table 3, the four benzoquinonoid ansamycins tested, herbimycins A and B, geldanamycin and macbecin I were observed to have anti-TMV activity. It has been reported that benzoquinonoid ansamycins have interesting activities[14] such as anti-leukemic activity (maytansine[9,15], ansamitocins[11] *etc.*), antiprotozoal activity (geldanamycin[3], macbecins[6] *etc.*) and anti-RNA tumor virus (geldanamycin[14]). However, our finding that benzoquinonoid ansamycins have anti-TMV activity is new.

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Table 3. Anti-TMV activity of herbimycins, geldanamycin and macbecin I.

Anti-TMV activity was determined by the local lesion method using leaves of *Nicotiana glutinosa*. Antibiotic solution was streaked on half of the leaf (treated group) and the other half was treated with water as control group. After one day, TMV prepared from an infected tomato leaf was inoculated over both sides of the tobacco leaf with the aid of carborundum and incubated at 25°C for 3 days. The following inhibition value (%) was calculated from local lesions formed on the leaf:

$$\left(1-\frac{\text{No. of lesions in treatment group}}{\text{No. of lesions in control group}}\right)\times 100.$$

Phytotoxicity of antibiotic on the tobacco leaf was observed at the same time.

Antibiotic	Concentration (ppm)	Inhibition (%)	Phytotoxicity
Herbimycin A	25.0	95	—
	12.5	90	—
	6.2	92	—
	3.1	85	—
Herbimycin B	25.0	100	—
	12.5	93	—
	6.2	88	—
	3.1	58	—
Geldanamycin	25.0	99	—
	12.5	98	—
	6.2	98	—
	3.1	80	—
Macbecin I	25.0	53	—

Table 4. Herbicidal activity of herbimycin A and macbecin I.

	Species	Pre-emergence system					Post-emergence system				
		Herbimycin A			Macbecin I		Herbimycin A			Macbecin I	
		50	25	12.5	50	25	50	25	12.5	50	25
Monocotyledon	*Oryza sativa*	0	0	0	0	0	0	0	0	0	0
	Echinochloa crus-galli	4	3	3	5	4					
	Digitaria adscendens	5	4	4	4	3	1	1	1	1	1
	Cyperus microiria	5	5	4	4	1	4	4	4	4	4
Dicotyledon	*Chenopodium ficifolium*	5	5	5	5	4	4	3	3	4	4
	Portulaca oleracea	5	5	5	5	4					
	Galinsoga ciliata	5	3	2	4	3	1	1	1	1	1
	Rorippa atrovirens	5	4	4	4	3					

For activity scale see Table 2.

ries, for the gift of macbecin I, and The Upjohn Company for the gift of geldanamycin.

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