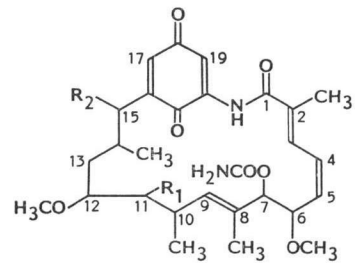
THE STRUCTURE AND CYTOCIDAL ACTIVITY OF HERBIMYCIN C

Sir:

Herbimycins A[1~3] (**1**) and B[4] (**2**), new benzoquinoid ansamycin antibiotics isolated from the culture broth of *Streptomyces hygroscopicus* AM-3672, possess herbicidal, anti-tobacco mosaic virus and antitumor activities. Recently, UEHARA *et al.*,[5] have reported that the antibiotics convert the transformed morphology of Rous sarcoma virus-infected rat kidney cells to the normal one. During the course of the investigation of production of **1** and **2**, an antibiotic, herbimycin C (**3**) was found in the culture broth. In this communication, we wish to describe the isolation, structure elucidation and cytocidal activity of **3**.

Fermentation was carried out under the conditions reported previously.[1] The cultured broth (15 liters) was centrifuged and the supernatant was extracted twice with EtOAc. After evaporation of the extract, the residue was chromatographed on an activated charcoal column using 20~30% aq acetone and then on a silica gel column using benzene - acetone, 5:1 to afford a yellowish amorphous powder of **3** (28.8 mg) in addition to **1** (650.1 mg). Antibiotic **3**: MP 203°C (dec); $[\alpha]_D^{23}$ +210° (*c* 0.5, $CHCl_3$); high resolution EI-MS *m/z* 560.273 (calcd for $C_{29}H_{40}N_2O_9$: 560.273), is considered to be structurally similar to **1** and **2** from its UV and IR spectra. The UV spectrum of **3** in MeOH exhibited absorptions at 269 nm (ε 19,000) and 395 nm (ε 2,300), corresponding to a benzoquinoid moiety. The IR spectrum (KBr) of **3** showed absorptions at 1730 cm^{-1} (carbamoyl), 1695 cm^{-1} (quinone carbonyl) and 1660 cm^{-1} (amide carbonyl). The ^{13}C NMR spectrum (100 MHz in $CDCl_3$) of **3** showed the presence of twenty nine carbon signals [seven methyls, one methylene, seven methines including five carbons bonded to an oxygen, ten olefinic

Chart 1.



Herbimycin A (**1**) R_1 = OCH_3 R_2 = OCH_3
Herbimycin B (**2**) R_1 = OH R_2 = H
Herbimycin C (**3**) R_1 = OH R_2 = OCH_3

Fig. 1. ^1H NMR spectrum of herbimycin C (**3**), 400 MHz, $CDCl_3$.

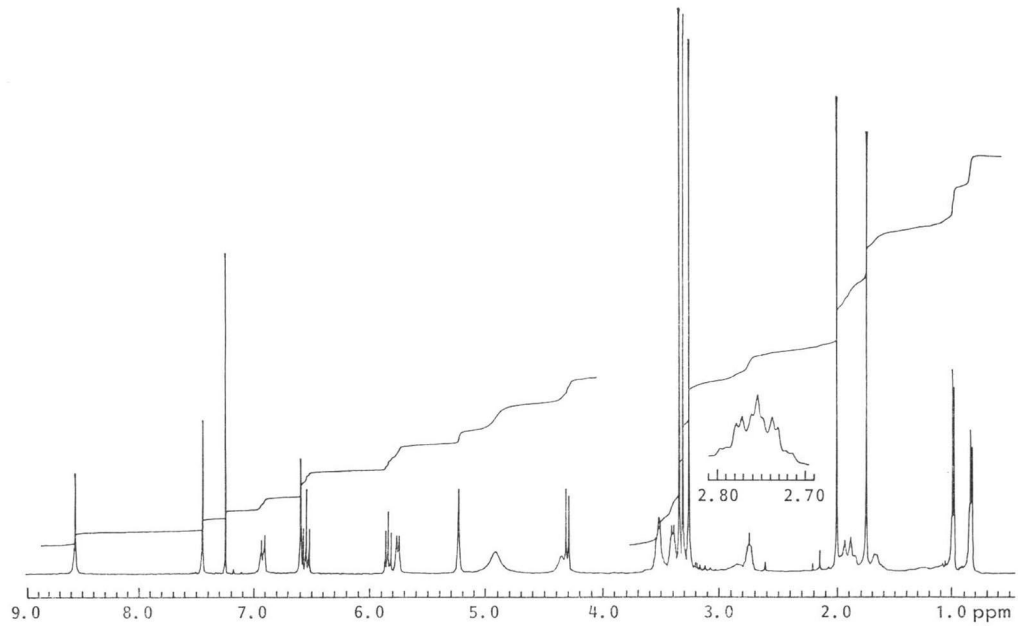


Table 1. ^{1}H and ^{13}C chemical shift values of herbimycins.

Carbon No.	Herbimycin C (3)*		Herbimycin A (1)	Herbimycin B (2)	Monoacetylherbimycin C (4)**	
	^{1}H	^{13}C	^{13}C	^{13}C	^{1}H	^{13}C
1	8.58 s	168.2	168.7	169.1	8.80 s	169.0
2		134.8	134.5	133.1		135.2
3	6.93 br d	127.2	128.2	128.3	6.97 br d	128.6
4	6.56 dd	126.3	125.6	125.6	6.50 t	126.2
5	5.85 t	136.2	136.7	133.7	5.86 dd	137.1
6	4.31 d	81.2	78.3	81.5	4.57 br d	76.0
7	5.24 s	80.7	79.2	80.6	5.72 br s	78.2
8		133.4	131.6	132.5		132.7
9	5.77 br d	132.7	130.1	131.8	5.36 br d	133.2
10	2.76 ddd	32.6	34.1	32.0	2.71 m	34.8
11	3.40 m	72.9	82.3	71.7	5.15 dd	75.1
12	3.53 m	81.2	83.4	80.0	3.62 m	78.6
13	1.95 m	33.8	34.0	30.4	1.80 m	34.1
14	1.68 m	32.1	36.7	26.1	1.60 m	37.5
15	4.37 br s	80.5	78.7	30.4	4.51 br s	81.0
16		143.9	144.6	145.1		145.3
17	6.61 dd	132.9	132.6	134.6	6.60 dd	133.2
18		187.7	187.7	187.8		187.8
19	7.45 d	113.6	112.9	112.2	7.32 d	113.0
20		138.3	138.2	140.2		138.5
21		185.0	183.9	182.4		184.2
2-CH_3	2.01 br s	12.6	12.4	12.4	2.02 br s	12.2
6-OCH_3	3.31 s	56.6	56.0	56.0	3.33 s	56.1
7-$OCONH_2$	4.93 br s	156.0	155.9	155.9	4.70 s	155.6
8-CH_3	1.77 br s	12.7	14.1	12.5	1.73 br s	14.2
10-CH_3	1.00 d	14.4	16.3	23.3	1.00 d	16.3
11-OCH_3			58.4			
12-OCH_3	3.26 s	57.1	57.6	56.5	3.29 s	57.8
14-CH_3	0.85 d	13.0	13.6	12.9	0.84 d	13.3
15-OCH_3	3.35 s	58.5	59.8		3.36 s	58.7

Chemical shifts in ppm are down field from $(CH_3)_4Si$. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ with a Varian XL-400.

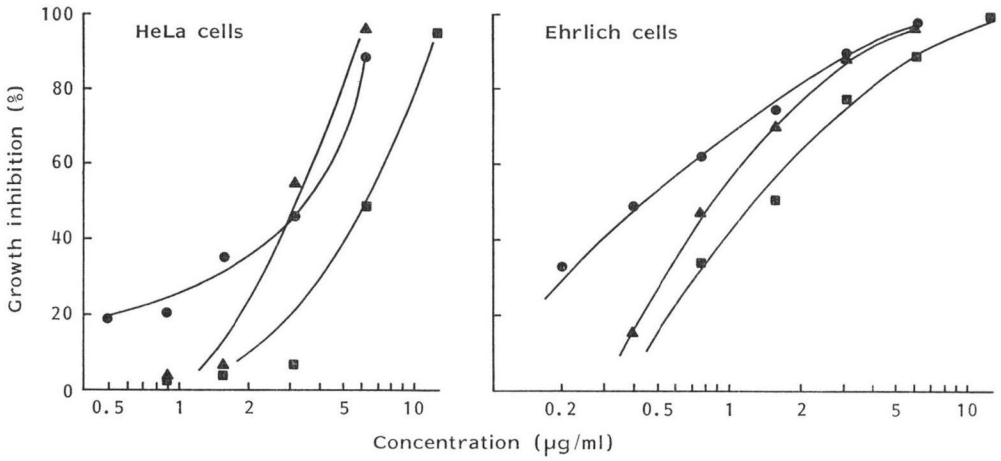
* 11-OH=δ_H 2.85. ** 11-$OCOCH_3$: δ_H 1.89, δ_C 21.1 and 171.0.

carbons and four carbonyl carbons (amide, carbamoyl and quinone)]. In the comparison of the ^{13}C NMR spectrum of **3** with that of **1**, the chemical shift value for each carbon signal arising from the ansa chain moiety in **3**, except for the signal at C-11, was coincident with that of **1**. The signal assigned to C-11 in **3** appeared to shift to a higher field at δ_C 72.9 (Δ 9.4 ppm) compared with that of **1** in which a methoxyl group was attached. This means that a hydroxyl group is located at the C-11 in **3** as in **2**. The structure of **3** was confirmed from the NMR spectrum of monoacetylherbimycin C (**4**): MP 169°C (dec); $[\alpha]_D^{23}$ +204° (*c* 0.5, $CHCl_3$); high resolution EI-MS *m/z* 602.283 (calcd for $C_{31}H_{42}N_2O_{10}$, 602.284); ^{1}H NMR δ_H 1.89 ($OCOCH_3$), which was obtained by acetylation of **3** with acetic anhydride in pyridine. In the ^{1}H NMR spectra of **3** and **4**, the signal at δ_H 3.40 assigned to the H-11 in **3** showed low field shift to δ_H 5.15 (Δ 1.65 ppm) by acetylation. A similar shift (Δ 2.2 ppm) from δ_C 72.9 at C-11 in **3** to δ_C 75.1 in **4** was observed. In the COSY spectrum of **4**, the signal at δ_H 2.71 (H-10) is correlated with the signals at 1.00 (d, J=6.8 Hz, 10-CH_3), 5.36 (br d, J=9.5 Hz, H-9) and 5.15 (dd, J=9.0 and 1.5 Hz, H-11). Further, the signal at δ_H 5.15 is correlated to the signal at δ_H 3.62 (m, H-12). Therefore, the structure of **3** was elucidated at 11-hydroxy-11-demethoxy-herbimycin A. Antibiotic **3** is the same compound with TAN 420-D which was obtained by

Fig. 2. Cytocidal activity of herbimycins on HeLa and Ehrlich cells.

HeLa S3 and Ehrlich cells were maintained in monolayers in EAGLE's minimum essential medium supplemented with 10% bovine serum and kanamycin (100 μg/ml) at 37°C. To determine the cytotoxicity of herbimycins, HeLa S3 or Ehrlich cells (5×10^4) in 1.5 ml of medium were placed in a tissue culture plate (Falcon, 24-well) and incubated for 24 hours at 37°C in a 5% CO_2 - 95% air atmosphere. Each culture well was added with 0.5 ml of fresh medium containing a different concentration of herbimycins, and reincubated for 72 hours. The cells were trypsinized to form a single cell suspension, and were counted in a hemocytometer.

●; Herbimycin A, ▲; herbimycin B, ■; herbimycin C.



oxidation of the EtOAc extract from the culture filtrate of *S. hygroscopicus*.[6~8] ^1H and ^{13}C chemical shift values of **3** and its related compounds were assigned as shown in Table 1.

Herbimycins A, B and C showed cytocidal activity on both HeLa cells and Ehrlich cells *in vitro* (Fig. 2). The IC_{50}s of herbimycins were as follows; A 3.5, B 3.2, C 7.3 on HeLa cells and A 0.4, B 0.8, C 1.2 μg/ml on Ehrlich cells. The exchange of hydroxyl group instead of methoxyl group at the C-11 position decreases the activity. Antibiotic **3** was weakly active against *Piricularia oryzae* and *Trichophyton rubrum* but inactive against various bacteria tested like other benzoquinoid ansamycins such as herbimycins A and B[4] and geldanamycin.[9] The herbicidal activity of **3** is under examination.

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KIYOSHI SHIBATA
SADAYOSHI SATSUMABAYASHI
AKIRA NAKAGAWA†
SATOSHI ŌMURA*,†

Nippon Dental University,
Chiyoda-ku, Tokyo 102, Japan
†The Kitasato Institute and
School of Pharmaceutical Sciences,
Kitasato University,
Minato-ku, Tokyo 108, Japan

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