CHEMICAL MODIFICATION OF HERBIMYCIN A

SYNTHESIS AND *IN VIVO* ANTITUMOR ACTIVITIES OF HALOGENATED AND OTHER RELATED DERIVATIVES OF HERBIMYCIN A

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Several halogenated and other related derivatives of herbimycin A have been synthesized and evaluated *in vivo* for their activities against Ehrlich ascites carcinoma. Some of these derivatives show higher activities than herbimycin A. Among them the derivatives modified at the 4, 5, 6, and 7-positions of the ansa chain showed particularly high activities.

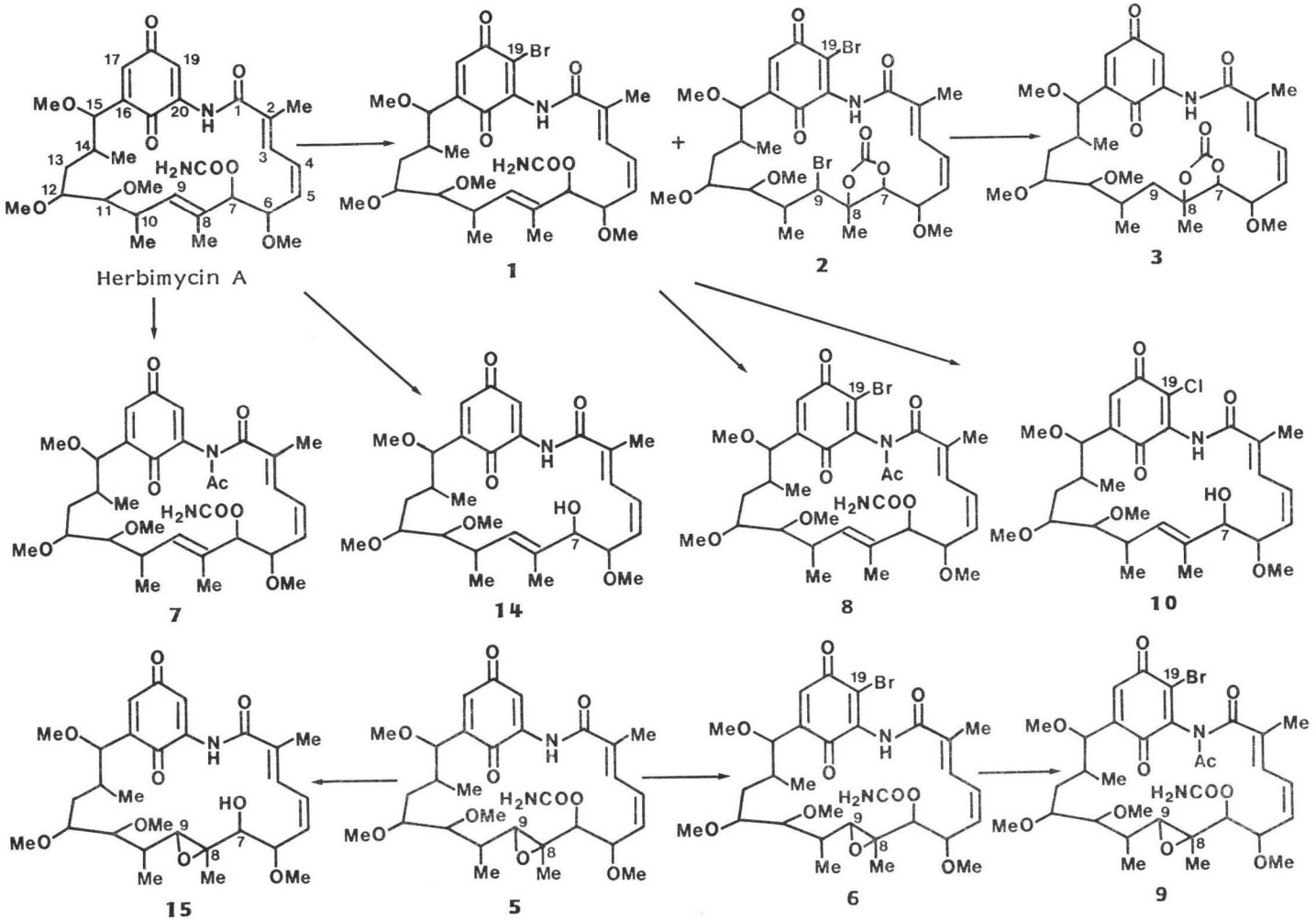
Herbimycin A[1], a new ansamycin antibiotic isolated from the culture broth of *Streptomyces hygroscopicus* AM-3672, shows herbicidal, anti-tobacco mosaic virus and antitumor activities[2].

The structure of herbimycin A has been confirmed as a benzoquinoid type ansamycin antibiotic[3,4], which is similar to the other antitumor antibiotics, geldanamycin[5,6], and macbecin[7,8]. In this paper, we wish to describe the synthesis of halogenated and other related derivatives of herbimycin A and their *in vivo* activities against Ehrlich ascites carcinoma.

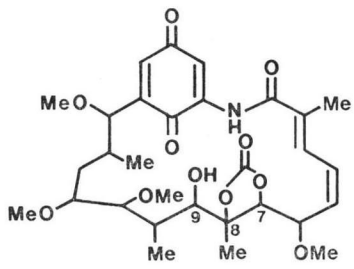
The hydrogen at the 19-position of herbimycin A is easily substituted by a nucleophile. For example, treatment of herbimycin A with pyridinium hydrobromide perbromide[9] (also, pyridinium hydrobromide perbromide is an electrophilic reagent which attacks nucleophilic centers in molecules) in a mixture of chloroform and methanol at −35°C gave 19-bromoherbimycin A (**1**) and an unexpected product, 9,19-dibromo-7-decarbamoyl-7,8-*O*-carbonyl-8,9-dihydro-8-hydroxyherbimycin A (**2**) in 85 and 5% yields, respectively. The yield of **2** was 15% when the reaction was performed at −10°C.

The structure of **1** was confirmed from a disappearance of the 19-proton signal observed in herbimycin A and a downfield shift (Δ 8.1 ppm) of the 19-carbon signal (δ_C 121.0) in the NMR spectrum, in addition to the indication of the substitution of a hydrogen atom by a bromine one in its mass spectrum. The structure of **2** was evidenced from the following data; the characteristic absorption of five-membered cyclic carbonate at 1760 cm^{-1}, in the IR spectrum, the disappearance of 19-proton signal in the ^1H NMR and the existence of only one nitrogen atom in the elemental analysis. The ^{13}C NMR spectrum showed the disappearance of olefinic carbon signals at 8 and 9-positions which were observed in the spectra of herbimycin A and **1**. Further, two new carbon signals at δ 85.9 (quarternary) and 67.8 (tertiary) were assigned to the carbons attached to oxygen and bromine, respectively. Treatment of **2** with *tert*-BuSnH[10] in toluene at refluxing temperature afforded debrominated product (**3**).

Scheme 1.



In the ^{13}C NMR spectrum of **3**, the signal at δ 67.8 observed in **2** disappeared and the new singal at δ 34.0, assignable to 9-methylene carbon by C-H selective decoupling and proton homo decoupling techniques was observed, confirming 7,8-*O*-carbonyl-8,9-dihydro structure for **2**. The transformation from **2** to **3** also supported the structure of **2**.



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We have assigned 7,9-cyclic carbamate for compound **4** in a previous report[11]. However, in the course of the structure determination of **2** and **3**, compound **4** was revised to be 7,8-cyclic carbonate structure, as shown above.

8,9-Epoxyherbimycin A (**5**)[11], which was obtained on treatment of **1** with *m*-chloroperbenzoic acid, was reacted with pyridinium hydrobromide perbromide in the similar manner described above to give 19-bromo-8,9-epoxyherbimycin A (**6**) as a sole product. Treatment of herbimycin A, **1** and **6** with silver acetate in acetic anhydride[12] gave each *N*-acetyl derivative (**7**~**9**) in which these structures were confirmed from the IR (disappearance of amide absorption at about 1550 cm^{-1} observed in mother compounds) and NMR (*N*-acetyl methyl at about 2.4 ppm) spectra.

Treatment of **1** with copper chloride in *N*,*N*-dimethylformamide at 90°C[13] resulted in a substitution of the bromine atom at 19-position by chlorine accompanying decarbamoylation, to afford 19-chloro-7-decarbamoylherbimycin A (**10**). Since this reaction seemed to be useful as decarbamoylation procedure in nonaqueous conditions, it was applied to herbimycin A and **5** to give decarbamoyl derivatives **14** and **15**, respectively.

Boron trichloride is well known to react with ether group giving the corresponding alkyl chloride

Scheme 2.

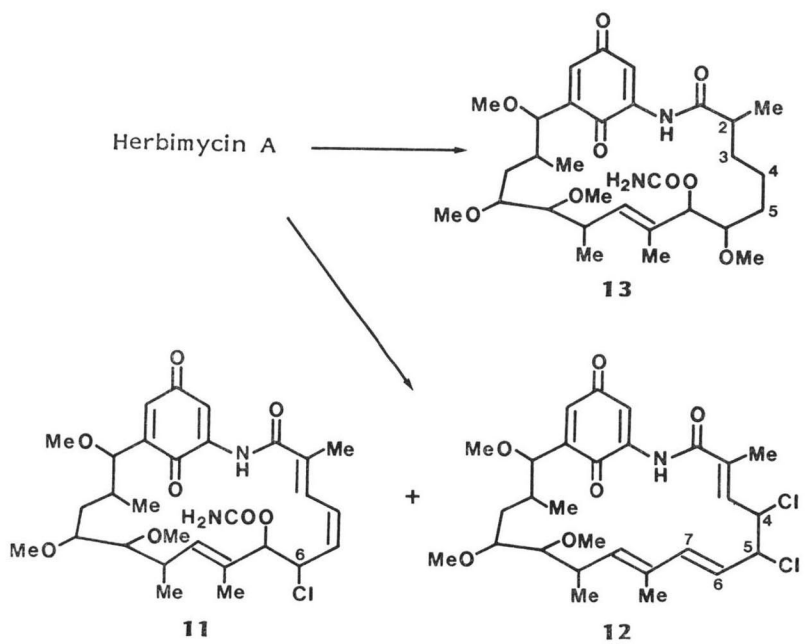


Table 1. ^{13}C NMR chemical shifts for herbimycin A derivatives.

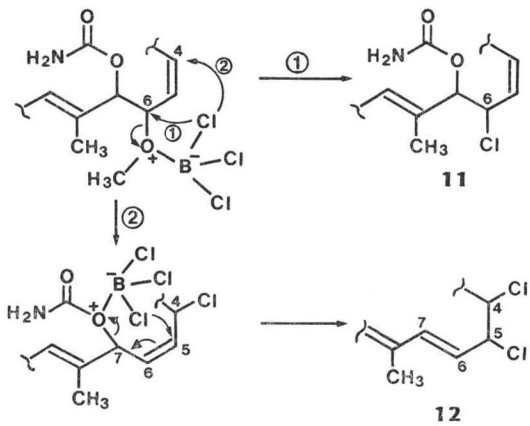
Carbon No.	Herbimycin A	**1**	**2***	**3****	**10**	**11**	**12**	**13**	**14**	**15**
CONH	168.7	173.3	166.1	167.8	173.4	168.6	167.2	175.0	168.2	168.8
2	134.5	138.7	134.5	136.3	138.3	135.7	137.1	43.2	135.3	138.1
2-CH_3	12.4	11.8	12.5	12.7	11.1	12.6	12.5	12.5	12.0	11.3
3	128.2	128.5	129.5	126.3	129.0	127.3	131.9	23.8	128.0	128.2
4	125.6	125.0	126.7	126.2	125.2	124.2	60.4	33.3	124.4	125.6
5	136.7	135.4	137.9	136.6	133.9	135.1	63.6	31.2	139.3	132.0
6	78.3	75.0	75.1	77.2	80.2	58.4	120.4	84.6	78.2	75.7
6-OCH_3	56.0	56.4	56.8	56.5	56.3	—	—	58.2	55.8	57.3
7	79.2	80.4	82.1	86.3	76.5	78.3	141.6	79.1	77.8	77.0
7-$OCONH_2$	155.9	156.4	—	—	—	155.7	—	156.1	—	—
8	131.6	128.5	85.9	87.0	131.3	131.0	132.9	136.2	134.0	59.1
8-CH_3	14.1	15.9	14.3	13.7	15.9	14.0	12.8	14.1	13.7	12.4
9	130.1	131.3	67.8	34.0	131.5	131.2	138.7	130.0	129.5	67.3
10	34.1	32.7	36.7	29.7	32.6	36.5	35.5	33.7	33.9	35.1
10-CH_3	16.3	18.4	19.4	17.7	16.3	16.1	17.7	18.0	16.2	14.6
11	82.3	81.3	83.2	78.1	80.4	82.4	83.5	81.2	82.2	80.7
11-OCH_3	58.4	57.1	58.4	58.6	57.1	58.4	58.2	59.1	58.1	58.5
12	84.4	82.6	84.6	79.2	82.8	83.3	84.2	81.4	83.1	78.6
12-OCH_3	57.6	56.7	57.5	58.0	56.7	56.1	55.4	57.3	56.9	56.2
13	34.0	25.3	35.1	31.0	25.2	34.3	33.9	35.5	33.6	34.1
14	36.7	35.8	42.1	36.2	35.7	33.8	37.8	34.4	36.4	35.5
14-CH_3	13.6	13.9	13.8	13.5	13.8	13.6	13.1	13.6	13.4	12.4
15	78.7	79.5	79.4	80.2	79.4	78.3	76.9	82.1	78.2	75.3
15-OCH_3	59.8	61.3	59.8	59.4	61.3	59.9	60.6	59.6	59.7	62.4
16	144.6	143.6	143.4	144.0	146.6	144.7	144.8	144.8	144.6	145.0
17	132.6	134.0	137.9	133.2	133.7	132.7	132.6	133.1	132.4	134.9
18	187.7	180.0	180.8	187.5	180.7	187.7	187.5	187.9	187.3	187.9
19	112.9	121.0	123.5	113.6	126.7	113.2	113.6	114.0	112.6	112.8
20	138.2	146.6	149.2	138.0	140.7	138.3	138.1	138.0	138.0	139.2
21	183.9	178.2	178.9	184.4	178.0	184.1	183.9	184.2	183.2	183.5

* 152.9 (7,8-OCOO).

** 154.0 (7,8-OCOO).

and alcohol[14]. Treatment of herbimycin A with trichloroboran (BCl_3) in chloroform at −40°C for 20 hours gave 6-chloro-6-demethoxyherbimycin A (**11**) and 4,5-dichloro-4,5-dihydro-7-decarbamoyloxy-6-demethoxy-6-enoherbimycin A (**12**) in 35 and 20% yields, respectively. The ^{13}C NMR spectrum of **11** indicated the substitution of a chlorine atom for the methoxy group at the 6-position. Consequently, the signal for the 6-position carbon atom (δ 58.4) was observed to shift upfield (Δ19.9 ppm) in comparison with that of herbimycin A supporting the structure of **11**. The structure of **12** was also assigned by proton homo decoupling and C-H selective decoupling techniques. These spectral data showed the disappearance of the carbamoyl carbon at the 7-position and the methoxy methyl at the 6-position and formation of additional two olefinic carbon signals at δ 120.4 and 141.6.

Scheme 3.



Compounds **11** and **12** seemed to be produced through intramolecular attack of a chlorine atom of BCl_3 bonded to 6-methoxy or 7-carbamoyl oxygen as shown in Scheme 3. Although the stereochemistry of **11** and **12** has not been determined yet, the reaction seems to proceed stereo-selectively.

The 4,5-saturated derivative (**12**) of herbimycin A was of interest because the antitumor activity was superior as described below. Thus, 2,3,4,5-tetrahydroherbimycin A (**13**) was synthesized by catalytic hydrogenation.

The ^{13}C NMR chemical shift values for herbimycin A derivatives were summarized in Table 1.

Antitumor Activities

Antitumor activities (T/C %) at optimal doses of herbimycin A derivatives against Ehrlich ascites carcinoma are given in Table 2. Among the various derivatives, the halogenated compounds (**1**, **11**

Table 2. Antitumor activity of herbimycin A derivatives against Ehrlich ascites carcinoma.

Compound	Total dose (mg/kg)	Dose (mg/kg×day)	T/C (%)	Number of* survival/total
1	250	50.0×5	190	4/4
2	125	25.0×5	134	1/4
3	250	50.0×5	150	2/4
6	250	50.0×5	144	1/4
7	125	25.0×5	89	0/4
8	62.5	12.5×5	91	0/4
9	31.3	6.3×5	92	0/4
10	250	50.0×5	129	1/4
11	125	25.0×5	200	4/4
12	250	50.0×5	215	4/4
13	125	25.0×5	193	3/4
14	250	50.0×5	200	3/4
15	125	25.0×5	146	2/4
Herbimycin A	6.3	1.3×5	126	1/4
Geldanamycin	62.5	12.5×5	123	1/4

* Number of surviving mice at day 31.

and **12**), the tetrahydro derivative (**13**) and decarbamoylherbimycin A (**14**) are notable for showing higher antitumor activity than that of herbimycin A. The carbamoyl group seems not to be necessary for activity because decarbamoyl derivatives **14** and **15** showed either similar or higher activity than the corresponding mother compounds.

The high activity of 4,5-dichloro (**12**) and 2,3,4,5-tetrahydro (**13**) derivatives indicate the possibility of additional improvement of activity by further chemical modification at the 4 and 5-position of herbimycin A. The acetylation of amide nitrogen in **7**~**9** resulted in a decrease in activity.

Experimental

NMR spectra were measured with a Jeol FX-90 and Bruker AM 400 spectrometer in $CDCl_3$ solution. Mass spectra were obtained with a Jeol D-100 and DX-300 spectrometer at 20 eV. Optical rotations were measured with a Jasco DIP-181 polarimeter. Thin-layer chromatography (TLC) was performed on pre-coated plates, Merck Kiesel gel 60 F_{254} with benzene - Me_2CO, 7: 3. Silica gel column chromatography was performed with Merck Kiesel gel 60.

Antitumor Activity

Ehrlich carcinoma cells (2.5×10^6) were inoculated ip to *dd*Y mice on day 0. Mice received various dose (<250 mg/kg) of herbimycin A derivatives for 5 successive days. Antitumor activity was expressed as T/C (%) at the optimal dose for each derivative: "T" is median survival days of the treated group and "C" is that of the control group.

19-Bromoherbimycin A (1) and 9,19-Dibromo-7-decarbamoyl-7,8-*O*-carbonyl-8,9-dihydro-8-hydroxyherbimycin A (2)

To a solution of herbimycin A (1.0 g) in $CHCl_3$ - MeOH, 1: 1, (20 ml), pyridinium hydrobromide perbromide (500 mg) was added under cooling at −35°C and held for 6 hours. The reaction mixture was poured into H_2O (100 ml) and extracted with $CHCl_3$ (100 ml×2). The $CHCl_3$ solution was washed with 5% aq $Na_2S_2O_3$ and satd solution of NaCl, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on a silica gel column with benzene - Me_2CO, 8: 2, to give a yellowish powder of **1**, 968 mg (85.0%) and **2**, 64 mg (5.0%).

1: TLC Rf 0.45; mp 178°C (dec); $[\alpha]_D^{23}$ +93° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 258 (18,600); ^1H NMR ($CDCl_3$) δ 6.92 (1H, d, J=0.9 Hz, H-17), 6.42 (1H, qd, J=1.1 and 11.5 Hz, H-3), 6.32 (1H, dd, J=11.5 and 11.5 Hz, H-4), 5.30 (1H, dd, J=10.6 and 11.5 Hz, H-5), 5.28 (1H, qd, J=1.0 and 9.8 Hz, H-9), 5.03 (1H, d, J=9.4 Hz, H-7), 4.49 (1H, dd, J=0.9 and 4.1 Hz, H-15), 4.00 (1H, dd, J= 9.4 and 10.6 Hz, H-6), 3.18 (1H, dd, J=1.8 and 10.0 Hz, H-11), 2.25 (1H, m, H-10), 1.26 (3H, d, J= 1.0 Hz, 8-CH_3).

Anal Calcd for $C_{30}H_{41}N_2O_9Br$: C 55.20, H 6.34, N 4.29, Br 12.10.
Found: C 54.89, H 6.32, N 4.26, Br 12.68.

2: TLC Rf 0.84; mp 132°C (dec); $[\alpha]_D^{23}$ +83° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 268 (18,000); ^1H NMR ($CDCl_3$) δ 7.28 (1H, qd, J=1.2 and 12.2 Hz, H-3), 6.81 (1H, d, J=1.6 Hz, H-17), 4.61 (1H, d, J=7.3 Hz, H-6), 4.47 (1H, s, H-7), 4.32 (1H, d, J=9.4 Hz, H-9), 2.39 (1H, m, H-10), 1.83 (3H, s, 8-CH_3).

Anal Calcd for $C_{30}H_{36}NO_{10}Br_2$: C 49.24, H 5.38, N 1.92, Br 21.16.
Found: C 48.97, H 5.31, N 1.93, Br 21.69.

7-Decarbamoyl-7,8-*O*-carbonyl-8,9-dihydro-8-hydroxyherbimycin A (3)

To a solution of **2** (200 mg) in toluene (4 ml), tributyltin hydride (0.80 ml) and α,α′-azobisisobutyronitrile (7.5 mg) were added and heated at 80°C for 3 hours under a nitrogen atmosphere. The reaction mixture was diluted with $CHCl_3$ (20 ml) and washed with H_2O. The $CHCl_3$ solution was dried over Na_2SO_4 and evaporated, to give a brown residue, which was chromatographed on a silica gel column with benzene - Me_2CO, 10: 1, giving 135 mg (86.0%) of **3**. TLC Rf 0.84; mp 120°C (dec); $[\alpha]_D^{23}$ +65° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 265 (17,300); high resolution MS 575.272 (Calcd for

$C_{30}H_{41}NO_{10}$: 575.273); ^{1}H NMR ($CDCl_3$) δ 7.42 (1H, d, J=2.5 Hz, H-19), 6.66 (1H, dd, J=1.6 and 2.6 Hz, H-17), 4.32 (1H, d, J=7.2 Hz, H-6), 4.28 (1H, s, H-7), 1.55 (3H, s, 8-CH_3), 1.28 (1H, m, H-9a), 0.89 (1H, m, H-9b).

Anal Calcd for $C_{30}H_{41}NO_{10}$: C 62.58, H 7.18, N 2.43.
Found: C 62.36, H 7.15, N 2.40.

8,9-Epoxy-19-bromoherbimycin A (**6**)

To a solution of 8,9-epoxyherbimycin A[11] (**5**, 500 mg) in $CHCl_3$ - MeOH, 1: 1 (10 ml), pyridinium hydrobromide perbromide (250 mg) was added and held for 2 hours at room temp. The reaction mixture was treated in a similar manner as with the preparation of **1**, to give a yellowish powder of **6**, 480 mg (85.0%). TLC Rf 0.40; mp 172°C (dec); $[\alpha]_D^{23}$ +78° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 262 (20,000); ^{1}H NMR ($CDCl_3$) δ 6.86 (1H, d, J=1.8 Hz, H-17), 6.48 (1H, dd, J=11.0 and 11.0 Hz, H-4), 5.73 (1H, dd, J=11.0 and 11.0 Hz, H-5), 4.58 (1H, br s, H-7), 4.52 (1H, d, J=11.0 Hz, H-6), 2.84 (1H, dd, J=3.5 and 9.0 Hz, H-9), 1.38 (3H, s, 8-CH_3).

Anal Calcd for $C_{30}H_{41}N_2O_{10}Br$: C 53.88, H 6.18, N 4.19, Br 11.81.
Found: C 53.52, H 6.09, N 4.12, Br 12.11.

N-Acetylherbimycin A (**7**)

To a solution of herbimycin A (250 mg) in acetic anhydride (4 ml), silver acetate (200 mg) was added and heated at 90°C for 2 days. The reaction mixture was poured into H_2O (100 ml) and extracted with $CHCl_3$ (100 ml×2). The $CHCl_3$ solution was washed with satd solution of NaCl and evaporated to give a solid, which was chromatographed on a silica gel column with benzene - Me_2CO, 10: 1, to give a yellowish powder of **7**, 190 mg (71.0%). TLC Rf 0.45; mp 119°C (dec); $[\alpha]_D^{23}$ +37° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 269 (21,000); ^{1}H NMR ($CDCl_3$) δ 6.76 (1H, dd, J=1.0 and 1.7 Hz, H-17), 6.70 (1H, d, J=1.0 Hz, H-19), 6.44 (1H, dd, J=11.5 and 11.6 Hz, H-4), 5.57 (1H, dd, J=10.0 and 11.5 Hz, H-5), 5.10 (1H, d, J=6.7 Hz, H-7), 4.05 (1H, dd, J=6.7 and 10.0 Hz, H-6), 2.43 (3H, s, N-$COCH_3$), 1.41 (3H, s, 8-CH_3).

Anal Calcd for $C_{32}H_{44}N_2O_{10}$: C 62.31, H 7.22, N 4.53.
Found: C 62.18, H 7.30, N 4.23.

N-Acetyl-19-bromoherbimycin A (**8**)

Compound **1** (250 mg) was treated with acetic anhydride (4 ml) and silver acetate (200 mg) in a similar manner described in the preparation of **7**, to give a yellowish powder of **8**, 135 mg (47.0%). TLC Rf 0.40; mp 135°C (dec); $[\alpha]_D^{23}$ +63° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 272 (18,200).

Anal Calcd for $C_{32}H_{43}N_2O_{10}Br$: C 55.31, H 6.24, N 4.03, Br 11.37.
Found: C 55.12, H 6.43, N 3.94, Br 11.83.

N-Acetyl-19-bromo-8,9-epoxyherbimycin A (**9**)

Compound **6** (250 mg) was treated with acetic anhydride (4 ml) and silver acetate (200 mg) in a similar manner with the preparation of **7**, to give a yellowish powder of **9**, 148 mg (60.0%). TLC Rf 0.38; mp 114°C (dec); $[\alpha]_D^{23}$ +71° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 260 (16,500).

Anal Calcd for $C_{32}H_{43}N_2O_{11}Br$: C 54.07, H 6.10, N 3.94, Br 11.11.
Found: C 53.81, H 6.35, N 3.79, Br 11.61.

7-Decarbamoyl-19-chloroherbimycin A (**10**)

To a solution of **1** (200 mg) in DMF (3 ml), CuCl (100 mg) was added and heated at 90°C for 20 hours. The reaction mixture was poured into H_2O (100 ml) and extracted with $CHCl_3$ (100 ml×2). The $CHCl_3$ solution was dried over Na_2SO_4 and evaporated to give a powder, which was chromatographed on a silica gel column with benzene - Me_2CO, 10: 1, to give a yellowish powder of **10**, 155 mg (91.0%). TLC Rf 0.55; mp 176°C (dec); $[\alpha]_D^{23}$ +49° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 251 (16,000); high resolution MS 565.244 (Calcd for $C_{29}H_{40}NO_8Cl$: 565.244); ^{1}H NMR ($CDCl_3$) δ 6.86 (1H, d, J=1.0 Hz, H-17), 5.27 (1H, dd, J=10.5 and 11.6 Hz, H-5), 5.14 (1H, qd, J=1.1 and 10.6 Hz, H-9), 4.48 (1H, dd, J=1.0 and 4.9 Hz, H-15), 3.85 (1H, dd, J=9.2 and 10.5 Hz, H-6), 3.78 (1H, d, J=9.2 Hz, H-7), 2.29 (1H, m, H-10), 1.29 (3H, d, J=1.1 Hz, 8-CH_3).

Anal Calcd for $C_{29}H_{40}NO_8Cl$: C 61.57, H 7.13, N 4.95, Cl 6.18.
Found: C 61.43, H 7.35, N 4.98, Cl 6.09.

6-Chloro-6-demethoxyherbimycin A (**11**) and 4,5-Dichloro-4,5-dihydro-7-decarbamoyloxy-6-demethoxy-6-enoherbimycin A (**12**)

To a solution of herbimycin A (1.0 g) in $CHCl_3$ (10 ml), 5% solution of BCl_3 (5 ml) was added under cooling at −40°C and held at −40°C for 20 hours. The reaction mixture was poured gradually into ice-water (100 ml) and extracted with $CHCl_3$ (100 ml×3). The $CHCl_3$ solution was dried Na_2SO_4 and evaporated to give a residue, which was chromatographed on a silica gel column with benzene - Me_2CO, 10: 1, to give a yellowish powder of **11**, 360 mg (35.0%) and **12**, 227 mg (21.0%).

11: TLC Rf 0.63; mp 188°C (dec); $[\alpha]_D^{23}$ +54° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 232 (18,500); high resolution MS 578.239 (Calcd for $C_{29}H_{39}N_2O_8Cl$: 578.239); ^1H NMR ($CDCl_3$) δ 7.23 (1H, d, *J*=2.3 Hz, H-19), 6.60 (1H, dd, *J*=2.3 and 3.0 Hz, H-17), 5.89 (1H, dd, *J*=7.6 and 11.6 Hz, H-5), 5.80 (1H, br s, H-7), 5.51 (1H, qd, *J*=1.0 and 7.1 Hz, H-9), 5.10 (1H, br d, *J*=7.6 Hz, H-6), 4.50 (1H, d, *J*=3.0 Hz, H-15), 1.66 (3H, d, *J*=1.1 Hz, 8-CH_3).

Anal Calcd for $C_{29}H_{39}N_2O_8Cl$: C 60.18, H 6.80, N 4.84, Cl 6.05.
Found: C 60.01, H 6.92, N 4.71, Cl 5.89.

12: TLC Rf 0.80; mp 199°C (dec); $[\alpha]_D^{23}$ +99° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 271 (23,500); high resolution MS 553.200 (Calcd for $C_{28}H_{37}NO_6Cl_2$: 553.200); ^1H NMR ($CDCl_3$) δ 7.33 (1H, d, *J*=2.5 Hz, H-19), 6.63 (1H, dd, *J*=2.0 and 2.5 Hz, H-17), 6.55 (1H, d, *J*=13.5 Hz, H-7), 5.86 (1H, dd, *J*=9.8 and 13.5 Hz, H-6), 4.99 (1H, dd, *J*=2.7 and 10.6 Hz, H-4), 4.66 (1H, dd, *J*=2.7 and 9.8 Hz, H-5), 1.75 (3H, d, *J*=1.3 Hz, 8-CH_3).

Anal Calcd for $C_{28}H_{37}NO_6Cl_2$: C 60.74, H 6.74, N 2.53, Cl 12.64.
Found: C 60.28, H 6.98, N 2.45, Cl 12.89.

2,3,4,5-Tetrahydroherbimycin A (**13**)

To a solution of herbimycin A (250 mg) in EtOH (10 ml), Pd-C (Pd 10%, 50 mg) was added and stirred under H_2 gas at atmospheric pressure for 1 hour. Solid was removed by filtration and the filtrate was poured into H_2O (100 ml). The solution extracted with $CHCl_3$ (100 ml×3) and the $CHCl_3$ solution was evaporated to afford the residual solid which was chromatographed on a silica gel column with benzene - Me_2CO, 10: 1, to give a yellowish powder of **13**, 195 mg (78.0%). TLC Rf 0.55; mp 208°C (dec); $[\alpha]_D^{23}$ +108° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 275 (14,500); high resolution MS 578.322 (Calcd for $C_{30}H_{46}N_2O_9$: 578.320); ^1H NMR ($CDCl_3$) δ 7.36 (1H, d, *J*=1.8 Hz, H-19), 6.66 (1H, dd, *J*=1.6 and 1.8 Hz, H-17), 5.60 (1H, qd, *J*=1.1 and 8.5 Hz, H-9), 1.58 (3H, d, *J*=1.1 Hz, 8-CH_3), 1.26 (3H, d, *J*=7.0 Hz, 2-CH_3).

Anal Calcd for $C_{30}H_{46}N_2O_9$: C 62.25, H 7.54, N 4.84.
Found: C 62.13, H 7.68, N 4.65.

7-Decarbamoylherbimycin A (**14**)

To solution of herbimycin A (250 mg) in DMF (3 ml), CuCl (150 mg) was added and treated in a similar manner with the preparation of **10**, to give a yellowish powder of **14**, 160 mg (71.0%). TLC Rf 0.43; mp 135°C (dec); $[\alpha]_D^{23}$ +68° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 267 (22,000); high resolution MS 531.282 (Calcd for $C_{29}H_{41}NO_8$: 531.283); ^1H NMR ($CDCl_3$) δ 7.18 (1H, d, *J*=2.0 Hz, H-19), 6.65 (1H, dd, *J*=2.0 and 2.0 Hz, H-17), 5.98 (1H, dd, *J*=8.5 and 11.5 Hz, H-5), 5.60 (1H, qd, *J*=0.9 and 10.0 Hz, H-9), 4.40 (1H, dd, *J*=2.0 and 8.5 Hz, H-6), 4.22 (1H, d, *J*=2.0 Hz, H-7), 1.54 (3H, d, *J*=0.9 Hz, 8-CH_3).

Anal Calcd for $C_{29}H_{41}NO_8$: C 65.50, H 7.78, N 2.64.
Found: C 65.38, H 7.99, N 2.51.

7-Decarbamoyl-8,9-epoxyherbimycin A (**15**)

To a solution of **5** (250 mg) in DMF (3 ml), CuCl (150 mg) was added and treated in a manner similar to that described above, to give a yellowish powder of **15**, 130 mg (57.0%). TLC Rf 0.41; mp 115°C (dec); $[\alpha]_D^{23}$ +53° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 266 (18,700); high resolution MS 547.277 (Calcd for $C_{29}H_{41}NO_9$: 547.278); ^1H NMR ($CDCl_3$) δ 7.33 (1H, d, *J*=2.0 Hz, H-19), 6.59 (1H, dd, *J*=1.8 and 2.0 Hz, H-17), 5.97 (1H, dd, *J*=10.0 and 10.0 Hz, H-5), 4.48 (1H, d, *J*=10.0 Hz, H-6), 2.95 (1H, br s, H-7), 1.27 (3H, d, *J*=1.8 Hz, 8-CH_3).

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