BIPHENOMYCINS A AND B, NOVEL PEPTIDE ANTIBIOTICS

II. STRUCTURAL ELUCIDATION OF BIPHENOMYCINS A AND B

ITSUO UCHIDA, NOBUHARU SHIGEMATSU, MASAMI EZAKI,
MASASHI HASHIMOTO*, HATSUO AOKI
and HIROSHI IMANAKA

Exploratory Research Laboratories,
Fujisawa Pharmaceutical Co., Ltd.,
2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan

(Received for publication June 21, 1985)

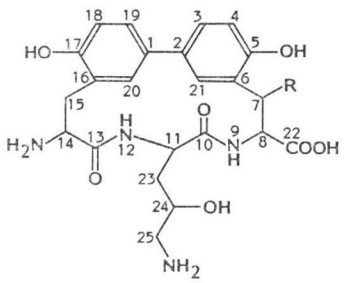
The structures of biphenomycins A and B, novel peptide antibiotics produced by a strain of *Streptomyces*, have been established as **1** and **2**, respectively, on the basis of spectroscopic and chemical evidence. They are unique in that they are cyclic peptides containing a biphenyl moiety included in a 15-membered ring and show potent antibacterial activities especially against Gram-positive bacteria.

Biphenomycin A (**1**), which was tentatively designated as WS-43708 A, is a novel peptide antibiotic with potent antibacterial activity especially against Gram-positive bacteria. Its discovery, isolation, and characterization were described in the preceding paper of this series.[1] In a previous communication,[2] we reported the structural elucidation of this antibiotic. This paper is devoted to a full account of that work. The strain producing biphenomycin A was found to co-produce a second antibiotic, biphenomycin B(**2**),[1] the structural elucidation of which is also the subject of this paper.

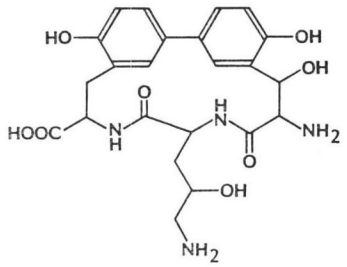
Biphenomycin A (**1**), $C_{23}H_{28}N_4O_8$, mp 205~209°C (dec) (HCl salt), $[\alpha]_D^{20}$ −22.5° (*c* 0.1, 1 N HCl), was isolated as a major component from the fermentation broth of *Streptomyces griseorubiginosus* No. 43708. From the same culture broth, biphenomycin B (**2**), $C_{23}H_{28}N_4O_7$, mp 206~209°C, $[\alpha]_D^{20}$ −10.6° (*c* 0.1, 1 N HCl), was isolated as a minor product.

Acetylation of **1** with Ac_2O in MeOH at 0°C and subsequent methylation with CH_2N_2 in MeOH at the same temperature gave the diacetyl monomethyl derivative **3**. After acetylation of **1** in the same manner, the product was treated with CH_2N_2 in MeOH at 5°C overnight to give the diacetyl trimethyl derivative **4**. These results indicated that **1** contains two amino, one carboxyl, and two weakly acidic hydroxyl groups.

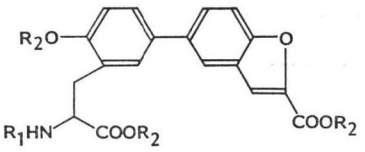
The ^1H NMR analysis (Table 1) of **1** and **3**, together with the ^{13}C NMR study (Table 2) of **1**, revealed all partial units of the structure of **1** (Fig. 1). The ^1H NMR spectrum of **1** in D_2O-DCl showed 17 protons, of which 3 protons at δ 5.02 (dd, J=7, 9 Hz), 4.91 (br s), and 4.47 (dd, J=3, 5 Hz), corresponding to δ 5.09 (dt, J=7.5, 8.8 Hz), 4.63 (br d, J=9.5 Hz), and 4.56 (dt, J=7.5, 3.3 Hz) in the spectrum of **3** in



1 R = OH
2 R = H

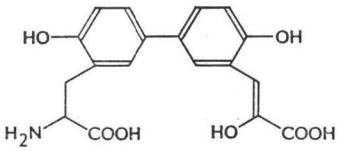


1'

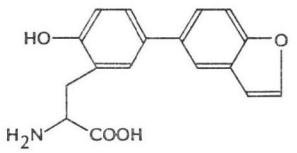


5 $R_1 = R_2 = H$

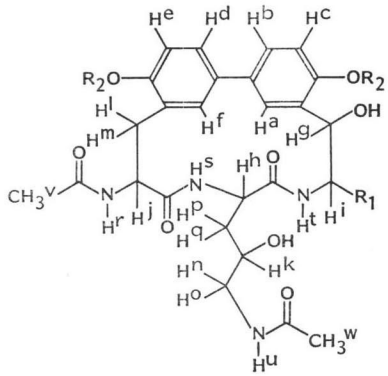
6 $R_1 = COCH_3$, $R_2 = CH_3$



7



8



3 $R_1 = COOCH_3{}^X$, $R_2 = H$

4 $R_1 = COOCH_3{}^X$, $R_2 = CH_3$

9 $R_1 = CH_2OH$, $R_2 = H$

DMSO-d_6, respectively, were assignable to the methine protons of amino acids (*e.g.* partial structures A, B, and C). These assignments were corroborated by the signals at 50.9 (d), 55.0 (d), and 57.4 (d) ppm in the ^{13}C NMR spectrum of **1**. Two protons at δ 5.84 (br s) and 4.09 (dddd, J=3, 4, 9, 10 Hz) in the ^1H NMR spectrum of **1** were attributable to the methine protons of secondary alcohols (*e.g.* partial structures A and C), which were supported by the signals at 64.4 (d) and 65.2 (d) ppm in the ^{13}C NMR spectrum of **1**. Taken together with the signals at 30.4 (t), 37.9 (t), and 44.9 (t) ppm in the ^{13}C NMR spectrum of **1**, three pairs of signals in the ^1H NMR spectrum of **1** at δ 3.55 (dd, J=5, 16 Hz) and 3.03 (dd, J=3, 16 Hz), 3.17 (dd, J=3, 13 Hz) and 2.97 (dd, J=10, 13 Hz), and 2.11 (ddd, J=4, 9, 14 Hz) and 1.95 (ddd, J=7, 9, 17 Hz) were assigned to the methylene protons (*e.g.* partial structures B and A). Two sets of signals at δ 7.39 (d, J=2.5 Hz), 7.17 (dd, J=2.5, 8.5 Hz), and 6.83 (d, J=8.5 Hz) and at δ 7.11 (dd, J=2.5, 8.5 Hz), 6.78 (d, J=8.5 Hz), and 6.88 (d, J=2.5 Hz) in the ^1H NMR spectrum of **3**, along with 12 signals in the ^{13}C NMR spectrum of **1** at 116.4 (d), 116.9 (d), 120.3 (s), 126.2 (d), 127.2 (d), 127.6 (d), 127.9 (s), 130.6 (d), 132.9 (s), 133.0 (s), 152.8 (s), and 154.6 (s) ppm, indicated that two tri-substituted phenyl rings (*e.g.* partial structures B and C) are present in **1**. The ^{13}C NMR spectrum showed three additional signals at 168.6 (s), 173.2 (s), and 174.0 (s) which were attributable to the carbonyls of amide and carboxy groups.

Confirmation of the partial unit A and extension of B and C to the partial structure B+C (Fig. 2)

Table 1. ^{1}H NMR (400 MHz) chemical shifts, multiplicities, and coupling constants (J=Hz) for biphenomycin A (**1**), **3**, and **6**.

H	1[a]	3[b]	6[c]
a	7.40, m (3H) and 6.93, m (2H)	7.39, d (2.5)	7.78, br d (2)
b		7.17, dd (2.5, 8.5)	7.61, dd (2, 8.5)
c		6.83, d (8.5)	7.62, br d (8.5)
d		7.11, dd (2.5, 8.5)	7.48, dd (2.5, 8.5)
e		6.78, d (8.5)	6.96, d (8.5)
f	6.87, br s	6.88, d (2.5)	7.33, d (2.5)
g	5.84, br s	5.70, br s	7.57, s
h	5.02, dd (7, 9)	5.09, dt (7.5, 8.8)	
i	4.91, br s	4.63, br d (9.5)	
j	4.47, dd (3, 5)	4.56, dt (7.5, 3.3)	4.80, dt (7.5, 7.5)
k	4.09, dddd (3, 4, 9, 10)	3.64, m	
l	3.55, dd (5, 16)	3.16[d]	3.22, d (7.5)
m	3.03, dd (3, 16)	2.77, dd (3.3, 15)	
n	3.17, dd (3, 13)	3.16[d]	
o	2.97, dd (10, 13)	3.07, m	
p	2.11, ddd (4, 9, 14)	1.79, m	
q	1.95, ddd (7, 9, 17)	1.50, m	
r		7.62, d (7.5)	6.23, d (7.5)
s		8.50, d (8.8)	
t		8.53, d (9.5)	
u		7.88, t (5.5)	
v			1.95, s (3H)
w		1.84, s (6H)	
x		3.71, s (3H)	4.10, s (3H)
y			3.73, s (3H)
z			3.91, s (3H)

[a]D_2O-DCl, [b]DMSO-d_6, [c]$CDCl_3$, [d]overlapping signals of H^l and H^n prevented the examination of their multiplicities.

Table 2. ^{13}C NMR chemical shifts (ppm) and multiplicities in D_2O-DCl for biphenomycins A (**1**) and B (**2**).

C	1	2	C	1	2
1	133.0[a] s	132.2[a] s	7	64.4 d	28.3 t
2	127.9[a] s	125.2[a] s	8	57.4 d	52.8 d
3	127.2[b] d	126.5[b] d	10	173.2[e] s	172.7[e] s
4	116.4[c] d	116.3[c] d	11	50.9 d	50.6 d
5	152.8 s	153.0 s	13	174.0[e] s	175.2[e] s
6	120.3 s	120.1 s	14	55.0 d	54.9 d
16	132.9 s	132.0 s	15	30.4 t	30.1 t
17	154.6 s	154.2 s	22	168.6 s	168.4 s
18	116.9[c] d	116.4 d	23	37.9 t	37.9 t
19	126.2[b] d	125.2[b] d	24	65.2 d	65.2 d
20	130.6[d] d	130.6[d] d	25	44.9 t	45.0 t
21	127.6[d] d	126.5[d] d			

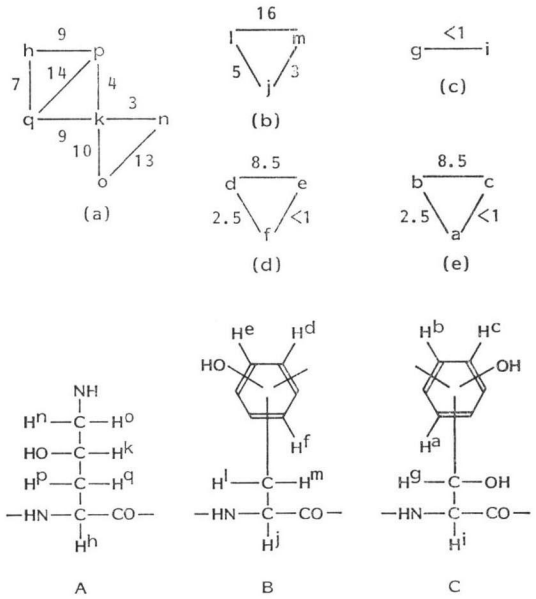
[a~e]: Assignments may be interchanged in **1** and **2**, respectively.

were obtained from the following acid-degradation experiment. Hydrolysis of **1** with 6 N HCl (110°C, 24 hours) gave, after chromatography on Toyopearl HW-40S, *erythro*-γ-hydroxyl-L-ornithine (HCl salt, mp 176~178°C (dec), $[\alpha]_D^{23}$ +10.9° (c 1.0, H_2O)) which was identified by comparison with an authentic sample,[3,4] confirming the presence of the partial unit A in the structure of **1**. The acid

Fig. 1. The partial structures A~C and the ^{1}H-^{1}H relationships (a)~(e).

The ^{1}H-^{1}H relationships (a), (b) and (c) were obtained by decoupling experiments on **1**, while (d) and (e) were derived by those on **3**.

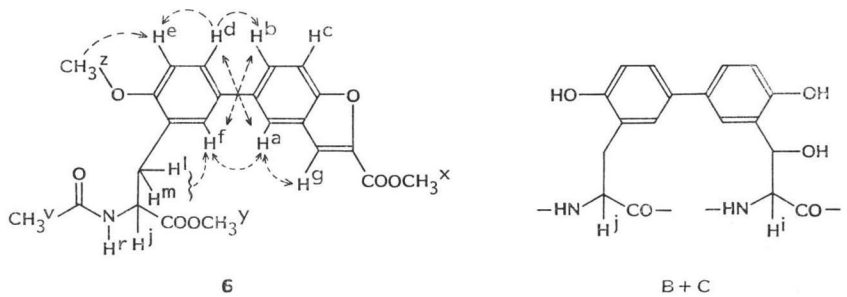
The vanishing value of the H^g-H^1 vicinal coupling constant is presumably owing to a restricted conformation of biphenomycin A which leads H^g and H^1 to a dihedral angle close to 90°. The phenyl groups of A and B may be interchanged.



hydrolysis also gave the fragment **5** (FD-MS: m/z 341 (M^+)), which was converted, by acetylation with Ac_2O in MeOH at room temperature followed by methylation with CH_2N_2 in MeOH at room temperature, to the monoacetyl trimethyl derivative **6**. The ^{1}H NMR analysis of **6** with the aid of spin-decoupling (Table 1) and nuclear Overhauser effect (NOE) (Fig. 2) experiments suggested the structure of **6** and accordingly the structure of **5**, which was supported by the IR and ^{13}C NMR spectra of **5** (see Experimental). The acid hydrolysis described above also gave two minor products **7** and **8**, whose structures were assigned by analysis of their IR and ^{1}H NMR spectra (see Experimental). The geneses of these products **5**, **7**, and **8** are rationalized by the following, plausible reaction mechanisms from the partial units B and C. Dehydration of the β-hydroxyl amino acid residue in C, followed by hydrolysis of the resulting dehydro amino acid, leads to the keto acid **7**. Dehydrative condensation of the phenolic hydroxyl group with the keto acid function in **7** gives the benzofuran structure **5**. Decarboxylation of **5** or dehydrative condensation after decarboxylation of **7** give the product **8**. This chemical evidence, together with the spectral data described above, thus leads to the partial structure B+C.

The problem remaining is to link the partial structures A and B+C for the full structure of biphenomycin A. In the ^{1}H NMR spectrum of **3** in DMSO-d_6, in addition to the acetamido NHs (probably δ 8.53 (d, J=9.5 Hz) and 7.88 (t, J=5.5 Hz)), two original amido protons coupled to H^h (δ 5.09, dt, J=7.5, 8.8 Hz) and H^1 (δ 4.63, br d, J=9.5 Hz) were observed at δ 7.62 (d, J=7.5 Hz) and

Fig. 2. The structure of **6** and the partial structure B+C.
The observed NOEs in **6** are shown by dotted line arrows.



8.50 (d, J=8.8 Hz), suggesting the presence of two peptide bonds in **1**. The most reasonable combination of the structural units A and B+C for these peptide bonds is an insertion of A between the two amino acid moieties of B+C, because a molecular model study indicated that an intramolecular cyclization of B+C itself is practically impossible

When a ^{1}H NMR spectrum of **1** was measured in D_2O-NaOD, H^i and H^j were shifted upfield by 0.36 and 0.67 ppm, respectively, as compared to their signals in D_2O-DCl (Table 1), while no shift was observed on H^h (δ 5.02 in both D_2O-DCl and D_2O-NaOD),[5] indicating that the α-amino acid group of A is incorporated into the cyclic peptide structure in **1**. Therefore, two structures **1** and **1'** can be proposed for biphenomycin A.

Reduction of **3** with $NaBH_4$ in MeOH gave alcohol **9** (FAB-MS: m/z 559 (M^++1)), in the ^{1}H NMR spectrum (CD_3OD) of which the singlet-like signal (δ 4.89, CD_3OD) corresponding to H^i in **1** was changed to a triplet (δ 4.20, J=7.5 Hz) coupled to the newly formed methylene group (δ 3.65 (dd, J=7.5, 11 Hz) and 3.78 (dd, J=7.5, 11 Hz)). This result indicated that the carboxylic acid function in **1** is bonded to C-8 bearing H^i. Consequently, the structure of biphenomycin A was thus established as being **1**.

By comparing the molecular formula of biphenomycin B (**2**) with that of biphenomycin A (**1**), **2** was surmised to be the deoxy derivative of **1**. The ^{13}C NMR spectrum of **2** in D_2O-DCl (Table 2) is superimposable on that of **1** except for the signals at 28.3 (t) and 52.8 (d) ppm in **2**, which correspond to those at 64.4 (d) and 57.4 (d) ppm in **1**, respectively. This fact shows that C-7 (28.2 ppm) in **2** consists of a methylene group and hence the signal at 57.4 ppm in **1** shifted upfield to 52.8 ppm in **2**. Upon treatment of **1** with PtO_2 in AcOH (4 atmospheric pressure of H_2), the benzylic hydroxyl group at C-7 in **1** underwent hydrogenolysis to give rise to a compound which was identical with **2** (IR, ^{1}H NMR and HPLC), thus confirming that the structure of biphenomycin B is **2**.

The structures of biphenomycins A and B were established as being **1** and **2**, respectively. Biphenomycin A showed high antibacterial activity especially against Gram-positive bacteria, while biphenomycin B was somewhat weaker (see the preceding paper[1]).

Experimental

Infrared spectra were recorded on a Jasco A-102 infrared spectrophotometer. UV spectra were measured on a Hitachi 220A double beam spectrophotometer. ^{1}H and ^{13}C NMR spectra are recorded by using Jeol JNM-GX400 and Jeol JNM-FX270 spectrophotometers.

NOE experiments were carried out by the technique of ^{1}H-[^{1}H] NOE difference spectra. Low-resolution and high-resolution EI mass spectra and FD mass spectra were measured on a Jeol JMS-D-300 mass spectrometer. FAB-MS spectra were determined with Jeol JMS-DX300 mass spectrometer.

Di-*N*-acetyl Monomethyl Ester (**3**)

To a stirred suspension of **1** (50 mg) in MeOH (5 ml) was added Ac_2O (1 ml) at 0°C. The resulting solution was stirred at the same temp for 30 minutes, and then diluted with H_2O. The solvent was removed *in vacuo* to give a residue, which was dissolved in MeOH (5 ml). To this solution was added a solution of CH_2N_2 in ether at 0°C. After standing for 10 minutes at the same temp, the solution was concd to dryness to give a residue, which was purified by preparative TLC on silica gel with $CHCl_3$-MeOH (5: 1) to afford **3** (25 mg): IR (Nujol) 3300, 1735, 1660 cm^{-1}; ^{13}C NMR (DMSO-d_6) 22.46 (q), 22.55 (q), 30.4 (t), 39.0 (t), 44.3 (t), 49.0 (d), 52.2 (q), 52.7 (d), 56.1 (d), 62.9 (d), 66.0 (d), 114.7 (d), 115.0 (d), 123.9 (s), 124.2 (d), 125.9 (d), 126.0 (d), 128.7 (d), 128.9 (s), 131.6 (s), 131.9 (s), 152.6 (s), 154.1 (s), 168.6 (s), 169.4 (s), 169.6 (s), 171.0 (s), 171.6 (s) ppm; ^{1}H NMR (CD_3OD) δ 1.79 (1H, ddd,

J=7.5, 8.5 and 14 Hz), 1.95 (6H, s), 2.00 (1H, m), 2.91 (1H, dd, J=3.5, 15 Hz), 3.26 (1H, dd, J=4.5, 15 Hz), 3.35 (1H, dd, J=6.0, 15 Hz), 3.45 (1H, dd, J=6.3, 15 Hz), 3.82 (3H, s), 3.88 (1H, m), 4.65 (1H, dd, J=3.3, 6.0 Hz), 4.89 (1H, br s), 5.10 (1H, dd, J=7.5, 8.5 Hz), 5.83 (1H, br s), 6.78 (1H, d, J=8.5 Hz), 6.81 (1H, d, J=8.5 Hz), 7.02 (1H, d, J=2.5 Hz), 7.19 (1H, dd, J=2.5, 8.5 Hz), 7.25 (1H, dd, J=2.5, 8.5 Hz), 7.57 (1H, d, J=2.5 Hz); FAB mass, m/z 587 (M^++1), 609 (M^++23).

Di-*N*-acetyl Trimethyl Derivative (**4**)

Acetylation of **1** (50 mg) was carried out with Ac_2O (1 ml) in MeOH (5 ml) in the same manner as the preparation of **3**. To a solution of the crude di-*N*-acetylbiphenomycin A (50 mg) in MeOH was added a solution of CH_2N_2 in ether at 0°C, and the solution was kept in the refrigerator overnight. After evaporation of the solvent, the residue was purified by preparative TLC on silica gel with $CHCl_3$ - MeOH (7: 1) to afford **4** (20 mg): ^{1}H NMR (CD_3OD) δ 1.60 (3H, s), 1.96 (1H, m), 2.10 (3H, s), 2.20 (1H, ddd, J=3.5, 3.8 and 14 Hz), 2.96 (1H, dd, J=5, 14 Hz), 3.25~3.50 (3H, m), 3.84 (3H, s), 3.86 (3H, s), 3.90 (3H, s), 4.10 (1H, m), 4.38 (1H, m), 4.61 (1H, d, J=2 Hz), 4.90 (1H, m), 5.92 (1H, d, J=2 Hz), 6.92 (1H, d, J=8.5 Hz), 6.94 (1H, d, J=8.5 Hz), 7.41 (1H, dd, J=2.5, 8.5 Hz), 7.43 (1H, dd, J=2.5, 8.5 Hz), 7.72 (1H, d, J=2.5 Hz), 7.85 (1H, d, J=2.5 Hz); FAB mass, m/z 615 (M^++1).

Acid Hydrolysis of Biphenomycin A (**1**)

A solution of **1** (50 mg) in 6 N HCl (5 ml) was heated for 24 hours at 110°C. After evaporation of the solvent *in vacuo*, the residue was dissolved in H_2O and the solution was adjusted to pH 7.0 with 1 N NaOH. The resulting solution was applied to a column of Diaion HP-20 (20 ml). The column was eluted with H_2O (50 ml) and then with MeOH (50 ml). The fractions eluted with H_2O were concd *in vacuo* to give a residue, which was chromatographed on CM-cellulose (15 ml) eluting with 0.05 M pyridine - AcOH buffer (pH 5.0). The fractions showing a positive ninhydrin reaction were collected and concd *in vacuo* to give a residue (13 mg), which was dissolved in H_2O (0.5 ml), adjusted to pH 5.0 with 6 N HCl and concd to give a syrup. This syrup was crystallized from H_2O - EtOH to afford γ-hydroxy-L-ornithine hydrochloride (7 mg): mp 176~178°C; $[\alpha]_D^{23}$ +10.9° (c 1.0, H_2O); ^{1}H NMR (D_2O) δ 2.05 (2H, m), 3.03 (1H, dd, J=8, 14 Hz), 3.26 (1H, dd, J=5, 14 Hz), 3.94 (1H, t, J= 7 Hz), 4.23 (1H, m); FD mass, m/z 149 (M^++1).

The fractions eluted with MeOH in the Diaion HP-20 chromatography were evaporated *in vacuo* to give a residue, which was chromatographed on Toyopearl HW-40S (100 ml) eluting with 50% aq MeOH to afford 3-[5-(2-carboxy-5-benzofuranyl)-2-hydroxyphenyl]alanine (**5**) (9.9 mg), 3-[4,4′-dihydroxy-3′-(2-hydroxy-2-carboxyvinyl)-3-biphenyl]alanine (**7**) (2.8 mg) and 3-[5-(5-benzofuranyl)-2-hydroxyphenyl]alanine (**8**) (3.1 mg). Compound **5**: IR (Nujol) 2800~2300, 1600, 1580 cm^{-1}; ^{1}H NMR (CD_3OD-D_2O) δ 3.19 (1H, dd, J=7.5, 14 Hz), 3.47 (1H, dd, J=5, 14 Hz), 4.29 (1H, dd, J=5, 7.5 Hz), 7.01 (1H, d, J=8.7 Hz), 7.49 (2H, m), 7.64 (1H, s), 7.66 (1H, d, J=8.7 Hz), 7.70 (1H, dd, J= 2, 8.7 Hz), 7.90 (1H, d, J=2 Hz); FAB mass, m/z 342 (M^++1); CD $[\theta]_{216nm}$+11,400°, $[\theta]_{260nm}$+2,400°. Compound **7**: IR (KBr) 1690, 1600 cm^{-1}; ^{1}H NMR (CD_3OD+D_2O) δ 3.07 (1H, dd, J=8.8, 14 Hz), 3.44 (1H, dd, J=4, 14 Hz), 3.97 (1H, dd, J=4, 8.8 Hz), 6.93 (1H, d, J=8.7 Hz), 7.12 (1H, s), 7.32 (1H, d, J=8.7 Hz), 7.42 (1H, dd, J=2.5, 8.7 Hz), 7.49 (1H, d, J=2.5 Hz), 7.59 (1H, dd, J=2.5, 8.7 Hz), 7.67 (1H, d, J=2.5 Hz); FAB mass, m/z 324 (M^++1). Compound **8**: IR (KBr) 3400, 3200~ 2200, 1600 cm^{-1}; ^{1}H NMR (CD_3OD-D_2O) δ 3.13 (1H, dd, J=7.5, 14 Hz), 3.48 (1H, dd, J=5, 14 Hz), 4.26 (1H, dd, J=5, 7.5 Hz), 6.88 (1H, d, J=2.5 Hz), 6.97 (1H, d, J=8.2 Hz), 7.44 (1H, d, J=2.3 Hz), 7.45 (1H, dd, J=2.3, 8.2 Hz), 7.49 (1H, dd, J=2.3, 8.2 Hz), 7.53 (1H, d, J=8.2 Hz), 7.77 (1H, d, J=2.5 Hz), 7.77 (1H, m); FD mass, m/z 297 (M^+).

Methyl *N*-Acetyl-3-[2-methoxy-5-(2-methoxycarbonyl-5-benzofuranyl)phenyl]alaninate (**6**)

To a solution of **5** (7 mg) in MeOH was added Ac_2O (0.5 ml), and the mixture was stirred at room temp for 4 hours. After evaporation of the solvent *in vacuo*, the residue was dissolved in MeOH and treated with a solution of CH_2N_2 in ether at 0°C. After the reaction was completed, the solvent was evaporated *in vacuo* to give a residue, which was purified by preparative TLC on silica gel with $CHCl_3$ - MeOH (95: 5) to afford methyl *N*-acetyl-3-[2-methoxy-5-(2-methoxycarbonyl-5-benzofuranyl)phenyl]-alaninate (**6**) (5.9 mg): IR ($CHCl_3$) 1720, 1660 cm^{-1}; UV λ_{max}^{EtOH} nm (ε) 265 (28,000), 271 (29,000), 283

(22,500), 320 (3,600); ^{13}C NMR ($CDCl_3$) 22.3 (q), 33.7 (t), 52.5 (q), 52.8 (q), 53.8 (d), 56.1 (q), 111.9 (d), 113.1 (d), 115.2 (d), 121.5 (d), 126.7 (s), 128.2 (d), 128.2 (d), 128.9 (s), 131.0 (d), 134.3 (s), 138.6 (s), 147.2 (s), 157.0 (s), 158.5 (s), 161.5 (s), 173.0 (s), 173.9 (s) ppm; high resolution EI mass, *m/z* 425.1457; Calcd for $C_{23}H_{23}NO_7$, 425.1472.

$NaBH_4$ Reduction of **3**

To a solution of **3** (30 mg) in MeOH (3 ml) was added $NaBH_4$ (100 mg) and the mixture was stirred at room temp for 4 hours. After evaporation of the solvent, the residue was dissolved in H_2O and neutralized with 1 N HCl. The resulting solution was desalted on Diaion HP-20 (20 ml) washing with H_2O and eluting with MeOH and then purified by preparative TLC on silica gel developing with $CHCl_3$ - MeOH - conc NH_3 (5: 3: 1) to afford **9** (10 mg): IR (Nujol) 3300, 1650 cm^{-1}; ^1H NMR (CD_3OD) δ 1.80 (1H, m), 1.92 (1H, m), 1.95 (3H, s), 1.96 (3H, s), 2.94 (1H, dd, J=3, 13.5 Hz), 3.24 (2H, m), 3.43 (1H, dd, J=6, 13.5 Hz), 3.65 (1H, dd, J=7, 11 Hz), 3.76 (1H, dd, J=7, 11 Hz), 3.78 (1H, m), 4.20 (1H, t, J=7 Hz), 4.64 (1H, dd, J=3, 6 Hz), 4.94 (1H, t, J=8 Hz), 5.40 (1H, s), 6.77 (1H, d, J=8.2 Hz), 6.78 (1H, d, J=8.2 Hz), 7.07 (1H, d, J=2 Hz), 7.17 (1H, dd, J=2, 8.2 Hz), 7.21 (1H, dd, J=2, 8.2 Hz), 7.55 (1H, d, J=2 Hz); FAB mass, *m/z* 559 (M^++1).

Catalytic Hydrogenation of Biphenomycin A (**1**)

A solution of **1** (20 mg) in AcOH (2 ml) containing 70% $HClO_4$ (20 μl) was hydrogenated on PtO_2 (30 mg) under medium pressure (3.5~4 atm) of H_2 for 3 hours. After removal of the catalyst by filtration, the filtrate was adjusted to pH 7 with 1 N NaOH. The resulting solution was concd to dryness *in vacuo* to give a residue, which was dissolved in H_2O and then subjected to preparative HPLC using the same conditions as in the preceding paper,[1] to afford biphenomycin B (**2**) (5 mg) together with biphenomycin A (**1**) (5 mg). The IR and NMR spectra and HPLC of these two compounds were identical with those of the natural biphenomycin B (**2**) and biphenomycin A (**1**), respectively.

References

1) Ezaki, M.; M. Iwami, M. Yamashita, S. Hashimoto, T. Komori, K. Umehara, Y. Mine, M. Kohsaka, H. Aoki & H. Imanaka: Biphenomycins A and B, novel peptide antibiotics. I. Taxonomy, fermentation, isolation and characterization. J. Antibiotics 38: 1453~1461, 1985
2) Uchida, I.; M. Ezaki, N. Shigematsu & M. Hashimoto: Structure of WS-43708A, a novel cyclic peptide antibiotic. J. Org. Chem. 50: 1341~1342, 1985
3) Mizusaki, K.; H. Yamamoto & S. Makisumi: Synthesis of *erythro*- and *threo*-γ-hydroxy-L-ornithine. Bull. Chem. Soc. Jpn. 53: 2605~2609, 1980
4) Mizusaki, K. & S. Makisumi: Synthesis of four stereoisomers of γ-hydroxyarginine *via* the corresponding isomers of γ-hydroxyornithine. Bull. Chem. Soc. Jpn. 54: 470~472, 1981
5) Sheinblatt, M.: Determination of amino acid sequence in di- and tripeptides by nuclear magnetic resonance techniques. J. Am. Chem. Soc. 88: 2845~2848, 1966