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EDIFICIO SISKI
CARRERA 4 No. 72 -
BOGOTA 8, COLOMI

Señora Jefe de la
DIVISION DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Trámite : 011
Evento : 379
Actuación : 444

Referencia : Expediente No. 05.003.061
Asunto : Solicitud de patente para: **"MACROCICLOS DE AMIDA ANTIBACTERIANOS"**
Solicitante : **AI CURIS GMBH & CO. KG**
Fecha de solicitud: 17 de enero de 2005

1. Yo, **Emilio FERRERO**, obrando como apoderado del solicitante en el asunto antes identificado, me refiero al Oficio No. **12188** notificado por fijación en lista del 26 de Septiembre de 2008, y atentamente presento junto con este escrito los siguientes artículos:

i) DATABASE CAPLUS 'Online!; CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; Database accession no. 1993:60099 XP002258609 abstract & U. SCHMIDT ET AL.: "Amino acids and peptides. 84. Synthesis of biologically active cyclopeptides. 24. Total sysnthesis of the biphenomycins. III. Synthesis of biphenornycin B" SYNTHESIS.,vol. 10, 1992, pages 1025-1030, THIEME VERLAG, STUTTGART., DE ISSN: 0039-7881
Cited in the application.

ii) DATABASE CAPLUS 'Online!; CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; Database accession no. 1987:637278 XP002258610 abstract & K. RAJAMOORTHI ET AL.: "Stereochenistry

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of the cyclic tripeptide antibiotic WS-43708A"; JOURNAL OF ORGANIC CHEMISTRY., vol. 52, no. 24, 1987, pages 5435-5437, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US; ISSN: 0022-3263.Cited in the application.

iii) DATABASE CAPLUS 'Online! ; CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; Database accession no. 1986:84995 XP002258611 abstract & I. UCHIDA ET AL.: "Biphenornycins A and B, novel peptide antibiotics. II. Structural elucidation of biphenornycins A and B" JOURNAL OF ANTIBIOTICS., vol. 38, no. 11, 1985, pages 1462-1468, JAPAN ANTIBIOTICS RESEARCH ASSOCIATION, TOKYO., JP; ISSN: 0021-8820.
Cited in the application.

iv) DATABAS E CAPLUS 'Online!; CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; Database accession no. 1985:167162 XP002258612 abstract & I. UTCHIDA ET AL.: "Structure of WS-43708A, a novel cyclic peptide antibiotic" JOURNAL OF ORGANIC CHEMISTRY., vol. 50, no. 8, 1985, pages 1341-1342, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0022-3263.
Cited in the application.

2. Puesto que se han atendido a cabalidad las peticiones hechas por su Despacho sobre la presente solicitud de patente, atentamente solicito que se continúe su tramitación.

Señora Jefe,

EMILIO FERRERO
T.P.A. No. 31.929

COLO-15807-843-001-0
COLO-14033-841-011-5
MGM\MGM\ID-139956\WPC15807
No. M-2008-139956\MGM

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)

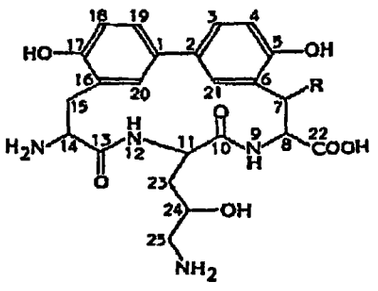
The structures of biphenomycins A and B, novel peptide antibiotics produced by a strain of *Streptomyces*, have been established as **1** and **2**, respectively, on the basis of spectroscopic and chemical evidence. They are unique in that they are cyclic peptides containing a biphenyl moiety included 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.

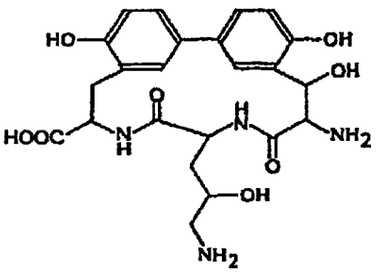
Biphenomycin A (1), $C_{23}H_{25}N_4O_8$, mp 205~209°C (dec) (HCl salt), $[\alpha]_D^{20}$ −22.5° (*c* 0.1, 1 N HCl), was isolated as a major component from the fermentation broth of *Streptomyces griseorubiginosus* No. 43708. From the same culture broth, biphenomycin B (2), $C_{23}H_{25}N_4O_7$, mp 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.

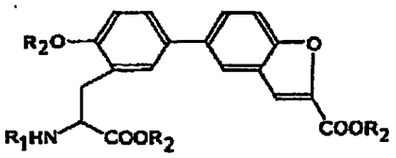
The ^1H NMR analysis (Table 1) of 1 and 3, together with the ^{13}C NMR study (Table 2) of 1, revealed all partial units of the structure of 1 (Fig. 1). The ^1H NMR spectrum of 1 in D_2O-DCl showed 17 protons, of which 3 protons at δ 5.02 (dd, J=7, 9 Hz), 4.91 (br s), and 4.47 (dd, J=3, 5 Hz), corresponding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



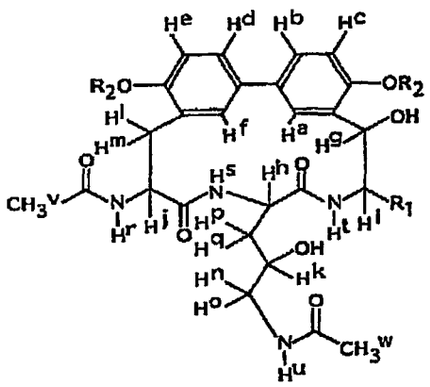
1 R = OH
2 R = H



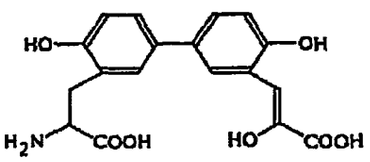
1*



5 $R_1 = R_2 = H$
6 $R_1 = COCH_3$, $R_2 = CH_3$



3 $R_1 = COOCH_3$[X], $R_2 = H$
4 $R_1 = COOCH_3$[X], $R_2 = CH_3$
9 $R_1 = CH_2OH$, $R_2 = H$



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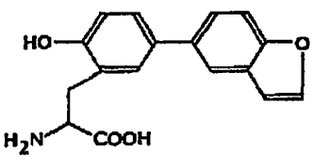

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DMSO-d_6, respectively, were assignable to the methine protons of amino acids (*e.g.* partial structures A, B, and C). These assignments were corroborated by the signals at 50.9 (d), 55.0 (d), and 57.4 (d) ppm in the ^{13}C NMR spectrum of **1**. Two 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			

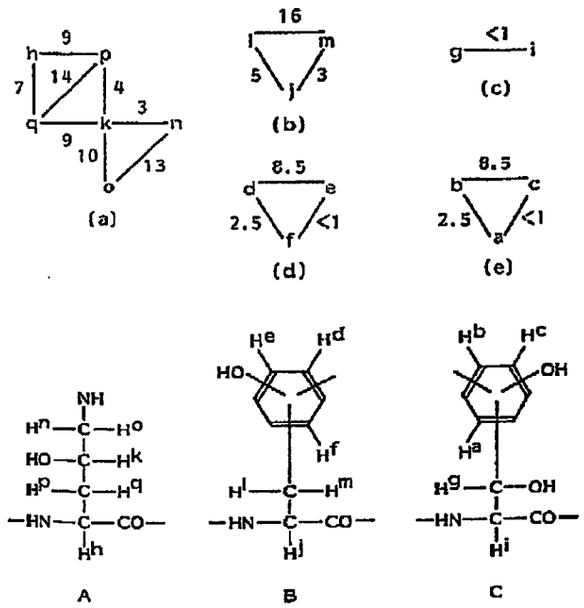
[a-e]: Assignments may be interchanged in 1 and 2, respectively.

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 ^1H-^1H relationships (a)~(e).

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

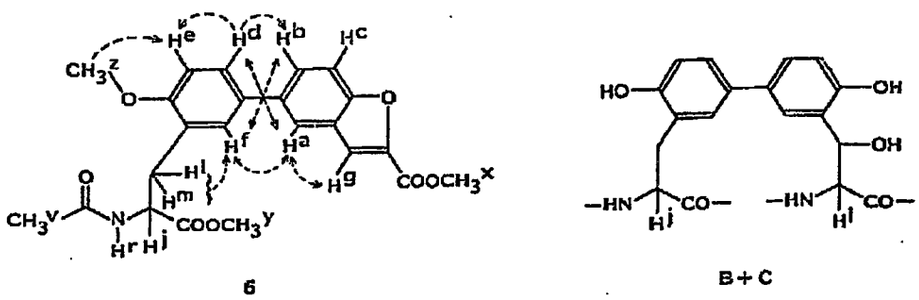
The vanishing value of the H^g-H^i vicinal coupling constant is presumably owing to a restricted conformation of biphenomycin A which leads H^g and H^i to a dihedral angle close to 90°. The phenyl groups of A and B may be interchanged.



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 ^1H 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 ^1H 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 ^1H 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^i (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; $[\alpha]_D^{28}$ +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

to ^{1}H NMR analysis consisted of 11 and 12 in a ratio of 4:1. The oil also contained a small amount (less than 6%) of dibenzylideneacetone as an impurity.

11: ^{1}H NMR (200 MHz) (assigned peaks in mixture with 12) δ 5.9–5.6 (m, 4 H, olefinic), 3.75 (s, 3 H, one of CO_2Me), 3.74 (s, 3 H, one of CO_2Me), 3.51 (d, J = 8.8 Hz, 1 H, $CH(CO_2Me)_2$), 3.18 (m, 1 H, $CHCH(CO_2Me)_2$), 2.45–2.3 (m, 2 H, allylic CH_2), 1.95–1.75 (m, 2 H, CH_2).

12: ^{1}H NMR (200 MHz) (assigned peaks in mixture with 11) δ 5.9–5.6 (m, 3 H, olefinic), 4.12 (s, 1 H, $CH(CO_2Me)_2$), 3.75 (s, 6 H, CO_2Me), 2.45–2.3 (m, 4 H, allylic CH_2), 1.95–1.75 (m, 2 H, CH_2).

Registry No. 1, 96039-90-6; 2, 96039-91-7; 3, 82736-52-5; 4, 110418-98-9; 5, 39495-94-8; 7, 110455-97-5; 8, 110455-98-6; 9, 15833-44-0; 10, 110418-99-0; 11, 91550-40-2; 12, 110419-00-6; $Pd(dppe)_2$, 31277-98-2; $Pd(dba)_2$, 81141-80-2; dimethyl malonate, 108-59-8; (*E*)-(*R**,*R**)-2-acetoxy-5-chloro-3-hexene, 95177-49-4; (*E*)-(*R**,*S**)-2-acetoxy-5-chloro-3-hexene, 95177-50-7; sodium dimethyl malonate, 18424-76-5; *cis*-1-acetoxy-4-chloro-2-cyclohexene, 82736-39-8; *cis*-1-acetoxy-4-chloro-2-cycloheptane, 82736-40-1; dimethyl diazomalonate, 6773-29-1; (*E*,*E*)-2,4-hexadiene, 5194-51-4.

Stereochemistry of the Cyclic Tripeptide Antibiotic WS-43708A

Rajamoorthi Kannan and Dudley H. Williams*

University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

Received April 27, 1987

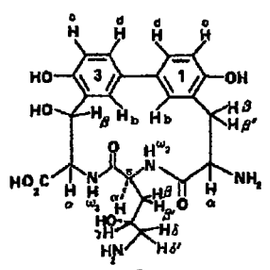
By the use of proton NMR spectroscopy, in particular of coupling constant and NOE data, it is shown that the cyclic tripeptide antibiotic WS-43708A has *S* stereochemistry at the α-carbon atom of all three amino acids and that the carbon atom at the benzylic position of residue 3 has *R* stereochemistry. The conformational preference of the biphenyl system has also been determined. Although WS-43708A appears to be the only reported antibiotic outside the vancomycin group to possess a biphenyl group, no evidence for binding of WS-43708A to the cell wall analogue N-Ac-D-Ala-D-Ala has been found. It is concluded therefore that WS-43708A exerts its antibacterial activity other than by binding to mucopeptide precursors terminating in D-Ala-D-Ala.

A covalent structure (1) for the cyclic tripeptide antibiotic WS-43708A has been reported by Hashimoto and co-workers.[1] The only stereochemical information which was available from their study was that residue 2 corresponds to *erythro*-γ-hydroxy-L-ornithine.[1] In view of a possible structural analogy between this structure and parts of the structures found for vancomycin group antibiotics,[2] we have fully determined and now report the stereochemistry of WS-43708A. Our interest in this stereochemistry was also initially aroused by the possibility that WS-43708A acts by inhibiting cell wall biosynthesis, as do also members of the vancomycin group.[2] We therefore wished to establish whether WS-43708A has a geometry suitable to bind mucopeptide precursors terminating in D-Ala-D-Ala.[2]

The earlier structural work on WS-43708A had been carried out in D_2O/DCl and CD_3OD/D_2O. Since we wished to utilize NOE and coupling data for the amide protons, it was desirable to work in DMSO-d_6 solution. As the dihydrochloride of the antibiotic (kindly provided by Dr. Hashimoto) was not soluble in DMSO-d_6, it was converted to the free base by dissolving in an aqueous solution of ammonium carbonate. The solution was then lyophilized several times to remove excess ammonium carbonate.

Proton NMR spectra of the above free base were obtained in DMSO-d_6 solution on a Bruker AM-400 spectrometer. Two and three bond proton–proton couplings were determined from a double quantum filtered phase-sensitive COSY spectrum. This spectrum also exposed the four bond couplings between benzylic protons and protons ortho to the benzylic position. Chemical shifts and coupling constants are summarized in Table I, by using the proton code given in 1, and are in accord with the reported structure. Phase-sensitive NOESY and CAMELSPIN[3]

Table I. ^{1}H NMR Chemical Shifts and Coupling Constants for WS-43708A[a]



1

proton	chem shift, δ	J, Hz
ω_2	8.04	9.2
ω_3	8.40	9.7
3b	7.44	2.6
1d, 3d	7.18	m
1b	6.94	2.2
1c, 3c	6.9	7.5
β_3	5.72	s
α_2	5.04	m
α_3	4.52	9.7
α_1	4.14	m
γ_2	3.92	m
β_1	3.24	15, 6
β_1'	3.02	15, 3
δ_2	2.98	13, 3
δ_2'	2.78	13, 8
β_2, β_2'	1.84	m

[a] Spectrum of the free base in DMSO-d_6 at 330 K: m = multiplet; s = singlet. The amino acid residues are coded 1, 2, and 3 from the N- to the C-terminus.

(1) Uchida, I.; Ezaki, M.; Shigematsu, N.; Hashimoto, M. *J. Org. Chem.* 1985, *50*, 1341–1342.
(2) Barna, J. C. J.; Williams, D. H. *Annu. Rev. Microbiol.* 1984, *38*, 339–357.

0022-3263/87/1952-5435$01.50/0

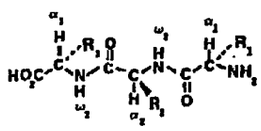
Table II. NOEs Observed from Phase Sensitive NOESY[a] and CAMELSPIN[b] Spectra of WS-43708A[c]

$\omega_2 \leftrightarrow \alpha_1$	1b $\leftrightarrow \beta_1'$	$\gamma_2 \leftrightarrow (\beta_2, \beta_2')$
$\omega_2 \leftrightarrow (\beta_2, \beta_2')$	$\beta_3 \leftrightarrow \alpha_3$	$\gamma_2 \leftrightarrow \delta_2$
$\omega_3 \leftrightarrow \alpha_2$	$\alpha_2 \leftrightarrow (\beta_2, \beta_2')$	$\beta_1 \leftrightarrow \beta_1'$
$\omega_3 \leftrightarrow$ 3b	$\alpha_1 \leftrightarrow \beta_1$	$\delta_2 \leftrightarrow \alpha_2$
3b $\leftrightarrow \alpha_3$	$\alpha_1 \leftrightarrow \beta_1'$	$\delta_2 \leftrightarrow \delta_2'$
3b $\leftrightarrow$ 1b		

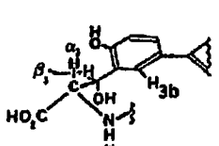
[a] Obtained in DMSO-d_6 at normal probe temperature (290–293 K). [b] Obtained in DMSO-d_6 containing 2% TFA (v/v) at 320 K, with a spin-locking duration of 200 ms; the experiment was repeated with a different transmitter offset to check for any false enhancement.[4] [c] All NOEs were negative in the NOESY spectrum; their magnitudes lay in the range 2–15%. The CAMELSPIN NOEs are positive.

spectra were obtained in the same solvent (but the latter after addition of 2% v/v TFA). The latter spectrum is useful in obtaining specific NOEs even in cases where spin diffusion is normally a problem, and in cases where the NOE would be near zero because $\omega_0\tau_c \simeq 1$. The NOE data obtained from these spectra are summarized in Table II. Since the pattern of NOEs was essentially the same from both types of experiment, it is clear that significant spin diffusion had been avoided at the mixing time (0.3 s) used in the NOESY experiment. It is also clear from these data that the conformation of the antibiotic, in the regions in which NOEs are observed, is essentially the same for the protonated form (after addition of TFA) and for the free base.

The vicinal coupling constants between the amide N*H* protons of amino acid residues 2 and 3 (ω_2 and ω_3) and their respective α-C*H*s both lie in the range 9–10 Hz.



2



3

These facts, coupled with the observation that neither of the α-CH protons shows an NOE to the amide NH of its own residue, indicate that the α-C*H*N*H* protons are approximately trans-orientated (see 2). Additionally, the strong NOEs $\alpha_1 \leftrightarrow \omega_2$ and $\alpha_2 \leftrightarrow \omega_3$ indicate that these two pairs of protons are on the same side of the molecule, as also indicated in 2.

Since the side chains of the oxidized phenylalanine units (R_1 and R_3 in 2) are connected (presumably via phenol oxidative coupling), then they must be orientated in the same direction from the peptide backbone unit 2. CPK models show that this direction must be downwards from the plane of the paper (see 2); if they orientated upwards from the plane of the paper, then the side chain of the γ-hydroxyornithine residue (R_2 in 2) cannot be accommodated within the 15-membered ring. Thus, both residues 1 and 3 have the *S* absolute configuration of their α-carbon atoms (see 2).

There is no discernible coupling between α_3 and β_3, and therefore the dihedral angle between these vicinal protons must be near to 90° (see also ref 1). CPK models show that this geometry can only be accommodated if the benzylic position of residue 3 has the *R* configuration, i.e., with the benzylic hydroxyl group approximately trans-orientated with respect to the C–$H_{\alpha 3}$ bond. Additionally, the fact that β_3 shows an NOE to α_3 but to no other proton (Table II) demands, when taken in conjunction with the other constraints, the *R* configuration at the benzylic center. This geometry is reproduced in 3 and is in accord with the observation that 3b shows NOEs to both ω_3 and α_3 but not to β_3.

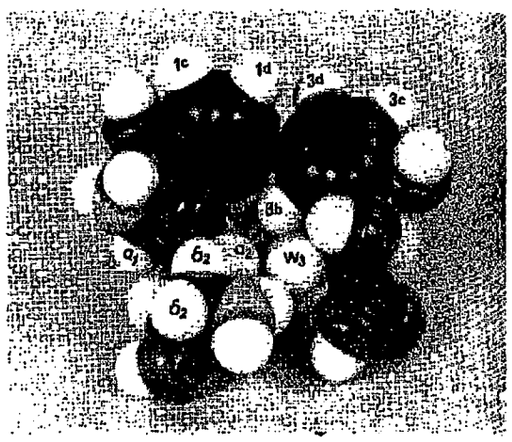
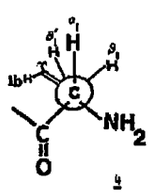
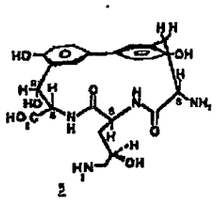


Figure 1. CPK model of WS-43708A.

The vicinal coupling constants of α_1 to β_1 and β_1' (6 and 3 Hz, respectively) establish that neither of these benzylic protons is held in a specific trans conformation relative to α_1. This conclusion, in conjunction with the observation of NOEs from α_1 to both β_1 and β_1', shows that these benzylic protons are on the same side of the structure as α_1. In the NOESY spectrum the cross peak between α_1 and β_1' is larger than that between α_1 and β_1; moreover, in the CAMELSPIN spectrum, only the $\alpha_1 \leftrightarrow \beta_1'$ NOE is observed. Hence α_1 is closer to β_1' than to β_1, which requires a smaller dihedral angle on average between α_1 and β_1' than between α_1 and β_1. Additionally, 1b shows an NOE to β_1' but not to β_1. These data demand a geometry of this part of the molecule which is close to that shown in 4. The original form of the Karplus equation would



4



5

of course require $J_{\alpha_1\beta_1'} > J_{\alpha_1\beta_1}$, but account must also be taken of the fact that vicinal coupling is reduced when one of the protons involved is approximately trans-coplanar to an electronegative substituents.[5] To take account of this type of effect (which operates here, since β_1' is almost trans-coplanar to the NH_2 substituent—see 4), we have used the equation of Gandour and co-workers.[6] The observed values of $J_{\alpha_1\beta_1'} = 3$ Hz and $J_{\alpha_1\beta_1} = 6$ Hz (Table I) in this equation give dihedral angles of 42° and 60°, respectively. Although the sum of these is somewhat less than the required 120°, their relative magnitudes are

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(5) Williams, D. H.; Bhacca, N. S. *J. Am. Chem. Soc.* **1964**, *86*, 2742–2743.
(6) Colucci, W. J.; Jungk, S. J.; Gandour, R. D. *Magn. Reson. Chem.* **1985**, *23*, 335–343.

consistent with the conclusions summarized in 4.

Other NOEs (Table II) give information about possible conformational states of the peptide. Proton 3b gives NOEs to both α_3 and ω_3 with the former being more intense. Protons α_3 and ω_3 are on opposite sides of the molecule—see 2); thus there is probably some conformational flexibility of the aromatic ring 3, with 3b on average being somewhat nearer to α_3 than to ω_3. Also noteworthy is the NOE $\alpha_2 \leftrightarrow \delta_2$ (Table II). This NOE establishes that the side chain of residue 2 spends some of its time coiled up such that the terminal carbon atom of the sidechain is proximate to its α-CH proton. The structure of WS-43708A is reproduced with stereochemical detail in 5; and, as a CPK model, in a conformation which is in accord with the above findings, in Figure 1. In this latter representation, we have rotated the covalent structure 1 through 180° about a vertical axis bisecting the C–C bond connecting the aromatic residues 1 and 3. This has been done because what transpires to be a concave face of the molecule is now presented upwards.

The site to which WS-43708A binds in exerting its antibacterial activity is unknown. However, addition of the cell wall analogue N-Ac-D-Ala-D-Ala[7] to a DMSO-d_6 solution of the antibiotic free base affected neither the chemical shifts nor the line shapes of the proton resonances. This situation still pertained as trifluoroacetic acid was then added stepwise to the solution to reduce the "pH". Additionally, the addition of N-Ac-D-Ala-D-Ala, or di-N-Ac-Lys-D-Ala-D-Ala[7] to a solution containing WS-43708A (0.1–0.2 mg/mL in 0.02 M sodium citrate buffer, pH 5.1) did not affect its original UV spectrum. We conclude that WS-43708A exerts its physiological action on cell wall biosynthesis other than by binding to mucopeptide precursors terminating in D-Ala-D-Ala.

(7) Nieto, M.; Perkins, H. R. *Biochem. J.* 1971, *123*, 789–803.

Experimental Section

^{1}H NMR spectra were obtained on a Brucker AM-400 spectrometer using approximately 20 mg/mL solutions in DMSO-d_6. One-dimensional spectra were usually recorded with a spectral width of 3500–4500 Hz and 16K data points. Two-dimensional double quantum filtered COSY (DQFCOSY), NOESY, and CAMELSPIN experiments were all run in the phase-sensitive mode. NOESY spectra were measured with various mixing times (0.3–0.5 s) to obtain the optimum NOES without spin diffusion. Two CAMELSPIN experiments were recorded with different transmitter offsets to check for false enhancements;[4] the same spin-locking durations of 200 ms was used in both cases. All the 2D data matrices consisted of 512 2K spectra, which yielded, after zero-filling in F1 and Fourier transformation, a 1-MW square matrix. A maximum of 32 scans were accumulated in each t_1 fid with proper phase cycling. The data were subjected to Lorentzian–Gaussian multiplications in t_2 and sine bell multiplication (SSB1 valve of 3) in t_1 prior to transformation. UV studies were carried out with a Pye Unicam PU 8800 UV/Vis spectrometer and followed a previously published procedure.[7]

Acknowledgment. We thank SERC for financial support and Dr. M. Hashimoto (Fujisawa Pharmaceutical Co. Ltd.) for a sample of WS-43708A.

Registry No. Antibiotic WS-43708A, 100296-21-7.

Formal Transfers of Hydride from Carbon–Hydrogen Bonds. Attempted Generation of Molecular Hydrogen by Intramolecular Reduction of Protons Bound by 2,3-Dihydro-1,3-dimethyl-2-(2-pyridinyl)-1*H*-benzimidazole

Socorro M. Ramos, Micheline Tarazi, and James D. Wuest*

Département de Chimie, Université de Montréal, Montréal, Québec H3C 3J7, Canada

Received May 4, 1987

2,3-Dihydro-1,3-dimethyl-2-(2-pyridinyl)-1*H*-benzimidazole (5) is designed to bind electrophilic substrates and reduce them by an internal transfer of hydride. The intended binding site is the nitrogen atom of the pyridine ring, and the source of hydride is the carbon–hydrogen bond at C_2 of the dihydrobenzimidazole ring. In the preferred conformation of compound 5, the carbon–hydrogen bond is activated as a donor of hydride by two antiperiplanar lone pairs, and the intended binding site is nearby. Protons bind primarily to the nitrogen atom of the pyridine ring as expected, but they are not reduced to molecular hydrogen. Instead, two other reactions are triggered by protonation. One is heterolysis of the carbon–carbon bond between the dihydrobenzimidazole and pyridinium rings, and the other is a 1,2-shift of hydride from the activated carbon–hydrogen bond to the pyridinium ring. The failure of compound 5 to reduce protons to molecular hydrogen can be attributed primarily to unfavorable thermodynamics; the hydridic carbon–hydrogen and acidic nitrogen–hydrogen bonds of salt 5-H$^+$ are inadequately activated, so alternative reactions are more rapid. Formation of hydrogen may also be disfavored because salt 5-H$^+$ adopts an unsuitable conformation or because an appropriate trajectory for intramolecular protonation of the activated carbon–hydrogen bond is unattainable.

We are interested in the design and synthesis of reducing agents that have some of the features of redox enzymes, including a receptor site that recognizes and binds reducible substrates, and an adjacent site that acts as a donor of hydride. In enzymatic reductions involving the coenzyme NADH, Cannizzaro reactions, Meerwein–Ponndorf–Verley reductions, and other important redox reactions, carbon–hydrogen bonds serve as the donors of hydride. The subdued reactivity of these bonds makes them particularly suitable when the substrate must be recognized, oriented, and activated by a receptor before the transfer of hydride takes place. We have therefore begun to study compounds in which a reducible substrate is held close to a suitably activated carbon–hydrogen bond.[1]

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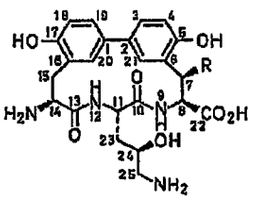
Total Synthesis of the Biphenomycins; III.[1] Synthesis of Biphenomycin B[2]

Ulrich Schmidt,* Regina Meyer, Volker Leitenberger, Helmut Griesser, Albrecht Lieberknecht
Institut für Organische Chemie und Isotopenforschung der Universität Stuttgart, Pfaffenwaldring 55, D-7000 Stuttgart 80, Germany
Received 9 April 1992

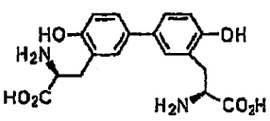
The total synthesis of the cyclopeptide biphenomycin B (**1b**), a compound exhibiting a potent antibacterial activity against Gram-positive bacteria, is described. The non-proteinogenic amino acid (*S,S*)-diisotyrosine (**2**) was prepared by enantioselective hydrogenation of the corresponding didehydroamino acids. The 15-membered ansa ring was obtained in 85% yield within 5 minutes by ring closure of the appropriate linear pentafluorophenyl ester in the two phase system chloroform–aqueous sodium hydrogen carbonate without dilution.

Biphenomycins A (**1a**) and B (**1b**) (W-S 43708 A and B) have been isolated[3] from *Streptomyces griseorubiginosus* No. 43708 and shown to exert a potent antibacterial activity against Gram-positive, β-lactam-resistant bacteria. The cyclic structure and the configuration of the *erythro*-γ-hydroxy-L-ornithine unit were elucidated by a Japanese group.[3] With the exception of members of the vancomycin group, these compounds seem to represent the only known antibiotics possessing a biphenyl unit. However, unlike the former compounds, they do not have any effect on the binding of mucopeptide precursors to the D-Ala-D-Ala units. The configurations at the biphenyl unit were assigned by Williams[4] and further substantiated by Brown[5] who determined the three-dimensional structure, including the axial chirality, of the biphenyl system in solution. A few years later, it was shown[6] that the antibiotics LL-AF 283 α and β, previously isolated from the fermentation of *Streptomyces filipinensis*,[7] are identical with biphenomycin A and arginylbiphenomycin A.

We reported the synthesis of diisotyrosine dimethyl ether and the construction[8] of a simple model of biphenomycin B and the first synthesis of biphenomycin B.[9] A few months later, the preparation of a cyclopeptide having diisotyrosine dimethyl ether and lysine as ring members was published.[10] An analogous cyclopeptide containing dityrosine and alanine has also been prepared.[11] In the present communication, we report the experimental details of the total synthesis. We have described the stereoselective construction of the hydroxyornithine in detail in a previous paper.[1,9]



1a R = OH
b R = H



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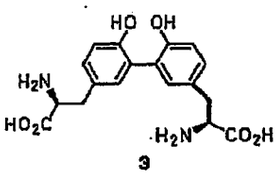

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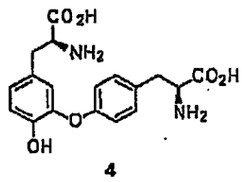

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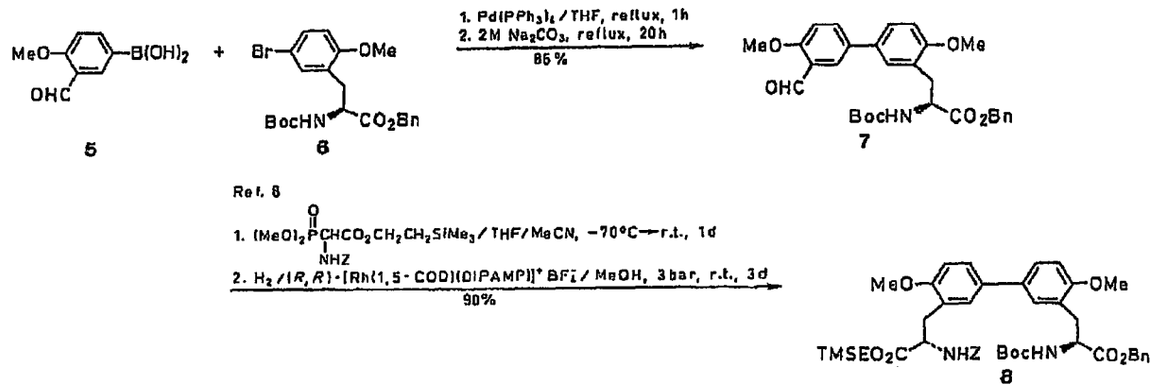
Dityrosine (**3**) and isodityrosine (**4**), which have been detected as metabolites of microorganisms, are plausible products of the dimerisation of tyrosine. However, there is as yet no convincing evidence that diisotyrosine (**2**) is a product of the oxidative dimerisation because *o*-hydroxyphenylalanine has only once been isolated[12] from a natural source to date. Hence, a subsequent hydroxylation of a dimeric phenylalanine cannot be discounted.

In the course of the total synthesis of biphenomycin B, it is necessary to mask two phenolic hydroxy groups, two amino groups, one hydroxy group, and one carboxy group with functions that are compatible with the protecting groups of the α-amino group of hydroxyorni-



Scheme 1

thine and a carboxy group of diisotyrosine which have to be cleaved during the peptide construction and ring closure steps. After numerous preliminary experiments, we decided to protect the δ-amino and γ-hydroxy groups of the hydroxyornithine as a benzyloxycarbonyloxazolidine function and the other groups as benzyl ether, benzyl ester, and benzyloxycarbonyl functions, respectively. We were fully aware of the difficulties inherent in the simultaneous cleavage of the five *O*-benzyl bonds in the last step.

For the construction of the biphenyl unit, we selected the palladium(0)-catalyzed coupling of two benzene derivatives with protected hydroxy groups and free or masked aldehyde groups as attachment points for the later construction of the two alanine units.

At first, we decided in favour of the palladium(0)-catalysed coupling of an arylboronic acid with an aryl bromide[13] for the formation of the biphenyl element. This concept was indeed realised with the methyl ethers **5** and **6**.[8] The second alanine unit was derived from the unprotected aldehyde group in **7** by way of the corresponding dehydroamino acid and its subsequent stereoselective hydrogenation (to **8**). We did not consider an analogous route for the preparation of the corresponding dibenzyl ether because the 4-benzyloxy-3-formylphenylboronic acid is only accessible in rather poor yields.

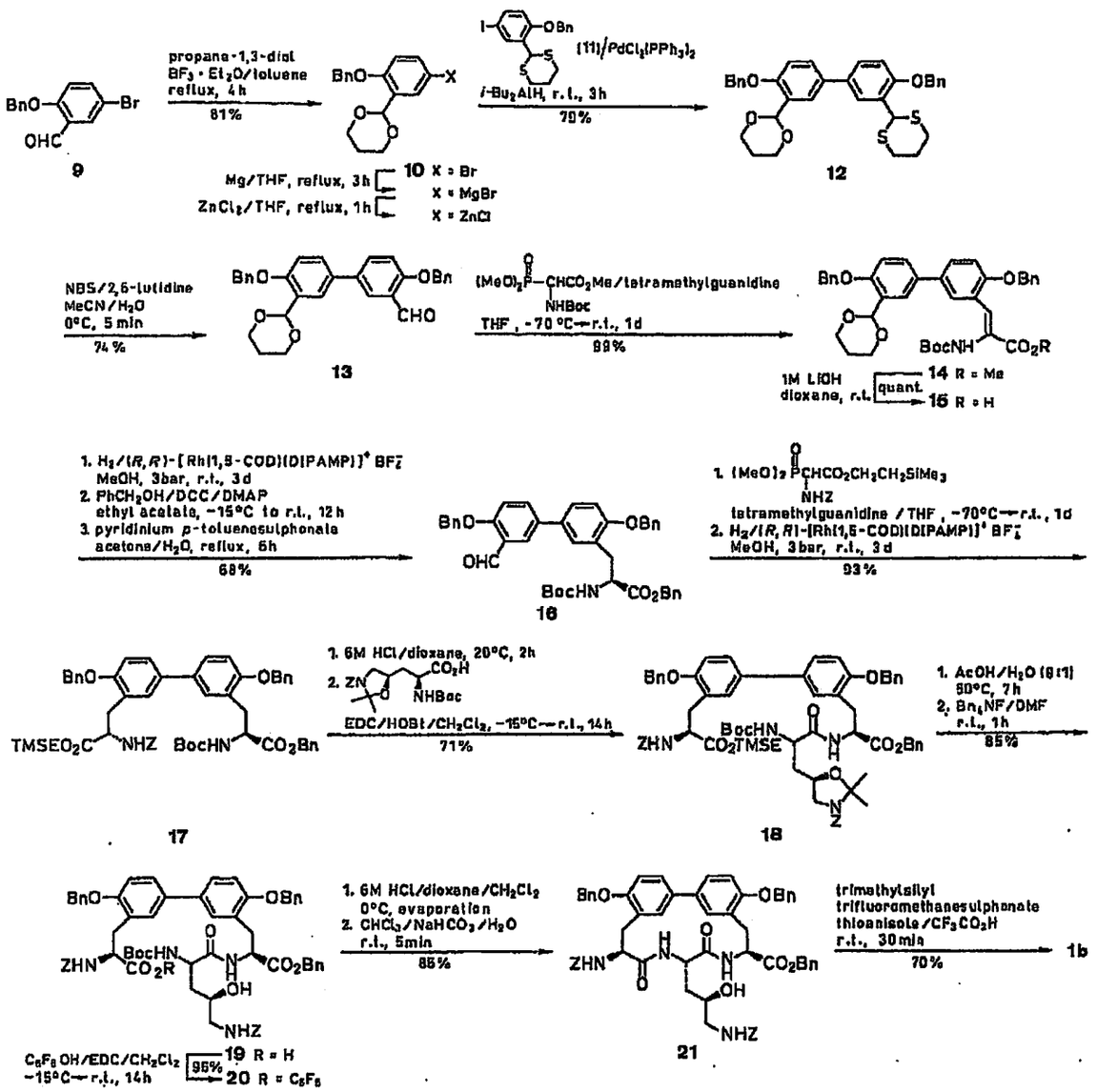
The construction of an (*S,S*)-diisotyrosine dibenzyl ether was achieved in good yield by the palladium(0)-catalysed coupling of the zinc compound (derived from **10** by way of the Grignard reagent) with the aryl iodide **11**. The two organometallic compounds are stable when the aldehyde group is protected as a 1,3-dioxane. The aldehyde groups can be demasked selectively for the subsequent formation of the two alanine elements.

Key reactions for the formation of the amino acid moieties are the condensations of the aldehydes **13** and **16** with an appropriate (dialkoxyphosphoryl)glycine ester[15] to furnish the didehydroamino acid derivatives. In contrast to our previous results,[9] the (*Z*)-didehydroamino acid derivatives were formed in high diastereoselectivities when 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or tetramethylguanidine was used as the base[16] (without lithium chloride). Both hydrogenations with the optically active catalyst[17] (*R,R*)-[Rh(1,5-cod) (dipamp)]$^+$[BF_4]$^-$ proceeded with high enantioselectivity or diastereoselectivity, respectively. The enantiomers or diastereomers could not be detected by spectroscopy (^{1}H NMR, ^{13}C NMR) and chromatography (HPLC on optically active columns). The diastereomer of **17** was available for comparison purposes from the synthesis of epibiphenomycin B. After cleavage of the Boc group, the diisotyrosine derivative **17** was coupled with the protected hydroxyornithine; we have described the stereoselective synthesis of the latter previously.[1,9]

Earlier experiments with a simple model had already shown[8] that ring closure at the two ring-amide groups is possible and that the ring closure of the ω-aminopentafluorophenyl ester takes place within a few minutes in the two phase system chloroform–aqueous sodium hydrogen carbonate[18] without dilution to furnish the ring product in yields in excess of 85%.

Corresponding attempts with the pentafluorophenyl ester of compound **18** (C_6F_5 in place of TMSE), however, were at first disappointing and indicative of an elimination of hydroxyornithine. Thus, we first opened the oxazolidine ring and, after cleavage of the Boc group of **20**, were then able to close the ring in 85% yield, even in the presence of the free hydroxy group of the hydroxyornithine. Preliminary attempts to cleave the five benzyl groups in the cyclopeptide **21** by hydrogenolysis with palladium black and hydrogen donors (cyclohexadiene, ammonium formate, formic acid) were disappointing; complex product mixtures were obtained which were suggestive of a competing hydrogenation of the biphenyl unit. Success was finally achieved by Yajima's method[19] using trimethylsilyl trifluoromethanesulphonate–thioanisole in trifluoroacetic acid. The trifluoroacetate of biphenomycin was precipitated from the reaction mixture by addition of absolute diethyl ether and purified by RP-HPLC with ammonium formate buffer–acetonitrile.

For further confirmation of the *S,S*-configuration of diisotyrosine in biphenomycin B, we have also prepared the cyclopeptide possessing the 8*S*,14*R*-configuration.[20]



Scheme 2

The ring closure step occurred with a somewhat lower yield (55%) in comparison to that of the *S,S*-compound (85%). HPLC analysis showed two signals of equal intensity and without base-line separation which changed into a single peak upon short warming. We thus assume that two rotamers are initially formed in approximately equal concentrations.

After cleavage of the protecting groups, the ^{13}C NMR spectrum of the purified epibiphenomycin B exhibited significant differences to the spectra of the naturally occurring and the synthesized biphenomycin B.[20] Hence, the *S,S*-configuration of diisotyrosine in the natural product has been confirmed by synthesis.

The ^1H NMR spectra were recorded on a Bruker WP 80 (80 MHz) and a Bruker CXP (300 MHz) respectively. Optical rotation values were determined with a Perkin-Elmer 241 polarimeter. Melting points (Reichert microscope) are uncorrected. TLC was done on silica gel (Merck Silica 60 F_{254} sheets) and medium pressure column chromatography used Merck LiChroprep Si 60 (15–25 μ). HPLC was done with a LKB Instrument and a silica gel column (Merck

LiChroCART (250–4 mm), LiChroSorb Si 60 7 μ). Compounds **9–18** and **21** gave satisfactory microanalyses (C ± 0.26, H ± 0.23, Br ± 0.08, N ± 0.15, S ± 0.12).

2-Benzyloxy-5-bromobenzaldehyde (9):
To a solution of 5-bromosalicylaldehyde (100 g, 497 mmol) in EtOH (300 mL), KOH (27.9 g, 497 mmol) in H_2O (60 mL) was added. After stirring for 10 min, benzyl chloride (75.6 g, 597 mmol) was added and the mixture was stirred under reflux for 10 h. The precipitated solid was separated and recrystallized from EtOH; yield: 138 g (95%); mp 74 °C.

^{1}H NMR (80 MHz, $CDCl_3$/TMS): δ = 5.16 (s, 2 H), 6.98 (d, 1 H, J = 10 Hz), 7.43 (s, 5 H), 7.60 (dd, 1 H, J = 2 Hz, 10 Hz), 8.00 (d, 1 H, J = 2 Hz), 10.40 (s, 1 H).

2-(2-Benzyloxy-5-bromophenyl)-1,3-dioxane (10):
A solution of **9** (50.0 g, 172 mmol), propane-1,3-diol (13.6 mL, 189 mmol) and $Et_2O \cdot BF_3$ (0.5 mL) in toluene (200 mL) was boiled in a Dean and Stark apparatus for 3 h to produce the theoretical amount of water. The mixture was washed with 1 M aq $NaHCO_3$ (100 mL) and the organic layer was dried ($MgSO_4$) and evaporated. The residue was recrystallized from EtOH; yield: 48.0 g (81%); mp 88–90 °C.

^{1}H NMR (80 MHz, $CDCl_3$/TMS): δ = 1.22–1.52 (m, 1 H), 1.90–2.50 (m, 1 H), 3.75–4.40 (m, 4 H), 5.08 (s, 2 H), 5.88 (s, 1 H), 6.78 (d, 1 H, J = 10 Hz), 7.25–7.60 (m, 6 H), 7.77 (d, 1 H, J = 2 Hz).

2-(2-Benzyloxy-5-iodophenyl)-1,3-dithiane (11):
To a solution of 5-iodosalicylaldehyde (30.0 g, 121 mmol) in DMF (500 mL) was added K_2CO_3 (20.1 g, 145 mmol) and benzylchloride (15.3 mL, 133 mmol) and the suspension was stirred at 120 °C for 15 h. The mixture was diluted with H_2O (500 mL). The precipitated 2-benzyloxy-5-iodobenzaldehyde was separated and recrystallized from EtOH; yield: 33.9 g (83%); mp 91 °C.

^{1}H NMR (80 MHz, $CDCl_3$/TMS): δ = 5.17 (s, 2 H), 6.82 (d, 1 H, J = 9 Hz), 7.41 (s, 5 H), 7.78 (dd, 1 H, J = 2, 9 Hz), 8.11 (d, 1 H, J = 2 Hz), 10.42 (s, 1 H).

To a solution of 2-benzyloxy-5-iodobenzaldehyde (12.0 g, 35.5 mmol) and propane-1,3-dithiol (4.28 mL, 42.6 mmol) in $CHCl_3$ (100 mL), $Et_2O \cdot BF_3$ (1 mL) was added at −20 °C. The mixture was stirred at r.t. for 1 d, washed with 10% aq KOH (50 mL) and brine (50 mL), dried ($MgSO_4$) and evaporated. The residue was recrystallized from EtOAc/hexane; yield: 13.9 g (92%); mp 167 °C.

^{1}H NMR (80 MHz, $CDCl_3$/TMS): δ = 1.80–2.15 (m, 2 H), 2.70–3.10 (m, 4 H), 5.10 (s, 2 H), 5.63 (s, 1 H), 6.63 (d, 1 H, J = 8 Hz), 7.22–7.53 (m, 6 H), 7.85 (d, 1 H, J = 2 Hz).

4,4'-Dibenzyloxy-3-(1,3-dioxane-2-yl)-3'-(1,3-dithiane-2-yl)biphenyl (12):
To magnesium turnings (1.06 g, 43.8 mmol) in THF (15 mL), one third of a solution of 2-(2-benzyloxy-5-bromophenyl)-1,3-dioxane (10) (15.3 g, 43.8 mmol) in THF (75 mL) was added. The mixture was heated and the reaction was initiated with a crystal of iodine. The remaining aryl halide solution was added dropwise under reflux. After a further 1 h of heating, the solution was cooled to r.t. To this Grignard reagent, dry $ZnCl_2$ (5.97 g, 43.8 mmol) was added and the mixture was refluxed for 1 h. In a second flask, a 1 M solution of diisobutylaluminum hydride (DIBAL-H) in hexane (2.92 mL) was added to a suspension of bis(triphenylphosphine)palladium(II) chloride (1.02 g, 1.46 mmol) in THF (75 mL). The mixture was stirred for 10 min, a solution of 2-(2-benzyloxy-5-iodophenyl)-1,3-dithiane (11) (12.5 g, 29.2 mmol) in THF (75 mL) was added and subsequently the arylzinc halide suspension prepared above. The reaction mixture was stirred for 3 h at r.t. and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (200 mL) and sat. aq NH_4Cl (200 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. The crude product was purified by silica gel chromatography (eluent: CH_2Cl_2/hexane 1:1); yield 11.5 g (69%); mp 198 °C.

^{1}H NMR (80 MHz, $CDCl_3$/TMS): δ = 1.20–1.55 (m, 1 H), 1.85–2.40 (m, 3 H), 2.70–3.15 (m, 4 H), 3.80–4.35 (m, 4 H), 5.12 (s, 2 H), 5.15 (s, 2 H), 5.67 (s, 1 H), 5.95 (s, 1 H), 6.91 (d, 1 H, J = 9 Hz), 6.94 (d, 1 H, J = 9 Hz), 7.20–7.53 (m, 12 H), 7.77–7.85 (m, 2 H).

4,4'-Dibenzyloxy-3-(1,3-dioxane-2-yl)biphenyl-3'-carbaldehyde (13):
A solution of the dithiane **12** (9.64 g, 17.0 mmol) in CH_2Cl_2 (80 mL) was added at 0 °C under efficient stirring to a solution of *N*-bromosuccinimide (18.0 g, 101 mmol) and 2,6-lutidine (23.6 mL, 202 mmol) in aqueous 80% acetonitrile (500 mL). After stirring for 10 min, the solvents were evaporated and the residue was dissolved in $CHCl_3$ (300 mL). The organic phase was washed with sat. aq $Na_2S_2O_3$ (100 mL) and brine (100 mL), dried ($MgSO_4$) and evaporated. The excess lutidine was removed by distillation in vacuo. The residue was dissolved in CH_2Cl_2 (20 mL) and quickly filtered through a short column of silica gel (2 × 10 cm) eluting with hexane/EtOAc (1:1). In the solvent mixture of the filtrate, the product precipitated spontaneously. After standing for 2 h the colorless solid was filtered off; yield: 6.00 g (74%); mp 142 °C.

^{1}H NMR (80 MHz, $CDCl_3$/TMS): δ = 1.25–1.50 (m, 1 H), 1.95–2.40 (m, 1 H), 3.80–4.40 (m, 4 H), 5.12 (s, 2 H), 5.18 (s, 2 H), 5.95 (s, 1 H), 6.75–7.55 (m, 13 H), 7.73 (dd, 1 H, J = 2, 9 Hz), 7.87 (d, 1 H, J = 2 Hz), 8.06 (d, 1 H, J = 2 Hz), 10.55 (s, 1 H).

3'-[(*Z*)-2-(*tert*-Butoxycarbonylamino)-2-(methoxycarbonyl)vinyl]-4,4'-dibenzyloxy-3-(1,3-dioxan-2-yl)biphenyl (14):
To a solution of methyl 2-*tert*-butyloxycarbonylamino-2-(dimethoxyphosphoryl)acetate (4.46 g, 15 mmol) and tetramethylguanidine (1.73 g, 15 mmol) in absol. THF (125 mL), biphenyl-3'-carbaldehyde **13** (5.90 g, 12.3 mmol) was added at −70 °C. The reaction mixture was allowed to warm to r.t. overnight and was stirred for a further 1 d at r.t. The solution was then diluted with EtOAc (500 mL), washed with water (50 mL) and 1 M H_2SO_4 (20 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was filtered through silica gel 60 (100 g eluent hexane/EtOAc 1/1) to yield **14** (8 g, quant.) which was used for the next reaction without further purification. *E*/*Z* Ratio 1.5/98.5; purity > 99% determined by HPLC (Rt_E = 5.5 min; Rt_Z = 7.9 min, eluent: hexane/ethyl acetate, 75:25) and by ^{1}H NMR (250 MHz).

^{1}H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.35 (s, 9 H), 1.88 (br, 1 H), 2.26 (m, 1 H), 3.84 (s, 3 H), 4.03 (dt, J_1 = 2.1, J_2 = 12.1 Hz, 2 H), 4.26 (dd, J_1 = 5, J_2 = 11 Hz, 2 H), 5.14 (s, 2 H), 5.17 (s, 2 H), 5.95 (s, 1 H), 6.54 (br s, 1 H), 6.95 (d, J = 8.7 Hz), 6.98 (d, J = 8.7 Hz, 1 H), 7.30–7.65 (m, 13 H), 7.71 (d, J = 2.2 Hz, 1 H), 7.81 (d, J = 2.4 Hz, 1 H).

The *E*-isomer was separated by MPLC from the product obtained by catalysis with DBU/LiCl. (*E*/*Z*) ratio 33/67.

3'-[(*Z*)-2-(*tert*-Butoxycarbonylamino)-2-carboxyvinyl]-4,4'-dibenzyloxy-3-(1,3-dioxan-2-yl)biphenyl (15):
To a solution of **14** (4.47 g, 6.86 mmol) in dioxane (30 mL), a solution of LiOH (574 mg, 13.7 mmol) in water (20 mL) was added. The mixture was stirred at r.t. When saponification was complete (checked by TLC), the solvent was evaporated in vacuo. The residue was suspended in sat. aq NH_4Cl (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. The product was purified by silica gel chromatography (hexane/EtOAc, 3:7); yield: 4.37 g (quant.).

^{1}H NMR (300 MHz, DMSO-d_6/TMS): δ = 1.23 (s, 9 H), 1.34–1.40 (m, 1 H), 1.99–2.08 (m, 1 H), 3.92 (t, 2 H, J = 12 Hz), 4.14 (dd, 2 H, J = 4.5, 11 Hz), 5.18 (s, 4 H), 5.85 (s, 1 H), 7.11 (d, 1 H, J = 8.9 Hz), 7.14 (d, 1 H, J = 9.1 Hz), 7.30–7.51 (m, 13 H), 7.64 (d, 1 H, J = 2.2 Hz), 7.80 (s, 1 H), 8.12–8.14 (s br, 1 H).

3-[(*S*)-2-(*tert*-Butoxycarbonylamino)-2-(benzyloxycarbonyl)ethyl]-4,4'-dibenzyloxybiphenyl-3'-carbaldehyde (16):
A solution of **15** (4.00 g, 6.27 mmol) in MeOH (100 mL), containing (*R*,*R*)-[Rh(1,5-cod)(dipamp)]$^+BF_4^-$ (20 mg) was hydrogenated (3 bar) at r.t. over 3 d. The mixture was evaporated and the residue chromatographed on silica gel with EtOAc; yield: 4.02 g (quant.); mp 135 °C; $[\alpha]_D^{20}$ + 10.5° (c = 0.73, $CHCl_3$).

^{1}H NMR (300 MHz), DMSO-d_6/TMS): δ = 1.27 (s, 9 H), 1.32–1.42 (m, 1 H), 1.99–2.11 (m, 1 H), 2.76–2.84 (m, 1 H), 3.22–3.30 (m, 1 H), 3.89–3.97 (m, 2 H), 4.16 (dd, 2 H, J = 4.4, 11.2 Hz), 4.27–4.36 (m, 1 H), 5.19 (s, 4 H), 5.86 (s, 1 H), 7.07 (d, 1 H, J = 8.5 Hz), 7.13 (d, 1 H, J = 8.7 Hz), 7.29–7.62 (m, 14 H), 7.69 (d, 1 H, J = 2.3 Hz), 12.1–12.6 (s br, 1 H).

Benzyl alcohol (0.53 mL, 5.10 mmol), dicyclohexylcarbodiimide (1.05 g, 5.10 mmol) and 4-dimethylaminopyridine (62 mg, 0.51 mmol) were dissolved in CH_2Cl_2 (20 mL) at 0 °C. After stirring at 0 °C for 10 min, the amino acid 3-[(*S*)-2-(*tert*-butyloxycarbonylamino)-2-carboxyethyl]-4,4′-dibenzyloxy-3′-(1,3-dioxan-2-yl)biphenyl (3.10 g, 4.85 mmol) was added. The mixture was stirred for 1 h at 0 °C, the solid was filtered off and the solvent was evaporated. After filtration of the residue on silica gel (petroleum ether/EtOAc, 7:3) the benzyl ester was recrystallized from i-PrOH; yield: 3.20 g (90 %); mp 137 °C; $[\alpha]_D^{20}$ + 11.5° (c = 0.92, $CHCl_3$).

^{1}H NMR (80 MHz, $CDCl_3$/TMS): δ = 1.32 (s, 9 H), 1.30–1.50 (m, 1 H), 2.00–2.40 (m, 1 H), 3.05–3.38 (m, 2 H), 3.85–4.35 (m, 4 H), 4.52–4.70 (m, 1 H), 5.05 (s, 2 H), 5.08 (s, 2 H), 5.12 (s, 2 H), 5.25 (s, br, 1 H), 5.93 (s, 1 H), 6.87 (d, 1 H, J = 9 Hz), 6.90 (d, 1 H, J = 9 Hz), 7.25–7.45 (m, 18 H), 7.79 (d, 1 H, J = 2 Hz).

A solution of 3-[(*S*)-2-(*tert*-butyloxycarbonylamino)-2-(benzyloxycarbonyl)ethyl]-4,4′-dibenzyloxy-3′-(1,3-dioxan-2-yl)biphenyl (3.20 g, 4.38 mmol) and pyridinium *p*-toluenesulfonate (330 mg, 1.32 mmol) in acetone (40 mL) and water (5 mL) was refluxed for 6 h. The solvent was evaporated, the residue was dissolved in $CHCl_3$ (50 mL) and the organic phase was washed with sat. aq $NaHCO_3$ (30 mL) and brine (30 mL), dried ($MgSO_4$) and evaporated. The residue was recrystallized from i-PrOH; yield: 2.47 g, 16 (84 %); $[\alpha]_D^{20}$ + 11.9° (c = 0.98, $CHCl_3$).

^{1}H NMR (80 MHz, DMSO-d_6/TMS): δ = 1.27 (s, 9 H), 2.75–3.10 (m, 2 H), 4.25–4.65 (m, 1 H), 5.11 (s, 2 H), 5.20 (s, 2 H), 5.35 (s, 2 H), 7.10–7.96 (m, 22 H), 10.72 (s, 1 H).

3-[(*S*)-2-Benzyloxycarbonylamino-2-(trimethylsilylethoxycarbonyl)-ethyl]-3′-[(*S*)-2-*tert*-butoxycarbonylamino)2-(benzyloxycarbonyl)-ethyl]-4,4′-dibenzyloxybiphenyl (17):
To a solution of trimethylsilylethyl 2-(benzyloxycarbonylamino)-2-(dimethoxyphosphoryl)acetate (1.8 g, 4.3 mmol) and tetramethylguanidine (495 mg, 4.3 mmol) in absol. THF (35 mL), 16 (2.40 g, 3.57 mmol) was added at − 70 °C. The reaction mixture was allowed to warm to r. t. overnight and was stirred for a further 1 d at r. t. The solution was then diluted with EtOAc (120 mL), washed with water (20 mL) and 0.5 M H_2SO_4 (5 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was filtered through silica gel 60 (20 g; eluent hexane/EtOAc, 1:1) to yield the didehydroamino acid derivative (3.4 g, quant.) which was used for the hydrogenation without further purification. *E*/*Z* Ratio 3/97; purity > 98 % determined by HPLC (Rt_E = 5.15 min; Rt_Z = 6.35 min, eluent: hexane/ethyl acetate 80/20) and by ^{1}H NMR (300 MHz), $[\alpha]_D^{20}$ + 8.29° (c = 2.09, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 0.05 (s, 9 H), 1.04 (t, 2 H, J = 8.4 Hz), 1.34 (s, 9 H), 3.05 (dd, 1 H, J = 8.9, 13.1 Hz), 3.22 (dd, 1 H, J = 4.6, 13.6 Hz), 4.31 (t, 2 H, J = 8.3 Hz), 4.62–4.68 (m, 1 H), 5.05–5.14 (m, 8 H), 5.39 (d br, 1 H, J = 7.2 Hz), 6.86 (d, 1 H, J = 8.3 Hz), 6.91 (s br, 1 H), 6.98 (d, 1 H, J = 8.6 Hz), 7.24–7.52 (m, 24 H), 7.67 (s, 1 H).

A solution of the didehydro amino acid derivative (1.80 g, 1.87 mmol) in MeOH (30 mL) containing (*R*,*R*)-[Rh(1.5-cod)(dippamp)]$^+$ BF_4^- (20 mg) was hydrogenated (3 bar) at r. t. over 3 d. The mixture was evaporated and the residue was filtered through silica gel with petroleum ether/EtOAc (1:1) to give 17; yield: 1.80 g (quant.), $[\alpha]_D^{20}$ + 11.2° (c = 1.44, $CHCl_3$); mp 121–124 °C.

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = − 0.03 (s, 9 H), 0.88 (t, 2 H, J = 8.3 Hz), 1.35 (s, 9 H), 3.06–3.25 (m, 4 H), 4.07–4.17 (m, 2 H), 4.58–4.65 (m, 2 H), 5.00–5.12 (m, 8 H), 5.37 (d, 1 H, J = 7.8 Hz), 5.60 (d, 1 H, J = 7.8 Hz), 6.92 (t, 2 H, J = 8.0 Hz), 7.23–7.47 (m, 24 H).

Dipeptide of 4,4′-dibenzyloxy-3-[(*S*)-2-benzyloxycarbonylamino-2-(trimethylsilylethoxycarbonyl)ethyl]-3′-[(*S*)-2-amino-2-(benzyloxycarbonyl)ethyl]biphenyl and (*R*)-3-benzyloxycarbonyl-5-[(*S*)-*N*-*tert*-butoxycarbonylalanin-3-yl]-2,2-dimethyl-1,3-oxazolidine (18):
To a solution of 17 (500 mg, 0.518 mmol) in dioxane (3 mL), 6 M HCl in dioxane (3 mL) was added at 0 °C. After stirring for 1 h, the solvent was evaporated, the residue was dissolved in EtOAc (20 mL) and washed with sat. aq. $NaHCO_3$ (20 mL). The organic layer was dried ($MgSO_4$) and evaporated. To a solution of the residue, (*R*)-3-benzyloxycarbonyl-5-[(*S*)-*N*-*tert*-butoxycarbonylalanin-3-yl]-2,2-dimethyl-1,3-oxazolidine (254 mg, 0.622 mmol) and hydroxybenzotriazole (119 mg, 0.777 mmol) in CH_2Cl_2 (3 mL), *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (119 mg, 0.622 mmol) was added at − 15 °C. The stirred solution was warmed to r. t. over 12 h, the solvent was evaporated and the residue dissolved in EtOAc (20 mL). The organic phase was washed with 1 M $KHSO_4$ (20 mL), brine (20 mL) and sat. aq. $NaHCO_3$ (20 mL), dried ($MgSO_4$) and evaporated. The product was purified by MPLC (hexane/EtOAc, 7:3); yield: 468 mg (71 %); $[\alpha]_D^{20}$ + 4.6° (c = 1.15, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = − 0.02 (s, 9 H), 0.89 (t, 2 H, J = 8.4 Hz), 1.38 (s, 9 H), 1.41 (s, 3 H), 1.53 (s, 3 H), 1.70–1.82 (m, 2 H), 2.96–3.27 (m, 5 H), 3.53–3.67 (m, 1 H), 4.08–4.19 (m, 4 H), 4.61–4.63 (m, 1 H), 4.87–4.89 (m, 1 H), 5.00–5.20 (m, 11 H), 5.65 (d, br, 1 H, J = 6 Hz), 6.90–7.46 (m, 32 H).

Protected Biphenomycin B (21):
Oxazolidin 18 (500 mg, 0.39 mmol) was dissolved in 90 % acetic acid (100 mL) and stirred for 2 h at 50 °C. The acid was evaporated and the residue dissolved in EtOAc (100 mL). The organic phase was washed with 1 M $KHCO_3$ (50 mL), dried ($MgSO_4$) and evaporated; yield 398 mg (83 %); mp 171 °C; $[\alpha]_D^{20}$ + 29.7° (c = 0.37, DMSO).

^{1}H NMR (250 MHz, $CDCl_3$): δ = − 0.03 (s, 9 H), 0.90 (t, J = 8.6 Hz, 2 H), 1.38 (s, 9 H), 1.35–1.60 (m, 2 H), 2.49 (s br, 1 H), 2.90–3.39 (m, 6 H), 3.57–3.80 (m, 1 H), 4.05–4.20 (m, 2 H), 4.64–4.78 (m, 1 H), 4.83–5.14 (m, 14 H), 5.83 (d, J = 7.7 Hz, 1 H), 6.91–6.96 (m, 2 H), 7.25–7.49 (m, 30 H).

The residue (117 mg, 0.095 mmol) was dissolved in DMF (10 mL). To this solution, tetrabutylammonium fluoride (59 mg, 0.189 mmol) was added. After 30 min, the reaction mixture was cooled to 0 °C and treated with H_2O (10 mL). This solution was diluted with EtOAc (50 mL), 1 M $KHSO_4$ (20 mL) was added and the acid was extracted into the organic phase. After drying ($MgSO_4$), the organic solvents were evaporated; yield 107 mg (95 %).

The carboxylic acid (107 mg, 0.095 mmol) was dissolved in CH_2Cl_2 (1 mL). To this solution, pentafluorophenol (18 mg, 0.098 mmol) was added. The solution was cooled to − 10 °C and EDC (18 mg, 0.098 mmol) was added. The stirred solution was warmed to r. t. over 12 h, the solvent was evaporated and the residue dissolved in EtOAc (20 mL). The organic phase was washed with brine (10 mL), dried and evaporated; yield 123 mg (98 %).

To a solution of the pentafluorophenyl ester (123 mg, 0.095 mmol) (see above) in dioxane (2 mL), 6 M HCl in dioxane (3 mL) was added at 0 °C. After stirring for 30 min, the solvent was evaporated and the residue was dissolved with $CHCl_3$ (200 mL). After being shaken (5 min) in a separating funnel containing 1 M $KHCO_3$ (100 mL), the organic phase was separated, dried ($MgSO_4$) and evaporated. The solid residue was washed with EtOAc (50 mL); yield 81 mg (85 %); mp > 300 °C; $[\alpha]_D^{20}$ + 25.8° (c = 0.15, dioxane).

^{1}H NMR (250 MHz, DMSO-d_6): δ = 1.54–1.65 (m, 1 H), 1.73–1.79 (m, 1 H), 2.86–3.12 (m, 4 H), 3.38–3.47 (m, 2 H), 3.50–3.67 (m, 1 H), 4.50–4.53 (m, 1 H), 4.68–5.24 (m, 13 H), 6.31 (d, J = 7.7 Hz, 1 H), 7.00–7.52 (m, 31 H), 8.80 (d, J = 9 Hz, 1 H), 8.89 (d, J = 8.5 Hz, 1 H).

^{13}C NMR (63 MHz): δ = 171.76, 171.17, 169.07, 156.29, 155.49, 154.86, 154.61, 137.13, 137.02, 136.74, 135.81, 131.59, 131.31, 128.92, 128.35, 128.31, 128.21, 127.93, 127.64, 127.51, 127.16, 127.04, 126.36, 125.57, 124.98, 124.28, 124.18, 112.32, 112.22,

112.11, 69.37, 66.18, 65.28, 65.16, 54.02, 50.68, 48.92, 45.99, 42.14, 42.07, 41.94, 41.71, 41.62, 41.22, 40.97, 30.49, 27.99.

Biphenomycin B (1b):
To the protected cyclopeptide **21** (53 mg, 0.052 mmol), 1-hydroxy-2-methylbenzene (56 µL, 0.52 mmol) was added at 0 °C. To this suspension, a cooled mixture (0 °C) of 1 M trimethylsilyltriflate/thioanisole in trifluoroacetic acid (7.9 mL) was added. After stirring for 2 h at 0 °C, the reaction mixture was quenched with Et_2O (20 mL) and a white solid precipitated. After filtration, the solid was washed with Et_2O (50 mL), dried under reduced pressure, dissolved in H_2O (20 mL) and NaF (10 mg) was added. After stirring for 2 h, the solution was freeze dried. The residue was purified by RP-HPLC (RP 8; ammonium formate buffer/acetonitrile (8:2) (buffer: 20 mmol $NH_4^+HCOO^-$ in 1 L H_2O); yield 16 mg (65 %); $[\alpha]_D^{20} + 4.3°$ ($c = 0.66$, 1 M HCl).

^{13}C NMR (63 MHz/D_2O/1,4-dioxane): δ = 175.75, 173.17, 168.89, 154.70, 153.44, 132.54, 132.23, 130.42, 127.03, 126.87, 125.66, 120.48, 116.85, 116.69, 65.64, 55.31, 53.20, 50.96, 45.44, 38.36, 30.56, 28.75

Ion-Spray-MS: 473.6 $(M+H)^+$

Support of this research work by BASF AG, the Fonds der Chemischen Industrie, the Land Baden-Württemberg and the Deutsche Forschungsgemeinschaft is gratefully acknowledged. We thank Prof. G. Jung and Dr. J. Metzger (Universität Tübingen) for mass spectra and Prof. P. Fischer for NMR spectra.

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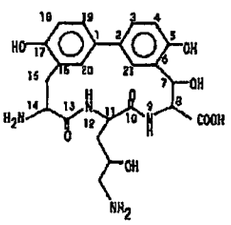
special destabilization of 7-norbornyl cations is much clearer in these data than it ever could be by considering solvolysis results, in which the effect of flattening at carbon is superimposed on the orbital symmetry effect.[7,8]

Registry No. 1, 51689-29-3; 1^{+}, 95484-94-9; 2, 88656-03-5; 2^{+}, 95484-95-0; 3, 95484-96-1; 8-(bicyclo[3.2.1]octan-8-ylidene)bicyclo[3.2.1]octane, 95484-97-2; 9-(bicyclo[3.3.1]nonan-9-ylidene)-bicyclo[3.3.1]nonane, 55993-21-0; 2-(2-adamantanylidene)-adamantane, 30541-56-1; tetracyclo[6.2.1.1^{3,6}.0^{2,7}]tetradec-2,7-ene, 73321-28-5; 1,2,3,4,5,6,7,8,9,10,11,12,13,14-tetradecahydro-1,5:3,7:8,12:10,14-tetramethanononalene, 30614-34-7.

Stephen F. Nelsen,* Daniel L. Kapp
S. M. McElvain Laboratories of Organic Chemistry
Department of Chemistry
University of Wisconsin
Madison, Wisconsin 53706

Received August 14, 1984



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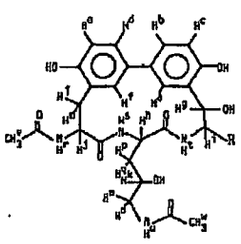

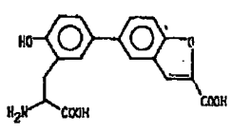

3

2 R = $COOCH_3$

5 R = CH_2OH

Structure of WS-43708A, a Novel Cyclic Peptide Antibiotic

Summary: On the basis of chemical and spectroscopic evidence, the antibiotic WS-43708A ($C_{23}H_{28}N_4O_8$) has been shown to be a cyclic peptide (1) containing a biphenyl moiety included in a 15-membered ring.

Sir: WS-43708A (1), recently isolated from *Streptomyces griseorubiginosus* No. 43078, is a novel cyclic peptide with potent antibacterial acctivity.[1] Herein, we report the structure eludication of this antibiotic on the basis of chemical and spectroscopic evidence.

WS-43708A was isolated as colorless needles from dilute HCl (pH 4): $C_{23}H_{28}N_4O_8 \cdot 2HCl$ (FABMS and elemental analysis);[2] mp 205–209 °C dec; $[\alpha]^{20}_D$ −22.5° (*c* 0.1, 1 N HCl); IR (KBr) 3200, 3000–2300, 1690, 1640 cm^{-1}; UV (H_2O) 264 (ε 18 600), 287 nm (sh); UV (0.1 N NaOH) 288 (ε 24 000), 303 nm (sh); positive ninhydrin test. Acetylation of 1 with Ac_2O in MeOH (0 °C), followed by methylation with CH_2N_2 in MeOH (0 °C), gave the diacetyl monomethyl ester 2 (FABMS, *m/z* 587 ($M^+ + 1$)). Hence, one carboxyl and two amino groups are present in 1. The ^{13}C NMR spectrum (D_2O–DCl) of 1 showed in the sp^3-carbon region eight signals including five methine signals attributable to two secondary alcohol carbons (64.4 (d) and 65.2 (d) ppm) and three α-amino acid carbons (50.9 (d), 55.0 (d), and 57.4 (d) ppm), the remainder being three methylene signals (30.4 (t), 37.9 (t), and 44.9 (t) ppm). In the sp^2-carbon region 15 signals were assignable to three carbonyl groups (168.6 (s), 173.2 (s), and 174.0 (s) ppm) and two phenyl rings substituted totally with six substituents (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 154.2 (s) ppm). Extensive spin decoupling (Table I) of the 400-MHz ^1H NMR spectra of 1 and 2 revealed ^1H–^1H relationships as shown in Figure 1, leading to partial structures A, B, and C, which are quite consistent with the ^{13}C NMR data described above.

The partial unit A was confirmed by the fact that hydrolysis of 1 with 6 N HCl (110 °C, 24 h) gave, after chromatography on Toyopearl HW40S, *erythro*-γ-hydroxy-L-ornithine (HCl salt; mp 176–178 °C dec; $[\alpha]^{25}_D$ +10.9° (*c* 1.0, H_2O)) which was identified by comparison with an authentic sample.[3] The partial units B and C can be combined and extended to partial structure B + C (Figure 2) based on the following grounds. The acid hydrolysis described above also gave compound 3 (FDMS, *m/z* 341 (M^+)),[4] which was converted, by acetylation with Ac_2O in MeOH followed by treatment with CH_2N_2 in MeOH, to the monoacetyl trimethyl derivative 4 (high-resolution EIMS, *m/z* 425.1457, calcd for $C_{23}H_{23}NO_7$ 425.1472). The ^1H NMR analysis of 4 with the aid of decoupling (Table I) and NOE experiments (Figure 2) revealed the structure of 3. The genesis of 3 is rationalized by the following, plausible reaction mechanism from the partial units B and C: (1) dehydration of the β-hydroxy amino acid residue in C to the dehydro amino acid; (2) hydrolysis to the keto acid;[5] (3) dehydrative condensation

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(2) FABMS, *m/z* 489 ($M^+ + 1$) (free base); elemental analysis. Anal. Calcd for $C_{23}H_{28}N_4O_8 \cdot 2HCl \cdot H_2O$: C, 47.65; H, 5.62; N, 9.67; Cl, 12.23. Found: C, 47.80; H, 5.85; N, 9.75; Cl, 12.11.

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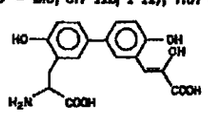
(4) ^1H NMR (CD_3OD–D_2O) δ 3.19 (dd, *J* = 7.5, 14 Hz, 1 H), 3.47 (dd, *J* = 5, 14 Hz, 1 H), 4.29 (dd, *J* = 5, 7.5 Hz, 1 H), 7.01 (d, *J* = 8.7 Hz, 1H), 7.49 (m, 2 H), 7.64 (s, 1 H), 7.66 (d, *J* = 8.7 Hz, 1 H), 7.70 (dd, *J* = 2, 8.7 Hz, 1 H), 7.90 (d, *J* = 2 Hz, 1 H).

(5) A subsequent inspection of the acid hydrolysate revealed the presence of keto acid i as a minor product, firmly supporting the mechanism leading to 3: ^1H NMR (CD_3OD–D_2O) δ 3.07 (dd, *J* = 8.8, 14 Hz, 1 H), 3.44 (dd, *J* = 4, 14 Hz, 1 H), 6.93 (d, *J* = 8.7 Hz, 1 H), 7.12 (s, 1 H), 7.32 (d, *J* = 8.7 Hz, 1 H), 7.42 (dd, *J* = 2.5, 8.7 Hz, 1 H), 7.49 (d, *J* = 2.5 Hz, 1 H), 7.59 (dd, *J* = 2.5, 8.7 Hz, 1 H), 7.67 (d, *J* = 2.5 Hz, 1 H).



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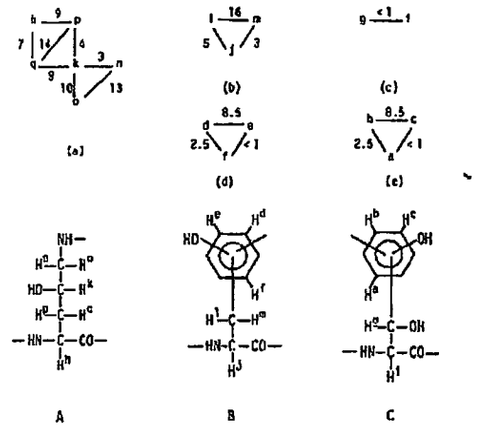

Figure 1. Partial structures A–C and the ^1H–^1H relationships a–e. The ^1H–^1H relationships a, b, and c were obtained by decoupling experiments on 1, while d and e were derived by those on 2. The very small vicinal coupling constant of the H^e–H^i is presumably due to a dihedral angle close to 90° caused by a restricted conformation of WS-43708A.

Table I. ^1H NMR (400 MHz) Chemical Shifts, Multiplicities, and Coupling Constants (J, Hz) for WS-43708A (1), 2, and 4

	1[a]	2[b]	4[c]
H^a	7.40, m (3 H)	7.39, d (2.5)	7.78, br d (2)
H^b	and	7.17, dd (2.5, 8.5)	7.61, dd (2, 8.5)
H^c	6.93, m (2 H)	6.83, d (8.5)	7.62, br d (8.5)
H^d		7.11, dd (2.5, 8.5)	7.48, dd (2.5, 8.5)
H^e		6.78, d (8.5)	6.96, d (8.5)
H^f	6.87, br s	6.88, d (2.5)	7.33, d (2.5)
H^g	5.84, br s	5.70, br s	7.57, s
H^h	5.02, dd (7, 9)	5.09, dt (8.8, 7.5)	
H^i	4.91, br s	4.63, br d (9.5)	
H^j	4.47, dd (3, 5)	4.56, dt (7.5, 3.3)	4.80, dt (7.5, 7.5)
H^k	4.09, dddd (3, 4, 9, 10)	3.64, m	
H^l	3.55, dd (5, 16)	3.16[d]	3.22, d (7.5)
H^m	3.03, dd (3, 16)	2.77, dd (3.3, 15)	
H^n	3.17, dd (3, 13)	3.16[d]	
H^o	2.97, dd (10, 13)	3.07, m	
H^p	2.11, ddd (4, 9, 14)	1.79, m	
H^q	1.95, ddd (7, 9, 17)	1.50, m	
H^r		7.62, d (7.5)	6.23, d (7.5)
H^s		8.50, d (8.8)	
H^t		8.53, d (9.6)	
H^u		7.88, t (5.5)	
H^v		1.84, s (6 H)	1.95, s (3 H)
H^w			
H^x		3.71, s (3 H)	4.10, s (3 H)
H^y			3.73, s (3 H)
H^z			3.91, s (3 H)

[a] D_2O–DCl. [b] Me_2SO-d_6. [c] $CDCl_3$. [d] Overlapping signals of H^l and H^o prevented the examination of their multiplicities.

with the phenolic hydroxy group to the benzofuran structure 3. These chemical evidence thus leads to the partial structure B + C.

The problem remaining is to link A and B + C for the full structure of WS-43708A. Since a molecular model study indicated that an intramolecular cyclization of B + C itself is practically impossible, insertion of fragment A was proposed. This was corroborated by the ^1H NMR spectrum of 1 in D_2O–NaOD, in which, in contrast to upfield shifts of 0.36 and 0.67 ppm, respectively, for H^j and H^l (δ 4.90 and 4.47 in D_2O–DCl), no shift was observed on

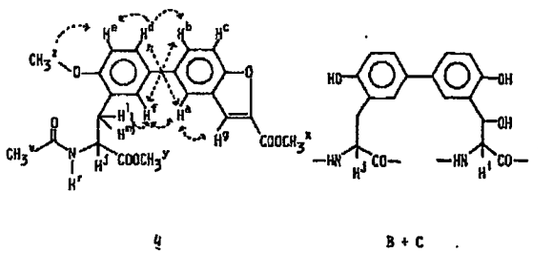

Figure 2. Structure of 4 and the partial structure B + C. The observed NOEs in 4 are shown by dotted line arrows.

H^h (δ 5.02 in both D_2O–DCl and D_2O–NaOD), indicating that the α-amino acid group of A is incorporated in a cyclic peptide structure.[6,7] Reduction of 2 with $NaBH_4$ in MeOH gave alcohol 5 (FABMS, m/z 559 ($M^+ + 1$)), in the ^1H NMR spectrum (CD_3OD) of which a singlet-like signal (δ 4.89, CD_3OD) corresponding to H^i in 2 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 indicates that the carboxylic acid function in 1 is bonded to C-8 bearing H^i. Consequently, peptide bonds between N-9/C-10 and N-12/C-13 are postulated to give the full structure of 1, without stereochemistry, for WS-43708A.[8]

The biphenyl group incorporated in the 15-membered cyclic peptide portion of WS-43708A is unique and such compounds are rarely found in nature.[9] The exceptional activity of WS-43708A against gram-positive bacteria will be reported separately.

Registry No. 1, 95485-50-0; 2, 95485-51-1; 3, 95485-52-2; 4, 95485-53-3; 5, 95485-54-4; *erythro*-γ-hydroxy-L-ornithine hydrochloride, 95485-55-5.

(6) For the pH-dependent chemical shifts of the α-methine protons of peptides, see: Sheinblatt, M. *J. Am. Chem. Soc.* 1966, *88*, 2845.

(7) The ^1H NMR spectrum (Me_2SO-d_6) of 2 showed a triplet (J = 5.5 Hz) corresponding to the amido H originated from the δ-amino group of A at δ 7.88 coupled to H^a and H^o (δ 3.07 (m) and 3.16 (m)), thus supporting that the δ-amino group of A in 1 is unsubstituted.

(8) Stereochemical deductions aside from that of the ornithine portion are the subject of future publications.

(9) As far as we are aware, the only antibiotics that contain a biphenyl group as part of a cyclic peptide system are those that belong to the vancomycin group of antibiotics, see: Barna, J. C. J.; Williams, D. H. *Annu. Rev. Microbiol.* 1984, *38*, 339.

Itsuo Uchida, Masami Ezaki
Nobuharu Shigematsu, Masashi Hashimoto*

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

Received December 18, 1984

Total Synthesis of (–)-Gilmicolin

Summary: The total synthesis of (–)-gilmicolin is disclosed; assignment of absolute stereochemistry is thereby secured.

0022-3263/85/1950-1342$01.50/0