Communications to the Editor

NEW ANTIFUNGAL ANTIBIOTICS, BENANOMICINS A AND B FROM AN *ACTINOMYCETE*

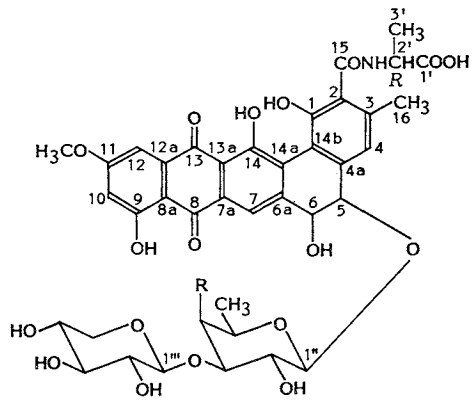
Sir:

New antifungal antibiotics, benanomicins A and B, possessing a benzo[*a*]naphthacene quinone skeleton have been found in the culture filtrate of *Actinomycete* sp. MH193-16F4. This strain is related to *Actinomadura*, but cannot be identified. Benanomicins A and B are active against fungi and some Gram-positive bacteria. In this communication, we report the isolation, characterization and structural elucidation of the antibiotics.

The seed culture (12 liters) of strain MH193-16F4 was transferred into a 570-liter fermentor containing 300 liters of medium (glycerol 2.0%, soybean flour (Ajinomoto) 1.5%, K_2HPO_4 0.0025%, KH_2PO_4 0.1125%, $CoCl_2 \cdot 6H_2O$ 0.0005%, silicon oil 0.03% and Adekanol (Asahi Denka) 0.01%, pH 7.0) and cultured at 28°C for 7 days under agitation of 300 rpm. The biological activity was determined by the paper-disc method using *Saccharomyces cerevisiae* F-7 as a test organism. The antibiotics in the filtrate (250 liters, pH 6.0) were adsorbed on a column of Diaion HP-20 (15 liters). After washing the column with water (100 liters) and 50% aq MeOH (45 liters), the antibiotics were eluted with 70% aq MeOH (45 liters) and then MeOH (90 liters). The biologically active eluate was cut into fractions I (53 liters), II (38 liters) and III (27 liters). Fraction I was concentrated to 3 liters and adjusted to pH 3.5 to give a precipitate (152 g) containing benanomicin A. The precipitate (150 g) was dissolved in DMF (600 ml) and kept at room temp for 3 days in a desiccator equilibrated with water vapor. The resulting dark red precipitate was filtered to give benanomicin A DMF solvate (29 g). Fraction II was treated in the same manner to afford benanomicin A DMF solvate (14 g). The solvate (1 g) was dissolved in DMSO (5 ml) and dropped slowly into MeOH (300 ml) under vigorous agitation to give pure benanomicin A (935 mg) as a dark red powder. Fraction III was concentrated to 1.5 liters and adjusted to pH 3.5 to give a precipitate (99 g) containing benanomicin B. The precipitate (1 g) was further purified by Sephadex LH-20 (1 liter) chromatography developed with DMF. The active eluate (54 ml) was concentrated to dryness to give a brownish powder (657 mg). To a MeOH solution (100 ml) of the brownish powder (300 mg) was added 1 N HCl (1 ml) and the solution was concentrated to dryness. The residue was dissolved in DMSO (3 ml) and dropped into $CHCl_3$ (200 ml) under agitation to give pure benanomicin B hydrochloride (258 mg) as a dark red powder.

Benanomicin A: MP >220°C, *Anal* calcd for $C_{39}H_{41}NO_{19} \cdot \frac{1}{2}CH_3OH$: C 56.23, H 5.14, N 1.66. Found: C 55.71, H 5.18, N 1.57; field desorption (FD)-MS *m/z* 827 (M^+); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 206 (718), 230 (sh, 600), 288 (482), 302 (sh, 390), 400 (sh, 120), 476 (197); $\lambda_{max}^{HCl\text{-}MeOH}$ 207 (649), 233 (629), 298 (561), 395 (sh, 140), 457 (233); $\lambda_{max}^{NaOH\text{-}MeOH}$ 214 (1,270), 249 (637), 320 (289), 498 (287); IR (KBr) cm^{-1} 3350, 1720, 1620, 1600, 1295, 1160, 1070, 1040. Benanomicin A is soluble in DMSO and DMF; slightly soluble in MeOH and $CHCl_3$; insoluble in water.

Benanomicin B hydrochloride: MP >220°C; $[\alpha]_D^{22}$ +360° (*c* 0.05, H_2O); *Anal* calcd for $C_{39}H_{42}N_2O_{18} \cdot HCl \cdot H_2O$: C 53.16, H 5.15, N 3.18, Cl 4.02. Found: C 53.57, H 5.58, N 3.01, Cl 3.80; secondary ion (SI)-MS *m/z* 827 (MH^+); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 205 (587), 233 (526), 296



Benanomicin A (**1**) R = OH
Benanomicin B (**2**) R = NH_2

Table 1. ^{1}H NMR data of benanomicins A (**1**) and B (**2**).

Proton No.	**1**			**2**		
	δ (ppm)[a]	m	J (Hz)	δ (ppm)	m	J (Hz)
1-OH	8.65[b]	br		ND		
4-H	7.21	br s		7.27	br s	
5-H	4.53	d	10.2	4.57	d	10.0
6-H	4.57	br d	10.2	4.62	br d	10.0
7-H	8.05	s		8.06	s	
9-OH	12.77	s		12.79	s	
10-H	6.86	d	2.3	6.90	d	2.3
11-OCH_3	3.92	s		3.94	s	
12-H	7.24	d	2.3	7.27	d	2.3
14-OH	13.69[b]	br		13.81[b]	br	
16-H	2.34	s		2.35	s	
1′-OH	12.47[b]	br		ND		
2′-H	4.43	dq	7.4, 7.0	4.44	dq	7.2, 7.0
2′-NH	8.45	d	7.0	8.45	d	7.0
3′-H	1.35	d	7.4	1.36	d	7.2
1″-H	4.65	d	7.8	4.75	d	7.8
2″-H	3.74	br		3.65	br	
3″-H	3.56	dd	9.8, 2.7	3.97	dd	9.8, 4.3
4″-H	3.63	br s		3.44	br	
5″-H	3.62	br q	6.3, <1	3.90	br q	6.6, <1
6″-H	1.14	d	6.3	1.20	d	6.6
1‴-H	4.43	d	7.0	4.57	d	7.0
2‴-H	3.13	dd	8.6, 7.0	3.19	m	
3‴-H	3.17	dd	8.6, 8.6	3.17	m	
4‴-H	3.32	ddd	10.6, 8.6, 5.1	3.34	ddd	10.2, 9.0, 5.1
5‴-H_{ax}	3.09	dd	10.6, 10.9	3.09	dd	11.3, 10.2
5‴-H_{eq}	3.72	dd	10.9, 5.1	3.75	dd	11.3, 5.1

[a] ppm from TMS (0 ppm) in DMSO-d_6 at 40°C as the internal reference.
[b] Tentative assignment.
m: Multiplicity.
ND: Not detected.

(426), 390 (sh, 100), 458 (169); $\lambda_{max}^{HCl\text{-}MeOH}$ 207 (514), 235 (530), 295 (442), 400 (sh, 114), 457 (173); $\lambda_{max}^{NaOH\text{-}MeOH}$ 214 (1,219), 247 (518), 317 (238), 496 (215); IR (KBr) cm^{-1} 3350, 1720, 1610, 1300, 1160, 1080, 1045. Benanomicin B hydrochloride is soluble in water, MeOH, DMSO and DMF; slightly soluble in Me_2CO and $CHCl_3$; insoluble in *n*-hexane.

The Rf values of benanomicins A and B (**1** and **2**) on TLC (Merck Art. No. 5715) developed with BuOH - AcOH - pyridine - water (6 : 1 : 4 : 3) were 0.57 and 0.45, respectively. They showed reddish purple spots on TLC plates and positive color reactions with $Mg(OAc)_2$, H_2SO_4 and Na_2MoO_4 reagents. ^{1}H and ^{13}C NMR data of **1** and **2** are shown in Tables 1 and 2, respectively.

Mild acid hydrolysis of **1** with 0.1 N HCl at 80°C for 18 hours gave an aglycone named benanomicinone (**3**) ($C_{28}H_{23}NO_{11}$, FD-MS *m/z* 549 (M^+)) and a mixture of sugars. Methanolysis of **1** with 1 N HCl - MeOH under reflux for 15 hours gave benanomicinone methyl ester (**4**) ($C_{29}H_{25}NO_{11}$, FD-MS *m/z* 563 (M^+)) and a mixture of methyl glycosides. The mixture was separated into methyl α-D-fucopyranoside ($[\alpha]_D^{22}$ +187°, *c* 0.57, H_2O)[1] and methyl α-D-xylopyranoside ($[\alpha]_D^{22}$ +145°, *c* 0.54, H_2O)[2] by preparative TLC of their triacetates followed by deacetylation with alkaline MeOH. Vigorous acid hydrolysis of **1** with a mixture of concd HCl and AcOH (1 : 1) at 110°C for 15 hours afforded a partially racemized D-alanine ($[\alpha]_D^{22}$ −8.2°, *c* 0.11, 1 N HCl). Structures of **3** and **4** were determined by ^{1}H-^{13}C shift correlation spectroscopy (^{1}H-^{13}C COSY), long range ^{1}H-^{13}C COSY and long

Table 2. ^{13}C NMR data of benanomicins A (**1**) and B (**2**) and their derivatives (**3**, **4**, **5** and **6**).

Carbon No.	**1** δ (ppm)[a]	m	**2** δ (ppm)	**3** δ (ppm)	**4** δ (ppm)	**5** δ (ppm)	**6** δ (ppm)
C-1	151.1	s	151.0	150.8	150.7	150.9	150.8
C-2	127.5	s	127.5	127.0	127.1	127.4	127.4
C-3	137.4	s	137.3	137.4	137.4	137.2	137.2
C-4[c]	118.6	d	118.9	117.5	117.5	118.8	118.9
C-4a	138.1[b]	s	137.8[b]	140.9[b]	141.1[b]	137.9[b]	138.0[b]
C-5[c]	81.7	d	81.0	71.3	71.3	81.1	81.1
C-6[c]	71.9	d	71.5	72.3	72.3	71.5	71.5
C-6a	147.7[b]	s	148.0[b]	149.9[b]	150.0[b]	147.9[b]	148.0[b]
C-7[c]	115.4	d	115.9	115.6	115.6	115.5	115.7
C-7a	131.3	s	131.2	131.1	131.0	131.2	131.2
C-8	184.9	s	184.9	184.9	184.8	184.9	184.9
C-8a	110.0	s	110.0	109.9	109.8	110.0	110.0
C-9	164.7	s	164.7	164.6	164.7	164.7	164.6
C-10	106.8	d	106.8	106.8	106.8	106.8	106.8
C-11	165.9	s	165.9	165.8	165.8	165.9	165.9
11-OCH_3	56.3	q	56.3	56.3	56.3	56.4	56.4
C-12	107.5	d	107.6	107.4	107.4	107.5	107.5
C-12a	134.2	s	134.2	134.1	133.9	134.2	134.2
C-13	187.3	s	187.4	187.3	187.3	187.4	187.4
C-13a	115.5	s	115.5	115.2	115.1	115.5	115.5
C-14	156.8	s	156.8	156.5	156.5	156.8	156.7
C-14a	125.6	s	125.7	125.8	125.7	125.7	125.6
C-14b	113.7	s	113.7	113.6	113.7	113.6	113.7
C-15	166.9	s	166.9	167.1	167.4	166.8	166.9
C-16	19.1	q	19.1	19.1	19.1	19.1	18.9
C-1′	173.9	s	173.9	173.9	173.0	173.8	172.8
1′-OCH_3	—	q	—	—	51.8	—	51.6
C-2′	47.6	d	47.6	47.6	47.8	47.6	47.6
C-3′	16.9	q	16.9	16.8	16.7	16.8	16.6
C-1″	104.4	d	104.1			104.6	104.7
C-2″	70.1	d	69.8			70.5	70.5
C-3″	83.0	d	77.4			69.8	69.9
C-4″	70.3	d	54.2			54.6	54.6
C-5″	70.1	d	67.0			67.1	67.1
C-6″	16.3	q	16.3			16.3	16.4
C-1‴	105.2	d	104.4				
C-2‴	73.6	d	73.3				
C-3‴	76.0	d	75.9				
C-4‴	69.4	d	69.4				
C-5‴	65.6	t	65.7				

[a] ppm from TMS (0 ppm) in DMSO-d_6 at 40°C as the internal reference.
[b] Exchangeable within each column.
[c] Broad signal.
m: Multiplicity.

range selective proton decoupling (LSPD) experiments. The assignments of all carbon signals of **3** and **4** are shown in Table 2.

The $^3J_{HH}$ coupling constants of the anomeric protons 1″-H (7.8 Hz) and 1‴-H (7.0 Hz) in ^1H NMR spectrum of **1** demonstrated the modes of sugar linkages as both β. By NMR studies mentioned above and nuclear Overhauser effects (NOE's) between 1″-H and 5-H, 1‴-H and 3″-H, 4-H and 5-H, and 6-H and 7-H in the ^1H-^1H NOE correlation spectrum of **1**, the positions of glycosidic linkages of D-fucopyranose and D-xylopyranose were determined at C-5 and C-3″, respectively.

Table 3. Antifungal activities of benanomicins A (**1**) and B (**2**).

Test organisms	MIC (μg/ml)	
	1	**2**
Candida tropicalis F-1	25	>100
C. pseudotropicalis F-2	6.25	6.25
C. albicans 3147	25	25
Candida Yu-1200	12.5	12.5
C. krusei F-5	6.25	6.25
Saccharomyces cerevisiae F-7	6.25	12.5
Cryptococcus neoformans F-10	3.13	1.56
Cochliobolus miyabeanus	>100	>100
Pyricularia oryzae	25	50
Pellicularia sasakii	25	50
Xanthomonas citri	>100	>100
X. oryzae	>100	>100
Aspergillus niger	50	>100
Trichophyton asteroides 429	50	25
T. mentagrophytes F-15	50	25

Treatment of **2** with 6 N HCl at 110°C for 12 hours afforded **3**, dexylosylbenanomicin B (**5**) ($C_{34}H_{34}N_2O_{14}$, FD-MS *m/z* 696 $(M+2)^+$), dealanylbenanomicinone ($C_{25}H_{18}O_{10}$, FD-MS *m/z* 478 (M^+)) and D-alanine ($[\alpha]_D^{25}$ −12°, *c* 0.2, 1 N HCl). Methanolysis of **2** with 1 N HCl - MeOH under reflux for 14 hours gave **4**, dexylosylbenanomicin B methyl ester (**6**) ($C_{35}H_{36}N_2O_{14}$, FD-MS *m/z* 708 (M^+)) and an anomeric mixture of methyl D-xylopyranosides which gave methyl 2,3,4-tri-*O*-acetyl-α-D-xylopyranoside ($[\alpha]_D^{22}$ +124°, *c* 1.0, $CHCl_3$)[2] and its β-anomer ($(\alpha]_D^{22}$ −55°, *c* 0.69, $CHCl_3$)[2] by acetylation followed by preparative TLC. Moreover, acid hydrolysis of **2** with 70% aq TFA at 90°C for 12 hours afforded thomosamine[3] in a low yield together with **3** and **5**. Treatment of thomosamine with 5% HCl - MeOH followed by acetylation with acetic anhydride in pyridine gave methyl 4-acetamide-2,3-di-*O*-acetyl-4-deoxy-α-D-fucopyranoside ($[\alpha]_D^{22}$ +78°, *c* 0.11, $CHCl_3$). The modes and positions of sugar linkages of **2** were determined by NMR studies to be the same as those of **1**.

The structures of benanomicins A and B are shown as **1** and **2**, respectively. The stereochemistry at C-5 and C-6 remains undefined.

Benanomicins A and B showed antifungal activities as shown in Table 3 and limited antibacterial activities against *Micrococcus luteus* FDA 16 (12.5 and 3.13 μg/ml) and *Corynebacterium bovis* 1810 (12.5 and 3.13 μg/ml). When tested in mice by intravenous injection, no acute toxicities of benanomicins A and B were observed at 600 mg/kg and 100 mg/kg, respectively. More details of the biological properties will be reported in due course.

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