ANTIFUNGAL AND ANTIVIRAL ACTIVITIES OF BENANOMICINS AND THEIR ANALOGUES†

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In vitro activities of benanomicins and their analogues against human immunodeficiency virus and fungi including *Candida*, *Cryptococcus* and *Aspergillus*, were examined. The free carboxyl group and at least one sugar moiety in the benanomicins were essential for their activities. Benanomicin A was most active and had low toxicity, and was selected as the best candidate for chemotherapy.

Benanomicins A and B were isolated from the culture filtrates of *Actinomadura* sp. MH193-16F4 as reddish powders.[2] These antibiotics showed excellent *in vitro* and *in vivo* activities against a wide range of fungi including *Candida, Cryptococcus* and *Aspergillus*.[3,4] They also inhibited *de novo* infection of human T-cells with human immunodeficiency virus type 1 (HIV-1), the causative agent of aquired immunodeficiency syndrome (AIDS), and syncytium formation of human T-cells after co-cultivation with HIV-producing cells.[5] Benanomicins have unique structures consisting of benzo[*a*]naphthacenequinone, D-alanine and disaccharide moieties,[6] and are classified into a new family of antibiotics together with pradimicins.[7]

In this paper, *in vitro* antifungal and anti-HIV activities of benanomicins and their various analogues are reported, and the structure-activity relationships are discussed.

Materials and Methods

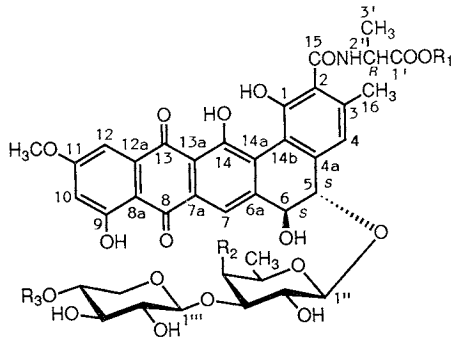
General

MP's were determined with a Yanaco MP-S3 micro melting point apparatus and were uncorrected. SI-, FD- and FAB-MS were measured on a Hitachi M-80B or a Jeol JMX-SX102 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM-GX400 or a Jeol JNM-GSX400 spectrometer.

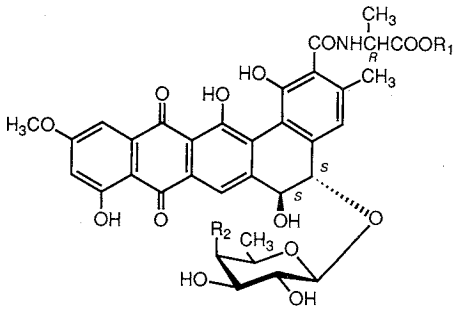
Compounds

Benanomicin A sodium salt (**A**), benanomicin B hydrochloride (**B**), dexylosylbenanomicin A (**A-DX**) and 7-methoxybenanomicinone (**AGL-OM**) were isolated from a culture filtrate of *Actinomadura* sp. MH193-16F4.[2,8] Dexylosylbenanomicin B hydrochloride (**B-DX**) and its methyl ester (**B-DXME**), and benanomicinone (**AGL**) and its methyl ester (**AGL-ME**) were derived from **B**.[6] The other compounds were prepared as follows.

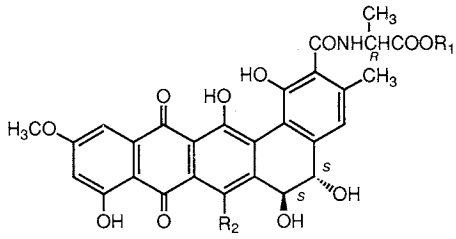
† A part of this work was presented at the 2nd International Symposium on the Chemical Synthesis of Antibiotics and the Related Microbial Products held in Oiso, Japan on Sept. 5, 1990.[1]



Benanomicin A (**A**)	$R_1=H$	$R_2=OH$	$R_3=H$
Benanomicin A methyl ester (**A-ME**)	$R_1=CH_3$	$R_2=OH$	$R_3=H$
Benanomicin A 4‴-sulfate (**A-SU**)	$R_1=Na$	$R_2=OH$	$R_3=SO_3Na$
Benanomicin B (**B**)	$R_1=H$	$R_2=NH_2$	$R_3=H$
Benanomicin B methyl ester (**B-ME**)	$R_1=CH_3$	$R_2=NH_2$	$R_3=H$
N-Acetylbenanomicin B (**B-AC**)	$R_1=H$	$R_2=NHCOCH_3$	$R_3=H$



Dexylosylbenanomicin A (**A-DX**)	$R_1=H$	$R_2=OH$
Dexylosylbenanomicin A methyl ester (**A-DXME**)	$R_1=CH_3$	$R_2=OH$
Dexylosylbenanomicin B (**B-DX**)	$R_1=H$	$R_2=NH_2$
Dexylosylbenanomicin B methyl ester (**B-DXME**)	$R_1=CH_3$	$R_2=NH_2$
N-Acetyldexylosylbenanomicin B (**B-DXAC**)	$R_1=H$	$R_2=NHCOCH_3$



Benanomicinone (**AGL**)	$R_1=R_2=H$	
Benanomicinone methyl ester (**AGL-ME**)	$R_1=CH_3$	$R_2=H$
7-Methoxybenanomicinone (**AGL-OM**)	$R_1=H$	$R_2=OCH_3$

Methyl Esters

A solution of **A** (55.8 mg) in 0.02 N HCl - MeOH (50 ml) was stirred at room temperature for 15 hours and then concentrated to dryness. The residue was dissolved in 1 ml of DMSO and purified on a column of Sephadex LH-20 (Pharmacia AB, 500 ml) developed with MeOH. The reddish eluate was concentrated to yield 38.3 mg of benanomicin A methyl ester (**A-ME**), mp > 220°C, FAB-MS *m/z* 841 (M^-).

Table 1. ^{1}H NMR data of benanomicin analogues.

Proton	A-ME		A-SU		A-DXME		B-ME		B-AC		B-DXAC	
	δ	m	δ	m	δ	m	δ	m	δ	m	δ	m
1-OH	8.60[a]	br	ND		ND		ND		ND		ND	
4-H	7.21	br s	7.18	br s	7.23	br s	7.26	br s	7.24	br s	7.26	br s
5-H	4.52	d	4.50	br d	4.48	d	4.57	d	4.56	d	4.52	d
	(9.8)		(12.0)		(10.6)		(9.8)		(10.0)		(10.2)	
6-H	4.58	br d	4.56	br d	4.56	br s	4.62	br d	4.61	br d	4.59	br d
	(9.8)		(12.0)		(10.6)		(9.8)		(10.0)		(10.2)	
7-H	8.09	br s	8.06	s	8.08	s	8.07	s	8.07	s	8.06	s
9-OH	12.82	s	12.80	s	12.80	s	12.80	s	12.80	s	12.79	s
10-H	6.94	d	6.92	d	6.91	d	6.93	d	6.91	d	6.89	d
	(2.3)		(2.7)		(2.0)		(2.3)		(2.3)		(2.3)	
11-OCH_3	3.96	s	3.93	s	3.95	s	3.95	s	3.95	s	3.93	s
12-H	7.31	d	7.27	d	7.28	d	7.29	d	7.28	d	7.25	d
	(2.3)		(2.7)		(2.0)		(2.3)		(2.3)		(2.3)	
14-OH	13.84[a]	br	ND		13.86[a]	br	13.82[a]	br	13.82[a]	br	13.80[a]	br
16-H_3	2.32	s	2.31	s	2.32	s	2.33	s	2.33	s	2.32	s
1'-OH	—		ND		—		—		ND		ND	
1'-OCH_3	3.68	s	—		3.69	s	3.68	s	—		—	
2'-H	4.42	dq	4.39	dq	4.48	dq	4.48	dq	4.43	dq	4.43	dq
	(7.2, 7.0)		(7.0, 7.0)		(7.0, 7.0)		(7.2, 7.0)		(7.2, 7.0)		(7.2, 7.0)	
2'-NH	8.60	d	8.56	d	8.61	d	8.60	d	8.45	d	8.46	d
	(7.0)		(7.0)		(7.0)		(7.0)		(7.0)		(7.0)	
3'-H_3	1.35	d	1.31	d	1.35	d	1.35	d	1.35	d	1.35	d
	(7.2)		(7.0)		(7.0)		(7.2)		(7.2)		(7.2)	
1''-H	4.64	d	4.64	d	4.55	d	4.75	d	4.64	d	4.59	br
	(7.8)		(7.0)		(7.4)		(7.8)		(7.4)			
2''-H	3.70	br	3.70	br	3.53	br	3.61	br	3.81	br	3.58	br
3''-H	3.54	dd	3.54	br dd	3.39	dd	3.95	m	3.74	dd	3.58	br
	(9.8, 3.1)		(9.0, 3.0)		(9.4, 3.1)				(10.0, 4.3)			
4''-H	3.61	m	3.60	br s	3.45	br d	3.43	br	4.21	br dd	4.07	br d
					(3.1)				(9.8, 4.3)		(9.4)	
4''-NH_3^+	—		—		—		7.95	br	—		—	
4''-NH	—		—		—		—		7.61	br d	7.53	br d
									(9.8)		(9.4)	
4''-NAc	—		—		—		—		1.92	s	1.95	s
5''-H	3.61	m	3.59	br q	3.57	br q	3.90	br q	3.71	br q	3.68	br dq
			(6.0)		(6.3)		(6.6)		(6.3)		(6.3, 1.0)	
6''-H	1.12	d	1.10	d	1.14	d	1.19	d	0.98	d	1.00	d
	(6.3)		(6.0)		(6.3)		(6.6)		(6.3)		(6.3)	
1'''-H	4.42	d	4.43	d			4.56	d	4.42	d		
	(7.0)		(8.0)				(7.0)		(6.6)			
2'''-H	3.12	dd	3.20	m			3.19	m	3.08	dd		
	(8.6, 7.0)								(8.2, 6.6)			
3'''-H	3.16	dd	3.31	dd			3.17	m	3.15	dd		
	(8.6, 8.6)		(8.0, 8.0)						(8.2, 8.2)			
4'''-H	3.31	m	3.92	m			3.32	m	3.29	ddd		
									(9.8, 8.2, 5.1)			
5'''-H_{ax}	3.08	dd	3.20	m			3.09	dd	3.06	dd		
	(11.3, 10.2)						(11.3, 10.2)		(11.3, 9.8)			
5'''-H_{eq}	3.71	dd	3.96	m			3.74	dd	3.70	dd		
	(11.3, 4.7)						(11.3, 5.1)		(11.3, 5.1)			

δ: ppm from TMS in DMSO-d_6 at 40°C. Coupling constants (Hz) are in parentheses.
m: Multiplicity.
[a] Tentative assignment.
ND: Not detected.

Table 2. ^{13}C NMR data of benanomicin analogues.

Carbon	A-ME	A-SU	A-DXME	B-ME	B-AC	B-DXAC
	δ m	δ	δ	δ	δ m	δ
C-1	150.9 s	151.0	150.9	150.9	151.1 s	151.0
C-2	127.4 s	127.6	127.4	127.5	127.5 s	127.5
C-3	137.2 s	137.4	137.3	137.2	137.3 s	137.3
C-4	118.4 d	118.4	118.6	118.4	118.8 d	118.9
C-4a	138.0 s	138.1	138.2	137.8	137.9 s	138.0
C-5	81.6 d	81.7	81.6	80.9	81.8 d	82.0
C-6	71.7 d	72.0	71.9	71.5	71.7 d	71.9
C-6a	147.7 s	147.8	147.9	147.9	147.8 s	147.9
C-7	115.4 d	115.6	115.3	115.5	115.5 d	115.5
C-7a	131.3 s	131.4	131.3	131.3	131.3 s	131.3
C-8	185.0 s	185.0	184.9	184.9	184.9 s	184.9
C-8a	110.1 s	110.1	110.0	110.0	110.0 s	110.0
C-9	164.7 s	164.8	164.7	164.7	164.7 s	164.7
C-10	106.8 d	106.9	106.8	106.9	106.9 d	106.9
C-11	166.0 s	166.0	165.9	166.0	166.0 s	166.0
11-OCH_3	56.4 q	56.5	56.3	56.4	56.4 q	56.4
C-12	107.5 d	107.7	107.6	107.6	107.6 d	107.6
C-12a	134.3 s	134.3	134.2	134.2	134.3 s	134.2
C-13	187.4 s	187.6	187.4	187.4	187.5 s	187.5
C-13a	115.5 s	115.6	115.5	115.5	115.5 s	115.5
C-14	156.8 s	156.9	156.7	156.8	156.8 s	156.8
C-14a	125.6 s	125.6	125.5	125.7	125.6 s	125.6
C-14b	113.7 s	113.5	113.6	113.7	113.7 s	113.7
C-15	166.9 s	167.0	167.0	166.9	166.9 s	166.9
C-16	18.9 q	19.2	19.0	18.9	19.1 q	19.1
C-1′	172.9 s	174.1	172.9	172.9	173.9 s	173.9
1′-OCH_3	51.6 q	—	51.6	51.6	—	—
C-2′	47.6 d	47.7	47.7	47.7	47.6 d	47.7
C-3′	16.6 q	16.9	16.6	16.6	16.9 q	16.9
C-1″	104.4 d	104.5	105.1	104.0	104.7 d	105.5
C-2″	70.0 d	70.1	71.1	69.7	70.5 d	71.3
C-3″	83.0 d	83.2	73.4	77.4	80.2 d	71.7
C-4″	70.3 d	70.3	70.9	54.2	52.0 d	52.7
4″-NAc	—	—	—	—	169.9 s, 22.5 q	170.3, 22.6
C-5″	70.0 d	69.5	70.2	66.9	69.9 d	69.8
C-6″	16.3 q	16.4	16.4	16.3	16.4 q	16.4
C-1‴	105.2 d	104.5		104.5	104.9 d	
C-2‴	73.6 d	73.8		73.3	73.3 d	
C-3‴	76.0 d	74.4		75.9	75.8 d	
C-4‴	69.4 d	74.4		69.4	69.3 d	
C-5‴	65.6 t	63.5		65.7	65.4 t	

δ: ppm from TMS in DMSO-d_6 at 40°C.
m: Multiplicity.

Benanomicin B methyl ester hydrochloride (**B-ME**) (45.3 mg) was obtained from **B** (50.3 mg) by a similar method to that described above. MP >200°C, FAB-MS *m/z* 840 (M^-).

Dexylosylbenanomicin A methyl ester (**A-DXME**) (77.9 mg) was obtained from **A-DX** (95.5 mg) by a similar method to that described above. MP >200°C, FD-MS *m/z* 709 (M^+).

NMR data of **A-ME**, **B-ME** and **A-DXME** are shown in Tables 1 and 2.

N-Acetyl Derivatives

To a solution of **B** (125 mg) in 0.1 M Na_2CO_3 (10 ml) was added Ac_2O (0.1 ml) and the solution was

stirred at room temperature for 20 minutes. The solution was adjusted to pH 4.0 with 1 N HCl and was passed through a column of Diaion HP-20 (Mitsubishi Chemical Ind., Ltd., 20 ml) and after washing with water, the column was eluted with 80% aq acetone to yield a reddish powder. The powder was re-chromatographed by Sephadex LH-20 column (300 ml) developed with MeOH to obtain 118 mg of *N*-acetylbenanomicin B (**B-AC**) as a reddish powder. MP >200°C, SI-MS *m/z* 868 (M^+).

N-Acetyldexylosylbenanomicin B (**B-DXAC**) (31.5 mg) was obtained from **B-DX** (35.0 mg) by a similar method to that described above. MP >200°C, FAB-MS *m/z* 736 (M^-).

NMR data of **B-AC** and **B-DXAC** are shown in Tables 1 and 2.

Benanomicin A 4‴-Sulfate (A-SU)

To a solution of **A** (free acid, 251 mg) in anhydrous pyridine (5 ml) was added sulfur trioxide-trimethylamine complex ($SO_3 \cdot NMe_3$, 84 mg). After warming at 50°C for 40 minutes, water (0.5 ml) was added to the reaction mixture to decompose the excess reagent. The solution was concentrated to give a solid, which was separated by centrifugal countercurrent partition chromatography (CPC Model NMF, Sanki Engineering) using the solvent system of BuOH-H_2O-pyridine (50:50:1). After development with mobile phase (upper layer), reverse-elution with lower layer gave 60 mg of **A-SU** (pyridinium salt), mp 172~178°C (dec), FAB-MS *m/z* 908 $(M+H)^+$, 906 $(M-H)^-$. NMR data are shown in Tables 1 and 2.

Antifungal Activities

MICs on a glucose-nutrient agar consisting of glucose 1.0%, peptone (Polypepton, Wako Pure Chemicals) 1.0%, meat extract (Kyokuto Seiyaku) 1.0%, NaCl 0.3% and agar (Kyoei Seiyaku) 2.0% (pH 7.0) were determined by the 2-fold agar dilution method after incubation at 27°C for 42 hours. The test organisms which were grown in Sabouraud bouillon medium at 27°C for 2 days, were used for inoculation.

Anti-HIV Activities

A human T-cell line, MT-4 cells (1×10^5 cells/ml) in 0.5 ml of RPMI-1640 culture medium were seeded into 48-well plates, and test compounds in Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) were added at concentrations of 100, 30, 10, 3 and 1 μg/ml (compounds were dissolved in DMSO at 10 mg/ml and the solution was diluted with PBS). Two hours later 50 μl of the HTLV-III_B strain of HIV-1 (2.5×10^5 to 5×10^5 PFU/ml) was inoculated onto the cells. After 4 days, MT-4 cells were smeared onto slide glasses, dried and fixed with acetone. The presence (%) of HIV antigen-positive cells were detected by the indirect immunofluorescence assay.[9]

The effects of test compounds and DMSO on growth of uninfected MT-4 cells were examined by counting viable cells using a dye exclusion method. MT-4 cells were seeded at 2×10^5/ml and counted after cultivation for 4 days.[5]

Syncytium formation assay[5] was performed as follows. Molt-4 human T-cells were seeded into Costar 48-well plates in an amount of 1×10^5 cells/well, and test compounds (50 μl) were added. After 2 hours, Molt-4 cells persistently infected with HIV-1 were added at 1.5×10^4 cells/well. After cultivation for 24 hours, numbers of syncytia formed were counted using a microscope after fixation with 5% formalin.

Activated Partial Thromboplastin Time (APTT)

APTT of plasma from a normal subject was examined in the presence of test compounds by using an automated machine. Platerin plus activator was obtained from Organon Teknika Corporation (Durham). Human plasma (90 μl) was mixed with platerin plus activator (100 μl) and test compounds (10 μl), and then APTT was measured.[10]

Acute Toxicity

LD_{50} were determined with five male Jcl : ICR mice (*ca.* 20 g weight) at each dose level. Test compounds in physiological saline or 10% DMSO aq soln (0.25 ml) were injected intravenously and mice were observed for 14 days.

Table 3. *In vitro* antifungal activity on glucose-nutrient agar.

Test organism	MIC (μg/ml)													
	A	**A-ME**	**A-DX**	**A-DXME**	**A-SU**	**B**	**B-ME**	**B-DX**	**B-DXME**	**B-AC**	**B-DXAC**	**AGL**	**AGL-ME**	**AGL-OM**
Candida albicans 3147	6.25	>100	6.25	100	12.5	12.5	100	6.25	>50	50	100	>50	>100	>100
C. tropicalis F-1	6.25	>100	6.25	>100	25	12.5	50	6.25	>50	50	100	>50	>50	100
C. pseudotropicalis F-2	3.13	>100	1.56	12.5	6.25	6.25	6.25	3.13	>50	12.5	12.5	>50	100	100
C. krusei F-5	3.13	>100	6.25	>100	25	6.25	6.25	12.5	>100	>100	>100	>100	>100	>100
Candida sp. Yu-1200	6.25	>100	6.25	100	12.5	6.25	50	6.25	>100	50	100	>100	>100	>100
Saccharomyces cerevisiae F-7	3.13	>100	3.13	12.5	6.25	6.25	25	6.25	>100	12.5	12.5	>50	>50	100
Cryptococcus neoformans F-10	1.56	>100	6.25	>100	6.25	1.56	3.13	3.13	>50	50	>100	>50	>50	>50
Aspergillus niger F-16	12.5	>100	50	>100	100	12.5	50	25	>50	>50	>100	>100	>100	>50
Trichophyton asterbides 429	50	100	100	>100	>100	50	100	50	100	50	>100	100	100	50
T. mentagrophytes F-15	50	>50	100	>100	100	50	>50	50	100	50	>100	100	100	50
Cochliobolus miyabeanus	50	100	100	>100	>100	12.5	100	50	>50	50	>100	>50	>50	50
Pyricularia oryzae	50	>100	100	>100	>100	>50	100	25	>50	>50	>100	>100	>50	>50
Pellicularia sasakii	12.5	100	6.25	>50	12.5	12.5	50	25	12.5	25	>50	>25	25	>25

Results

Antifungal Activities

As shown in Table 3, **A** and **B** showed good *in vitro* antifungal activities against *Candida*, *Saccharomyces*, *Cryptococcus* and *Aspergillus* on glucose-nutrient agar medium. **A-DX** and **B-DX** have also good activities: **B-DX** is more active than **B** against *Candida*, but not against *Aspergillus*. **A-SU** and **B-AC** have moderately active against yeasts and yeast like fungi. The methyl esters (**A-ME**, **A-DXME**, **B-ME** and **B-DXME**) and benanomicinone derivatives (**AGL**, **AGL-ME** and **AGL-OM**) were nearly inactive.

Anti-HIV Activities

As shown in Table 4, **A** and **B** inhibited infection of HIV-1 at concentration of 10 μg/ml, but did not inhibit HIV reverse transcriptase (data are not shown). Growth of MT-4 cells (Table 5) was affected by 100 μg/ml of **B-ME**, **B-DX**, **B-DXME** and DMSO. Although all sample solutions contained DMSO, we concluded that **A** and **B** had little or no effect on growth of cells at 100 μg/ml. As already reported,[5] **A** and **B** also inhibited syncytium formation induced by HIV-1 at concentrations of 10~100 μg/ml (Table 6).

Table 4. Effects on infection of MT-4 cells with HIV-1.

Concentration (μg/ml)	Viral antigen-positive cells (%)										
	A	**A-ME**	**A-DX**	**A-SU**	**B**	**B-ME**	**B-DX**	**B-DXME**	**B-AC**	**B-DXAC**	**AGL**
100	<1	>90	CT	<1	<1	CT	CT	CT	82	CT	>90
30	<1	>90	60	53	<1	85	80	24	>90	>90	>90
10	23	>90	>90	>90	1	>90	>90	70	>90	>90	>90
3	>90	>90	>90	>90	83	>90	>90	>90	>90	>90	>90
1	>90		>90	>90	>90	>90	>90	>90		>90	
0	>90										

CT: Cytotoxic.

Table 5. Effects on growth of MT-4 cells.

Concentration (μg/ml)	Number of cells ($\times 10^{-4}$/ml)							
	DMSO[a]	**A**	**B**	**B-ME**	**B-DX**	**B-DXME**	**B-DXAC**	**AGL**
100	29	53	42	3	7	20	32	42
30	71	79	68	51	50	76	75	78
10	74	78	78	68	63	76	79	82
3	79	78	76	71	75	76	73	80
0	79							

[a] Solvent control.

Table 6. Effects on syncytium formation induced by HIV-1.

Concentration (μg/ml)	Number of syncytia/5 × 5 mm^2						
	A	**B**	**B-ME**	**B-DX**	**B-DXME**	**B-DXAC**	**AGL**
100	0	0	0	1	0	67	71
30	0	0	0	7	0	92	88
10	0	0	77	68	78	101	101
3	78	68	92	95	82	106	103
0	104						

Table 7. Effects on APTT.

Compound	APTT (seconds)
A	47.6
B	49.4
B-ME	48.9
B-DX	49.2
B-DXME	46.3
B-AC	47.8
B-DXAC	48.1
AGL	50.3
Heparin (10 μg/ml)	>276
DMSO	47.2
PBS	46.4

Test compounds were determined at 100 μg/ml. DMSO (1% in PBS) is the solvent control.

Table 8. Intravenous acute toxicity in mice.

Compound	**A**	**A-DX**	**A-SU**	**B**	**B-DX**	**B-AC**
LD_{50} (mg/kg)	>600	>300	>500	150	180	>300

APTT

Although heparin markedly prolonged APTT, all test compounds were negative at concentration of 100 μg/ml (Table 7). These findings suggested that compounds would not have anti-coagulant activity *in vivo*.

Acute Toxicity

As shown in Table 8, compounds having a free amino group, **B** and **B-DX** showed acute LD_{50} values (mice, iv) of 150 and 180 mg/kg, respectively. However, compounds possessing no positive charge had very low toxicity. In particular, **A** was not toxic by iv injection of 600 mg/kg.

Discussion

Studies on structure-activity relationships among various analogues of benanomicins revealed that the carboxyl group and at least one sugar moiety were essential for antifungal and anti-HIV activities.

The important role of the free carboxyl group in the benanomicins was evident, because all ester derivatives had reduced activities. Interestingly, dexylosyl derivatives showed good activities against *Candida* and *Cryptococcus*, but decreased anti-*Aspergillus* and anti-HIV activities. A free amino group of **B** increased the acute toxicity in mice.

From the above-mentioned results, **A**, which has very low toxicity was selected as the best candidate not only for antifungal chemotherapy but also for anti-AIDS therapy. Because *Candida*, *Cryptococcus* and *Aspergillus* have been frequently detected in patients with AIDS, when **A** is used as an anti-HIV agent, the administration may be especially advantageous for patients with AIDS or AIDS-related complex who are infected with fungi or are at risk for fungal infection.

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References

1) KONDO, S.; D. IKEDA, S. GOMI & T. TAKEUCHI: A new target for antifungal and antiviral agents: Benanomicins. Book of Abstracts of the 2nd International Symposium on the Chemical Synthesis of Antibiotics and the Related Microbial Products, No. G-18, p. 116, Oiso, Sept. 4~7, 1990
2) TAKEUCHI, T.; T. HARA, H. NAGANAWA, M. OKADA, M. HAMADA, H. UMEZAWA, S. GOMI, M. SEZAKI & S. KONDO: New antifungal antibiotics, benanomicins A and B from an *Actinomycete*. J. Antibiotics 41: 807~811, 1988
3) TAKEUCHI, T.; T. HARA, M. HAMADA, H. YAMAMOTO, S. GOMI, Y. ORIKASA, M. SEZAKI, S. KONDO & H. YAMAGUCHI: Benanomicins A and B, novel antifungal antibiotics. Program and Abstracts of the 28th Intersci. Conf. on Antimicrob. Agents Chemother., No. 1007, p. 288, Los Angeles, Oct. 23~26, 1988
4) YAMAGUCHI, H.; K. UCHIDA, Y. ORIKASA, T. MATSUMOTO, H. YAMAMOTO, S. INOUYE, S. KONDO & T. TAKEUCHI: Antifungal activity of benanomicin A, a novel antibiotic. Program and Abstracts of the 29th Intersci. Conf. on

Antimicrob. Agents Chemother., No. 715, p. 221, Houston, Sept. 17~20, 1989

5) HOSHINO, H.; J. SEKI & T. TAKEUCHI: New antifungal antibiotics, benanomicins A and B inhibit infection of T-cell with human immunodeficiency virus (HIV) and syncytium formation by HIV. J. Antibiotics 42: 344~346, 1989
6) GOMI, S.; M. SEZAKI, S. KONDO, T. HARA, H. NAGANAWA & T. TAKEUCHI: The structures of new antifungal antibiotics, benanomicins A and B. J. Antibiotics 41: 1019~1028, 1988
7) OKI, T.; M. KONISHI, K. TOMATSU, K. TOMITA, K. SAITOH, M. TSUNAKAWA, M. NISHIO, T. MIYAKI & H. KAWAGUCHI: Pradimicin, a novel class of potent antifungal antibiotics. J. Antibiotics 41: 1701~1704, 1988
8) KONDO, S.; S. GOMI, K. UOTANI, S. INOUYE & T. TAKEUCHI: Isolation of new minor benanomicins. J. Antibiotics 44: 123~129, 1991
9) TAKEUCHI, Y.; M. INAGAKI, N. KOBAYASHI & H. HOSHINO: Isolation of human immunodeficiency virus from a Japanese hemophilia B patient with AIDS. Gann 78: 11~15, 1987
10) HANDA, A.; H. HOSHINO, K. NAKAJIMA, M. ADACHI, K. IKEDA, K. ACHIWA, T. ITOH & Y. SUZUKI: Inhibition of infection with human immunodeficiency virus type 1 by sulfated gangliosides. Biochem. Biophys. Res. Commun. 175: 1~9, 1991