THE STRUCTURES OF NEW ANTIFUNGAL ANTIBIOTICS, BENANOMICINS A AND B

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Structures of new antifungal antibiotics, benanomicins A and B were determined to be *N*-[[5-[6-deoxy-3-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyloxy]-5,6-dihydro-1,6,9,14-tetrahydroxy-11-methoxy-3-methyl-8,13-dioxobenzo[*a*]naphthacen-2-yl]carbonyl]-D-alanine and *N*-[[5-[4-amino-4,6-dideoxy-3-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyloxy]-5,6-dihydro-1,6,9,14-tetrahydroxy-11-methoxy-3-methyl-8,13-dioxobenzo[*a*]naphthacen-2-yl]carbonyl]-D-alanine, respectively, by spectral analyses and chemical degradation studies.

Benanomicins A and B have been found in the culture of *Actinomycete* sp. MH193-16F4 and the antibiotics are active against fungi.[1] In this paper, we report the structural elucidation of benanomicins A and B by spectroscopic and chemical degradation studies.

The molecular formulae of benanomicins A (**1**) and B (**2**) were determined to be $C_{39}H_{41}NO_{19}$ and $C_{89}H_{42}N_2O_{18}$, respectively, from the elemental analyses, mass spectra [field desorption mass spectrum (FD-MS) of **1**: *m/z* 827 (M^+), 828 (MH^+), fast atom bombardment mass spectrum (FAB-MS, positive) of **1**: 829 $(M+2)^+$, 851 $(MH+Na)^+$, 867 $(MH+K)^+$; FAB-MS (negative) **1**: 827 (M^+); secondary ion mass spectrum (SI-MS) of **2**: 826 (M^+), 827 (MH^+), 828 $(M+2)^+$]; ^1H and ^{13}C NMR spectra (Tables 1, 2, 3 and 4). UV spectral data of **1** and **2** as shown in Table 5 suggested a polyhydroxyanthraquinone-like structure similar to that in the G-2N[2], G-2A[2], KS-619-1[3,4] and SF2446 antibiotics.[5,6] Visible absorption bands of **1** and **2** in alkaline solution exhibited characteristic bathochromic shifts (**1**: UV $\lambda_{max}^{HCl-MeOH}$ 457 nm, λ_{max}^{MeOH} 476 nm and $\lambda_{max}^{NaOH-MeOH}$ 498 nm; **2**: UV $\lambda_{max}^{HCl-MeOH}$ 457 nm, λ_{max}^{MeOH} 458 nm and $\lambda_{max}^{NaOH-MeOH}$ 496 nm).

The absorption band at 1720 cm^{-1} in the IR spectrum of **1** was assigned to a carboxyl group and disappeared in that of the sodium salt. The ^1H and ^{13}C NMR spectra of **1** revealed the presence of two sugar moieties [4.65 (1″-H), 104.4 (C-1″), 4.43 (1‴-H) and 105.2 (C-1‴) ppm]. By the analyses of ^1H-^1H shift correlation spectrum (COSY) and long range ^1H-^1H COSY, the two sugars were deduced to be fucopyranose and xylopyranose which were both β-glycosides ($J_{1'',2''}$=7.8 Hz and $J_{1''',2'''}$=7.0 Hz). Methanolysis of **1** gave methyl α-D-fucopyranoside ($[\alpha]_D^{22}$ +187°, literature[7] $[\alpha]_D^{26}$ +192.7°) and methyl α-D-xylopyranoside ($[\alpha]_D^{22}$ +145°, literature[8] $[\alpha]_D$ +153.9°). Furthermore, the observation of a low-field shift of the C-3″ signal in the ^{13}C NMR spectrum of **1** suggested that the xylopyranose was connected at the C-3″ position of fucopyranose as a β-glycosidic linkage. This was supported by the observed nuclear Overhauser effect (NOE) between 1‴-H and 3″-H in the ^1H-^1H NOE correlation spectrum (NOESY) of **1**.

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

Proton	1	2	3	4	5	6
1-OH	8.65*br	8.45*br	8.54*br	8.42*br	8.60*br	8.92*br
4-H	7.21 br s	7.27 br s	7.08 br s	7.07 br s	7.25 br s	7.27 br s
5-H	4.53 d (10.2)	4.57 d (10.0)	4.27 s	4.24 d (11.3)	4.53 d (9.8)	4.53 br d (9.6)
6-H	4.57 br d (10.2)	4.62 br d (10.0)	4.27 s	4.25 d (11.3)	4.60 br d (9.8)	4.60 br d (9.6)
7-H	8.05 s	8.06 s	8.08 s	8.03 s	8.08 s	8.07 s
9-OH	12.77 s	12.79 s	12.76 s	12.72 s	12.82 s	12.80 s
10-H	6.86 d (2.3)	6.90 d (2.3)	6.82 d (2.3)	6.75 d (2.3)	6.94 d (2.3)	6.92 br d (2.0)
11-OCH_3	3.92 s	3.94 s	3.90 s	3.84 s	3.96 s	3.95 s
12-H	7.24 d (2.3)	7.27 d (2.3)	7.15 d (2.3)	7.03 d (2.3)	7.31 d (2.3)	7.28 br d (2.0)
14-OH	13.69*br	13.81*br	13.82*br	13.82*br	13.81*br	13.80*br
16-H	2.34 s	2.35 s	2.36 s	2.35 s	2.33 s	2.33 s
1′-OH	12.47*br	ND	12.43*br	—	ND	—
1′-OCH_3	—	—	—	3.70 s	—	3.68 s
2′-H	4.43 dq (7.4, 7.0)	4.44 dq (7.2, 7.0)	4.44 dq (7.4, 7.0)	4.49 dq (7.4, 7.0)	4.43 dq (7.4, 7.0)	4.47 dq (7.4, 7.0)
2′-NH	8.45 d (7.0)	8.45 d (7.0)	8.47 d (7.0)	8.72 d (7.0)	8.45 d (7.0)	8.62 d (7.0)
3′-H	1.35 d (7.4)	1.36 d (7.2)	1.36 d (7.4)	1.35 d (7.4)	1.34 d (7.4)	1.35 d (7.4)

δ: ppm from TMS in DMSO-d_6 at 40°C. Coupling constants (J=Hz) are in parentheses. **2**, **5** and **6** are hydrochlorides.
* Tentative assignment.
ND: Not detected.

Table 2. ^{1}H NMR data of the sugar parts of benanomicins A (**1**) and B (**2**) and derivatives (**5** and **6**) of benanomicin B.

Proton	1	2	5	6
1″-H	4.65 d (7.8)	4.75 d (7.8)	4.68 d (7.8)	4.68 d (7.8)
2″-H	3.74 br	3.65 br	3.47 br	3.48 br
3″-H	3.56 dd (9.8, 2.7)	3.97 dd (9.8, 4.3)	3.74 br dd (9.4, 4.3)	3.75 br dd (9.6, 4.1)
4″-H	3.63 br s	3.44 br	3.26 br	3.28 br
4″-NH_3^+	—	7.99 br	7.87 br	7.92 br
5″-H	3.62 br q (6.3, <1)	3.90 br q (6.6, <1)	3.86 br q (6.3, <1)	3.86 br q (6.3, <1)
6″-H	1.14 d (6.3)	1.20 d (6.6)	1.18 d (6.3)	1.18 br d (6.3)
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 (10.9, 10.6)	3.09 dd (11.3, 10.2)		
5‴-H_{eq}	3.72 dd (10.9, 5.1)	3.75 dd (11.3, 5.1)		

δ: ppm from TMS in DMSO-d_6 at 40°C. Coupling constants (J=Hz) are in parentheses. **2**, **5** and **6** are hydrochlorides.

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

Carbon	1	2	3	4	5	6
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*	118.6 d	118.9	117.5	117.5	118.8	118.9
C-4a	138.1 s	137.8	140.9	141.1	137.9	138.0
C-5*	81.7 d	81.0	71.3	71.3	81.1	81.1
C-6*	71.9 d	71.5	72.3	72.3	71.5	71.5
C-6a	147.7 s	148.0	149.9	150.0	147.9	148.0
C-7*	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

δ: ppm from TMS in DMSO-d_6 at 40°C. **2**, **5** and **6** are hydrochlorides.
* Broad signal.

Table 4. ^{13}C NMR data of the sugar parts of benanomicins A (**1**) and B (**2**), derivatives (**5** and **6**) and methyl glycosides.

Carbon	1	2	5	6	F	T	X
C-1″	104.4 d	104.1	104.6	104.7	100.2	99.8	
C-2″	70.1 d	69.8	70.5	70.5	70.2	68.1	
C-3″	83.0 d	77.4	69.8	69.9	68.6	66.8	
C-4″	70.3 d	54.2	54.6	54.6	72.5	56.4	
C-5″	70.1 d	67.0	67.1	67.1	67.1	63.1	
C-6″	16.3 q	16.3	16.3	16.4	15.9	16.1	
C-1‴	105.2 d	104.4					100.2
C-2‴	73.6 d	73.3					71.9
C-3‴	76.0 d	75.9					73.8
C-4‴	69.4 d	69.4					70.0
C-5‴	65.6 t	65.7					61.6
OCH_3	— q	—	—	—	55.8	56.0	55.8

F: Methyl α-D-fucopyranoside.
T: Methyl 4-amino-4-deoxy-α-D-fucopyranoside.
X: Methyl α-D-xylopyranoside.
δ: ppm; **1**, **2**, **5** and **6** were measured in DMSO-d_6 at 40°C. **F**, **T** and **X** were measured in D_2O at 25°C. **2**, **5**, **6** and **T** are hydrochlorides.

Table 5. UV spectral data of benanomicins A (**1**) and B (**2**) and their derivatives (**3**, **4**, **5**, **6** and **7**).

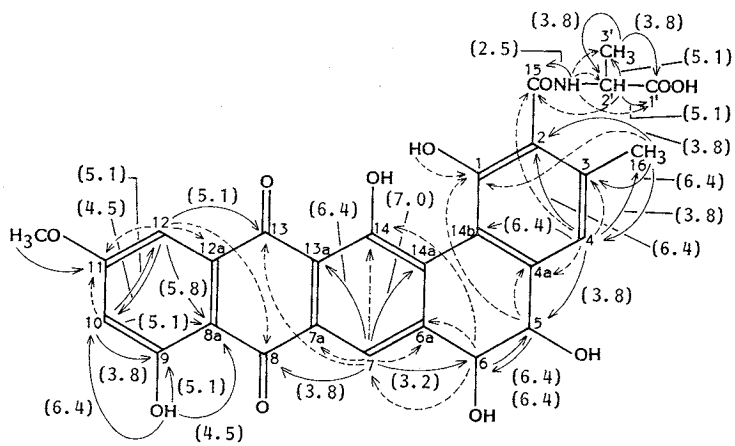
	1	**2**	**3**	**4**	**5**	**6**	**7**
MeOH	206 (718), 230 (sh, 600), 288 (482), 302 (sh, 390), 400 (sh, 120), 476 (197)	205 (587), 233 (526), 296 (426), 390 (sh, 100), 458 (169),	206 (571), 232 (553), 267 (sh, 350), 291 (453), 300 (sh, 430), 400 (sh, 115), 466 (185)	204 (515), 227 (438), 270 (sh, 290), 289 (329), 300 (sh, 280), 400 (sh, 75), 474 (125)	204 (569), 234 (517), 290 (431), 300 (sh, 395), 400 (sh, 110), 463 (170)	204 (636), 232 (492), 270 (sh, 325), 290 (384), 300 (sh, 330), 400 (sh, 90), 471 (145)	219 (543), 237 (488), 250 (sh, 440), 270 (342), 303 (359), 441 (219)
HCl - MeOH	207 (649), 233 (629), 298 (561), 395 (sh, 140), 457 (233)	207 (514), 235 (530), 295 (422), 400 (sh, 114), 457 (173)	208 (482), 234 (561), 270 (sh, 350), 301 (509), 400 (sh, 135), 458 (200)	206 (388), 234 (410), 270 (sh, 240), 301 (354), 400 (sh, 85), 459 (133)	209 (522), 234 (557), 296 (487), 400 (sh, 130), 459 (196)	206 (422), 235 (449), 270 (sh, 285), 293 (sh, 405), 300 (406), 400 (sh, 105), 458 (164)	220 (519), 238 (483), 250 (sh, 450), 272 (350), 304 (381), 441 (225)
NaOH - MeOH	214 (1,270), 249 (637), 320 (289), 498 (287)	214 (1,219), 247 (518), 317 (238), 496 (215)	213 (1,234), 248 (606), 320 (279), 496 (275)	214 (1,199), 236 (530), 246 (532), 260 (sh, 450), 318 (255), 495 (225)	213 (1,205), 249 (575), 260 (sh, 500), 319 (259), 496 (251)	212 (1,120), 248 (474), 260 (sh, 460), 319 (215), 497 (209)	214 (1,235), 251 (509), 272 (sh, 420), 313 (269), 500 (192)

UV λ_{max} nm ($E^{1\%}_{1cm}$).

Benanomicinone (**3**), the aglycone, was obtained by acid hydrolyses of **1** and **2**. In the ^1H NMR spectrum of **3**, a methine proton at 4.44 ppm (2′-H) was coupled to methyl protons at 1.36 ppm (3′-H) and a substituted amide proton at 8.47 ppm (2′-NH). This sequence was assigned to D-alanine which was obtained by vigorous acid hydrolyses of **1** and **2**, A *meta*-coupling between aromatic protons 10-H (6.82 ppm) and 12-H (7.15 ppm) was also observed.

The structures of **3** and its methyl ester (**4**) were determined by the aid of ^1H-^{13}C COSY, long range ^1H-^{13}C COSY, a heteronuclear multiple bond correlation spectrum[9] (HMBC) and long range selective proton decoupling (LSPD) experiments. The NMR spectra of **3** and **4** closely resembled each other. As shown in Fig. 1, the aromatic proton 10-H of **3** was coupled to four carbons C-8a (109.9 ppm), C-9 (164.6 ppm), C-11 (165.8 ppm) and C-12 (107.4 ppm), and the *meta*-coupled proton 12-H was coupled to five carbons C-8a, C-10 (106.8 ppm), C-11, C-12a (134.1 ppm) and C-13 (187.3 ppm as a quinone carbonyl carbon). The C-8a and C-11 were further coupled to a hydrogen bonded phenolic hydroxyl proton at 12.76 ppm (9-OH) and methoxyl protons at 3.90 ppm (11-OCH_3), respectively. The 9-OH was also coupled to C-9 and C-10. It was deduced that another quinone carbonyl carbon at 184.9 ppm (C-8) was located at the *peri* position to C-9 because of the presence of a hydrogen bond to 9-OH. This was confirmed by a W-type coupling between C-8 and 12-H. The most low-field aromatic proton at 8.08 ppm (7-H) was strongly coupled to C-8 and weakly coupled to C-13 by a W-type coupling. Consequently, the 7-H was located at the *peri* position to C-8 and was further coupled to three carbons C-6 (72.3 ppm), C-13a (115.2 ppm) and C-14a (125.8 ppm). The remaining aromatic proton at 7.08 ppm (4-H) was clearly coupled to C-2 (127.0 ppm), C-5 (71.3 ppm), C-14b (113.6 ppm) and aromatic methyl carbon C-16 (19.1 ppm). The strong correlations between 16-H (2.36 ppm) and three carbons C-2, C-3 (137.4 ppm) and C-4 (117.5 ppm) were observed in the HMBC, but no correlation between 16-H and C-4 was observed in the long range ^1H-^{13}C COSY because of the broadened signal of C-4. Furthermore, weak W-type couplings were observed between 4-H and C-15 (167.1 ppm as a substituted amide carbon), and between 16-H and C-1 (150.8 ppm). The above-mentioned results indicated that the methyl group, substituted amide group and phenolic

Fig. 1. Proton-carbon correlation map of benanomicinone (**3**) by long range ^1H-^{13}C COSY, HMBC and LSPD experiments.



Solid arrows indicate the remarkable coupling and the values in the parentheses represent the coupling constants (Hz). Dotted arrows indicate the low coupling constant (J=*ca.* 1 Hz) and/or positive NOE.

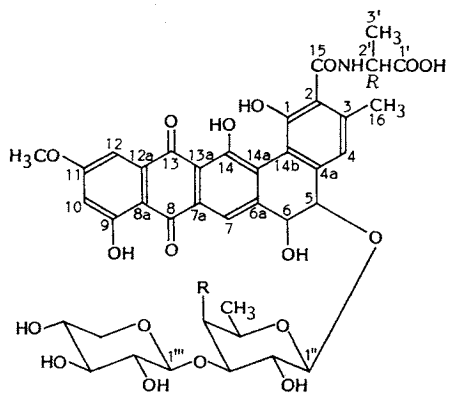
hydroxyl group were located at the *ortho*, *meta* and *para* positions to C-4, respectively. From these results, the sequence of C-1 to C-14 was established, and finally, the structure of **3** was given by connecting the remaining bond between C-14a and C-14b as shown in Fig. 1.

The position of attachment of the disaccharide moiety to **3** was determined to be at C-5 by observation of an NOE between 1″-H and 5-H in the ^{1}H-^{1}H NOESY of **1**. The stereochemistry of the C-5 and C-6 positions of **1** remains undefined. Therefore, the structure of **1** was as shown in Fig. 2.

Acid hydrolysis of **2** gave **3**, dexylosylbenanomicin B (**5**) and D-alanine, and methanolysis of **2** gave **4**, dexylosylbenanomicin B methyl ester (**6**) and methyl xylopyranoside. As stated above, **2** and **1** might differ only in the structure of the first sugar attached to the aglycone. Furthermore, comparison of the molecular formula, ^{1}H and ^{13}C NMR spectra of **2** with those of **1**, indicated that the C-3″ position of **2** was substituted by an amino group instead of a hydroxyl group as in **1**. The treatment of **2** with 70% aqueous trifluoroacetic acid followed by methylation and then acetylation afforded methyl 4-acetamido-2,3-di-*O*-acetyl-4-deoxy-*α*-D-fucopyranoside.[10] In the ^{13}C NMR spectrum of **2**, the C-3″ appeared at 7.6 ppm lower field than that of **5** so that xylopyranose was bonded at C-3″ position. The attached positions of 4-amino-4-deoxy-D-fucopyranose (thomosamine[11]) and xylopyranose were determined to be at C-5 and

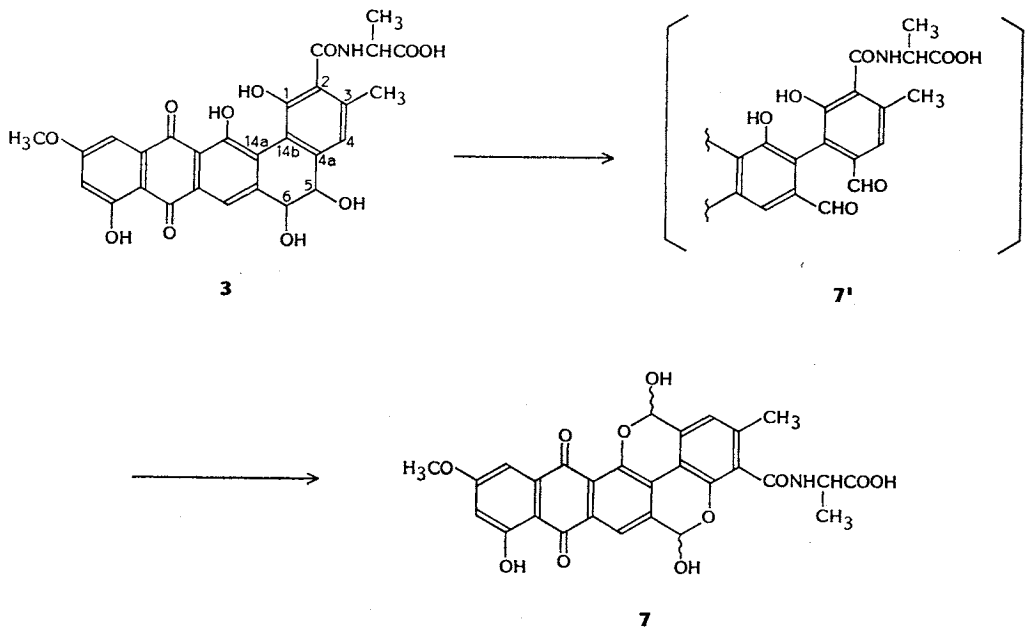
Fig. 2. Structures of benanomicins A and B.

The carbon numbers apply to the assignments of NMR.



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

Fig. 3. Oxidation of benanomicinone (**3**) with $NaIO_4$.



3 **7'** **7**

C-3″, respectively, by the observation of NOE's between 1″-H and 5-H, and between 1‴-H and 3″-H in the NOESY of **2**. From these results, the structure of **2** was as shown in Fig. 2.

In order to support the structure of **3**, an oxidative rearrangement reaction was carried out as follows. Treatment of **3** with $NaIO_4$ in alkaline water gave a dihemiacetal (**7**) in 79% yield. This reaction was assumed to progress through the dialdehyde **7′** as shown in Fig. 3. Accordingly, the structure of **3** was confirmed to be as shown in Fig. 1.

In FD-MS or SI-MS of benanomicins and their derivatives, the intensities of M^+, MH^+ and $(M+2)^+$ ion peaks altered remarkably under measurement conditions. The $(M+2)^+$ ion peak may originate from a hydroquinone form. This phenomenon has been observed with the ubiquinone group.[12]

Experimental

General

UV and IR spectra were recorded on a Shimadzu UV-260 spectrophotometer and a Hitachi 260-10 IR spectrophotometer, respectively. MS were measured on a Hitachi M-80B mass spectrometer. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. MP's were determined with a Yanaco MP-S3 micro melting point apparatus and were uncorrected. ^1H and ^{13}C NMR spectra were recorded on Jeol JNM-GX400 and Jeol JNM-GSX400 spectrometers.

Mild Acid Hydrolysis of Benanomicin A (1)

A suspension of **1** (50.1 mg) in 0.1 N HCl (50 ml) was heated at 80°C for 18 hours. After cooling, the reaction mixture was filtered. The precipitate obtained, was washed with water and dried under reduced pressure at 50°C for 24 hours to give benanomicinone (**3**) [25.7 mg, 77.3%; mp >220°C; $C_{28}H_{23}NO_{11}$, FD-MS *m/z* 549 (M^+); IR (KBr) cm^{-1} 3380, 1720, 1620 (sh), 1600] as a dark red powder. The filtrate was concentrated to dryness and the residue was purified by charcoal column chromatography (3 ml) developed with water as the eluant to afford a mixture of fucose and xylose (5.2 mg).

Acid hydrolysis of **1** (500 mg) with 6 N HCl (100 mg) at 100°C for 10 hours gave **3** (302 mg, 91.0%).

Vigorous Acid Hydrolysis of 1

A suspension of **1** (104 mg) in a mixture of concd HCl and acetic acid (1 : 1, 10 ml) was heated at 105°C for 15 hours and then evaporated to dryness. The residue was washed with water (20 ml) and filtered. The filtrate was concentrated to 1 ml and charged on a column of Diaion PK-208 (H^+, 5 ml) which was washed with water (20 ml) and developed with 0.5 N NH_4OH. The ninhydrin-positive fractions were combined and evaporated to afford a partially racemized D-alanine [1.1 mg, $[\alpha]_D^{22}$ −8.2° (*c* 0.11, 1 N HCl)].

Methanolysis of 1

A suspension of **1** (200 mg) in 1 N HCl - MeOH (60 ml) was refluxed for 15 hours. After cooling below 5°C, the reaction mixture was filtered and a precipitate was washed with chilled MeOH. The precipitate was dried at 50°C under reduced pressure yielding benanomicinone methyl ester [**4**: 111 mg, 81.5%; mp >220°C; *Anal* calcd for $C_{29}H_{25}NO_{11}$: C 61.81, H 4.47, N 2.49, found: C 61.49, H 4.34, N 2.41; FD-MS *m/z* 563 (M^+); IR (KBr) cm^{-1} 3480, 1730, 1635, 1600] as a dark red powder. The filtrate was concentrated to dryness to give a crude oil. The crude oil was further purified by silica gel column chromatography (5 g, $CHCl_3$ - MeOH, 10 : 1) to afford **4** (21.0 mg, 15.4%) and a mixture of methyl glycosides (28.7 mg). The methyl glycosides was treated with acetic anhydride (0.1 ml) in pyridine (2 ml) for 15 hours at room temp. To the reaction mixture was added MeOH (0.5 ml) and the mixture was stirred for 30 minutes and then evaporated to dryness. The residual oil was purified by preparative TLC (hexane - acetone, 3 : 1) to give methyl 2,3,4-tri-*O*-acetyl-α-D-fucopyranoside (13.2 mg, 18.0% from **1**), methyl 2,3,4-tri-*O*-acetyl-α-D-xylopyranoside (14.5 mg, 20.7% from **1**) and a mixture of their β-anomers (16.2 mg).

Methyl 2,3,4-Tri-*O*-acetyl-α-D-fucopyranoside: $[\alpha]_D^{22}$ +146° (*c* 1.0, $CHCl_3$); FD-MS *m/z* 305

(MH^+); ^1H NMR ($CDCl_3$) δ 4.94 (d, J=3.6 Hz, 1-H), 3.40 (s, 1-OCH_3), 5.15 (dd, J=11.0 and 3.6 Hz, 2-H), 5.36 (dd, J=11.0 and 3.3 Hz, 3-H), 5.30 (dd, J=3.3 and 1.0 Hz, 4-H), 4.13 (dq, J=6.7 and 1.0 Hz, 5-H), 1.16 (d, J=6.7 Hz, 6-H), 1.99, 2.09 and 2.17 (2, 3 and 4-OAc).

Methyl 2,3,4-Tri-*O*-acetyl-α-D-xylopyranoside: $[\alpha]_D^{22}$ +121° (*c* 1.0, $CHCl_3$); FD-MS *m/z* 291 (MH^+); ^1H NMR ($CDCl_3$) δ 4.88 (d, J=3.6 Hz, 1-H), 3.40 (s, 1-OCH_3), 4.84 (dd, J=10.3 and 3.6 Hz, 2-H), 5.48 (dd, J=10.3 and 9.5 Hz, 3-H), 4.97 (ddd, J=10.5, 9.5 and 5.9 Hz, 4-H), 3.59 (dd, J=11.0 and 10.5 Hz, 5-H_{ax}), 3.80 (dd, J=11.0 and 5.9 Hz, 5-H_{eq}), 2.03×2 and 2.08 (2, 3 and 4-OAc).

Deacetylation of Triacetates

To a solution of methyl 2,3,4-tri-*O*-acetyl-α-D-fucopyranoside (10.6 mg) in methanol (1 ml) was added 1 N NaOH - MeOH (0.2 ml) and the mixture was stirred for 5 minutes at room temp. After neutralization with dry ice, the mixture was concentrated to dryness and extracted with acetone. The extract was concentrated and the residue was chromatographed on a silica gel (1 g) column using $CHCl_3$ - MeOH (10:1) as a developing solvent to give methyl α-D-fucopyranoside [5.7 mg, 91.8%; $[\alpha]_D^{22}$ +187° (*c* 0.57, H_2O); FD-MS *m/z* 179 (MH^+); ^1H NMR (D_2O) δ 4.79 (d, J=3.6 Hz, 1-H), 3.42 (s, 1-OCH_3), 3.78~3.85 (m, 2, 3 and 4-H), 4.06 (br q, J=6.7 and <1 Hz, 5-H), 1.24 (d, J=6.7 Hz, 6-H)]. ^{13}C NMR data are shown in Table 4.

Treatment of methyl 2,3,4-tri-*O*-acetyl-α-D-xylopyranoside with the same manner mentioned above gave methyl α-D-xylopyranoside [5.4 mg, 88.4%; $[\alpha]_D^{22}$ +145° (*c* 0.54, H_2O); FD-MS *m/z* 165 (MH^+); ^1H NMR (D_2O) δ 4.82 (br d, J=3.6 Hz, 1-H), 3.45 (br s, 1-OCH_3), 3.47~3.85 (m, 2, 3, and 4-H, 5-H_{ax} and 5-H_{eq})]. ^{13}C NMR data are shown in Table 4.

Conversion of **4** into **3**

Compound **4** was treated with 0.1 N NaOH (5 ml) for 5 minutes at room temp and then acidified by using 1 N HCl (1 ml). The resulting precipitate was removed by filtration and washed with water and dried under reduced pressure to afford **3** (9.7 mg, 88.8%).

Acid Hydrolysis of Benanomicin B (**2**)

To a solution of **2** hydrochloride (130 mg) in water (10 ml) was added concd HCl (10 ml) and the suspension was heated at 110°C for 12 hours. After cooling, the mixture was filtered. The precipitate so obtained was purified by preparative TLC (butanol - acetic acid - pyridine - water, 6:1:4:3) and then chromatographed on a column of Diaion HP-20 using acidic MeOH as the eluant to give **3** (57.0 mg, 68.9%) and **5** hydrochloride [17.8 mg, 16.2%; mp >180°C (partially dec); $[\alpha]_D^{24}$ +396° (*c* 0.05, 0.05 N HCl); $C_{34}H_{34}N_2O_{14}$, FD-MS *m/z* 696 $(M+2)^+$; IR (KBr) cm^{-1} 3400, 1730, 1610]. The filtrate was concentrated to dryness and the residue was dissolved in water (1 ml) and charged on a column of Diaion PK-208 (H^+, 5 ml). The column was washed with water and developed with 0.5 N NH_4OH. The ninhydrin-positive fractions were combined and passed through a charcoal column. The effluent was concentrated to afford D-alanine [2.0 mg, $[\alpha]_D^{25}$ −12.0° (*c* 0.2, 1 N HCl)].

Thomosamine and Its Derivative

A solution of **2** (45.0 mg) in 70% aqueous trifluoroacetic acid (5 ml) was heated at 90°C for 12 hours and then concentrated to dryness. The residue was dissolved in water (5 ml) and applied on a column of Diaion HP-20 (15 ml) which was washed with water and developed with MeOH. The eluate containing **3** and **5** was further purified by preparative TLC (butanol - acetic acid - pyridine - water, 6:1:4:3) followed by a column of Sephadex LH-20 (50 ml) to give **3** (4.3 mg, 15.0%) and **5** (20.5 mg, 53.8%). The effluent and washing water were pooled, concentrated to a small volume, and subjected to a column of Amberlite CG-50 (NH_4^+ - H^+, 7:3, 5 ml) which was washed with water and eluted with 0.5 N NH_4OH. The ninhydrin-positive fractions were combined and evaporated to dryness to give thomosamine (1.5 mg). A solution of this sugar in 5% HCl - MeOH (1 ml) was refluxed for 30 minutes and concentrated to dryness. Treatment of the residue with acetic anhydride (0.1 ml) in pyridine (1 ml) at 37°C for 3 hours followed by silica gel column chromatography afforded methyl 4-acetamido-2,3-di-*O*-acetyl-4-deoxy-α-D-fucopyranoside[9] [1.1 mg, $[\alpha]_D^{22}$ +78° (*c* 0.11, $CHCl_3$); FD-MS 304 (MH^+); ^1H NMR ($CDCl_3$) δ 4.91 (m, 1-H), 3.38 (s, 1-OCH_3), 4.91 (m, 2-H), 5.26 (m, 3-H), 4.52 (ddd, J=9.7, 4.1 and 1.5 Hz, 4-H), 5.65 (d, J=9.7 Hz, 4-NH), 4.21 (dq, J=6.4 and 1.5 Hz, 5-H),

1.16 (d, J=6.4 Hz, 6-H), 1.99, 2.07 and 2.10 (2 and 3-OAc, 4-NAc).]

Methanolysis of **2**

A suspension of **2** hydrochloride (217 mg) in 1 N HCl - MeOH (40 ml) was heated under reflux for 12 hours and then concentrated to dryness. The residue was dissolved in water (30 ml) and applied to a column of Diaion HP-20 (100 ml) which was washed with water and eluted with MeOH. The eluate was concentrated to dryness and the residue was chromatographed on a silica gel column (5 g) using $CHCl_3$ - MeOH (10:1) and MeOH as developing solvents to give **4** (15.3 mg, 10.8%) and **6** hydrochloride [146 mg, 78.0%; mp >180°C (partially dec); $[\alpha]_D^{24}$ +316° (c 0.05, 0.05 N HCl); $C_{35}H_{38}N_2O_{14}$, FD-MS m/z 708 (M^+), 709 (MH^+), SI-MS m/z 710 $(M+2)^+$; IR (KBr) cm^{-1} 3400, 1730, 1610]. The effluent and washing of Diaion HP-20 column were concentrated to about 2 ml and subjected to a column of Amberlite CG-50 (NH_4^+ - H^+, 7:3, 5 ml) which was washed with water and eluted with 0.5 N NH_4OH. The ninhydrin-positive eluate was concentrated to dryness to afford an anomeric mixture (α:β, 5:1) of methyl 4-amino-4-deoxy-D-fucopyranoside (1.8 mg, 4.0%). The effluent and washing of Amberlite CG-50 column were pooled and concentrated to dryness to afford an anomeric mixture of methyl xylopyranoside (35.4 mg) which was acetylated with acetic anhydride (0.2 ml) in pyridine (2 ml) yielding a mixture of triacetates. The mixture was purified by preparative TLC (hexane - acetone, 3:1) to give methyl α-D-xylopyranoside (39.2 mg, 53.7% from **2**) and methyl β-D-xylopyranoside (17.1 mg, 23.4% from **2**).

Conversion of **6** into **5**

To a solution of **6** hydrochloride (125 mg) in water (20 ml) was added 1 N NaOH (5 ml) and the reaction mixture was stirred for 10 minutes at room temp. The mixture was acidified with 1 N HCl (7 ml) and concentrated to dryness. The residue was dissolved in water (20 ml) and charged to a column of Diaion HP-20 (100 ml) which was washed with water and eluted with MeOH. The eluate containing **5** was concentrated to a small volume and applied to a column of Sephadex LH-20 which was developed with MeOH. The fractions containing **5** were concentrated to dryness. The residue was dissolved in water, adjusted to pH 2 with 1 N HCl, and concentrated to dryness to give **5** hydrochloride (110 mg, 89.3%).

Oxidation of **3** with $NaIO_4$

To a solution of **3** (15.0 mg) in 0.02 N NaOH (10 ml) was added $NaIO_4$ (41 mg). After stirring for 10 minutes, the mixture was adjusted to pH 3 with 0.1 HCl and extracted with ethyl acetate (50 ml ×6). The extract was chromatographed on a silica gel column (2 g, $CHCl_3$ - MeOH, 2:1) yielding diacetal **7** [11.8 mg, 79.0%; FD-MS 549 $(M+2)^+$].

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