AMINO ACID ANALOGS OF BENANOMICIN A THROUGH DESALANINEBENANOMICIN A

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Desalaninebenanomicin A has been synthesized in good yield by the cleavage of the amido bond of benanomicin A using MEERWEIN's reagent. This is a useful intermediate to prepare amino acid analogs of benanomicin A. MEERWEIN's reagent reacts with totally protected benanomicin A to give a stable imino ether. After deprotection, the imino ether is treated with aqueous acetone at reflux to afford a methyl ester of desalaninebenanomicin A. Desalaninebenanomicin A was coupled with a variety of amino acids by the active ester method to afford new benanomicin analogs.

Benanomicin A (**1**), isolated from the culture broth of *Actinomadura* sp. MH193-16F4,[1] exhibits excellent therapeutic effects against systemic fungal infections in mice and inhibits the infection of T-cell with human immunodeficiency virus and the syncytium formation by the virus.[2,3] Antibiotic **1** consists of a benzo[*a*]naphthacenedione, D-alanine and 3-*O*-(D-xylopyranosyl)-D-fucopyranose.[4] Pradimicin antibiotics produced by *Actinomadura hibisca* P157-2 are included in this group.[5]

Among naturally occurring benanomicins, two amino acid analogs, 2'-demethylbenanomicin A (glycine analog)[6] and 2'-hydroxybenanomicin A (D-serine analog),[7] show comparable antifungal activities to **1**. SAWADA and coworkers also isolated 2'-hydroxypradimicins (pradimicins FA-1 and FA-2) by the addition of D-serine to the culture medium.[8]

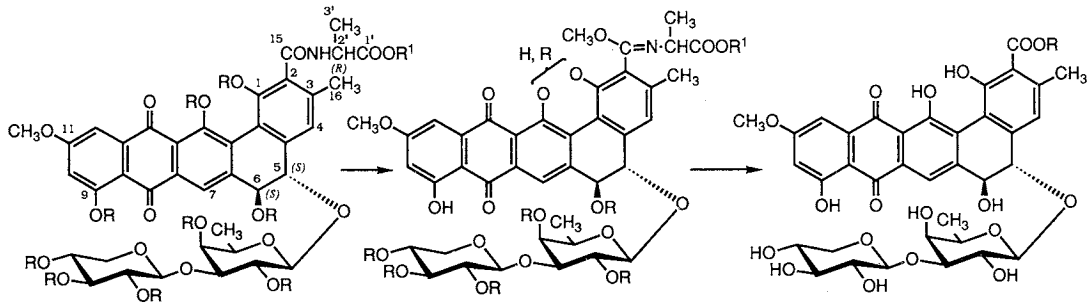
In our studies on benanomicins, our attention was focused on the relationship between the structure of the amino acid moiety and the antifungal activity. Herein, we wish to report the chemical transformation of **1** into new amino acid analogs.

Chemistry

Our initial efforts were directed toward preparing desalaninebenanomicin A (**6**). On acid hydrolysis of **1**, the cleavage of glycosidic bonds preceded the amido cleavage.[4] Alkaline hydrolysis of **1** gave no desired product. The amido bond was extremely stable under the alkaline condition. Treatment of **1** with diazodiphenylmethane in a mixture of *N*,*N*-dimethylformamide and methanol, followed by the acetylation with acetic anhydride in pyridine afforded nona-*O*-acetyl-diphenylmethyl ester **2** in a good yield. An excess of MEERWEIN's reagent (trimethyloxonium tetrafluoroborate is preferable to triethyloxonium tetrafluoroborate for this reaction) smoothly reacted with **2** in dry dichloromethane to yield a stable methyl imino ether-diphenylmethyl ester **3a** along with methyl ester **3b** and carboxylic acid **3c**. ^{1}H NMR spectrum of **3a** showed the presence of a new methoxy group. Alkaline hydrolysis of a mixture of **3a**~**3c** with 1 M sodium hydroxide at room temperature, followed by the acidification gave an orange powder of benanomicin A methyl imino ether (**4**). On refluxing **4** in aqueous acetone for 3 hours, desalaninebenanomicin A methyl ester (**5**) was obtained in 44% yield from **1**. By the hydrolysis with hydrochloric acid, imino ether **4** reverted to amido **1**. ^{1}H NMR spectrum of **5** revealed the presence of an aromatic methyl ester at δ 3.82. The

negative FAB-MS of **5** showed a parent peak at *m/z* 770. The methyl ester **5** was hydrolyzed with 2 M sodium hydroxide at 70°C gave **6**, which was an important key intermediate for the preparation of amino acid analogs of **1**. (Scheme 1)

Scheme 1. Synthesis of desalaninebenanomicin A.

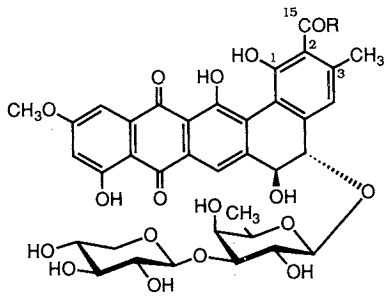


	R	R_1
Benanomicin A (**1**)	H	H
2	Ac	$CHPh_2$

	R	R_1
3a	Ac	$CHPh_2$
3b	Ac	CH_3
3c	Ac	H
4	H	H

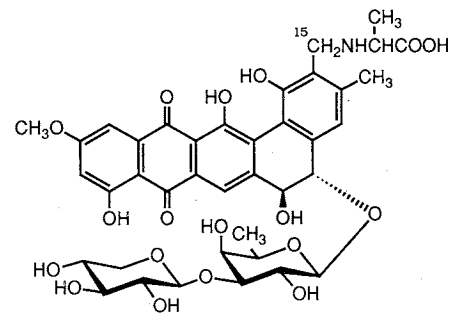
	R
5	CH_3
Desalaninebenanomicin A (**6**)	H

Fig. 1. Amino acid analogs of benanomicin A.



	R
8a	L-Ala
8b	D-Thr
8c	D-aThr
8d	L-Thr
8e	L-aThr
8f	D-Asp
8g	$NHCHCO_2H$ (D-Butyrine) CH_2CH_3
8h	D-Phe
8i	D-Ser
8j	$NHCH(CO_2H)_2$ (Aminomalonic acid)
8k	$NHCH_2CO_2H$ (Glycine)
8l	$NHCH_2CH_2CO_2H$ (β-Alanine)
8m	$NHCH_2CH_2CH_2CO_2H$ (γ-Aminobutyric acid)
8n	$N(CH_3)CH_2CO_2H$ (Sarcosine)
8o	$N(CH_2CO_2H)_2$ (Iminodiacetic acid)
8p	$NHCH_2SO_3H$ (Aminomethanesulfonic acid)

aThr = allothreonine



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After total *O*-acetylation of **6**, nonaacetate **7** was coupled with a variety of amino acids by the active ester method using i) *N*-hydroxybenzotriazole (HOBT) and *N*,*N*'-dicyclohexylcarbodiimide (DCC), ii) *p*-hydroxyphenyldimethylsulfonium methylsulfate and DCC or iii) HOBT and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. The protected new benanomicin analogs were treated with 0.2 M potassium carbonate and chromatographed on silica gel to give new benanomicin analogs **8**. (Fig. 1)

The reduction of **4** with sodium borohydride in an aqueous ethanol gave 15-deoxobenanomicin A (**9**). ^{1}H NMR of **9** hydrochloride in DMSO-d_6 revealed a singlet methylene signal at δ 4.22. In ^{13}C NMR, a new methylene carbon instead of the amido carbon at C-15 position was observed at δ 43.3.

In Vitro Antifungal Activity

Minimum inhibitory concentrations (MICs) of new benanomicin analogs, **8a**, **8b**, **8c**, **8d**, **8e**, **8f**, **8g**, **8j** and **8l** against various fungi are summarized in Table 1. The D-amino acid analogs retain the antifungal activity. The L-amino acid analogs, **8a**, **8d** and **8e**, are essentially devoid of antifungal activity. D-Allothreonine (D-aThr) and D-butyrine analogs, **8c** and **8g** respectively, are slightly less active than **1**. Interestingly, **8c** is superior to D-threonine analog, **8b**. However, D-phenylalanine analog, **8h**, markedly decreases the antifungal activity. Aminomalonic acid analog, **8j**, which has no asymmetric center in the amino acid moiety is weaker than **1**. When the distance between the amino group and carboxyl group in the amino acid moiety is increased, in the cases of β-alanine **8l** and γ-aminobutyric acid **8m**, the antifungal activity falls. *N*-Substituted analogs, **8n** and **8o**, and sulfonic acid analog, **8p**, in place of carboxylic acid, have no antifungal activity. The reduction of amido carbonyl at the C-15 position leads to a completely

Table 1. Antifungal activity of benanomicin A (**1**) and amino acid analogs.

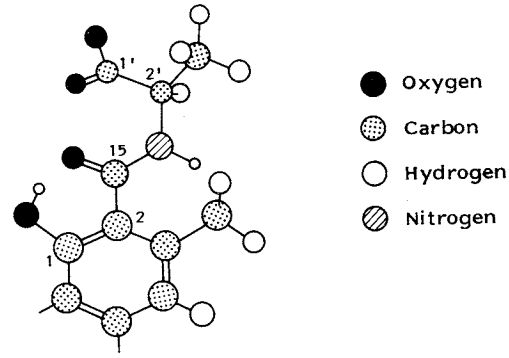
Microorganism	MIC (μg/ml)				
	1	**8a**	**8b**	**8c**	**8d**
Candida albicans 3147	6.25	>100	25	12.5	>100
C. tropicalis F-1	6.25	>100	25	12.5	>100
C. pseudotropicalis F-2	3.13	25	6.25	3.13	25
C. krusei F-5	3.13	>100	12.5	6.25	>100
Candida sp. YU-1200	6.25	>100	12.5	12.5	>100
Saccharomyces cerevisiae F-7	3.13	25	12.5	6.25	50
Cryptococcus neoformans F-10	1.56	>100	12.5	3.13	>100
Aspergillus niger F-16	12.5	>100	>100	>100	>100
Trichophyton asteroides 429	50	>100	>100	>100	>100
T. mentagrophytes F-15 (833)	50	>100	>100	>100	>100

Microorganism	MIC (μg/ml)				
	8e	**8f**	**8g**	**8j**	**8l**
Candida albicans 3147	>100	50	12.5	25	>100
C. tropicalis F-1	>100	50	12.5	25	>50
C. pseudotropicalis F-2	25	12.5	3.13	6.25	>50
C. krusei F-5	>100	50	12.5	25	>100
Candida sp. YU-1200	>100	50	12.5	25	>100
Saccharomyces cerevisiae F-7	25	12.5	3.13	6.25	>50
Cryptococcus neoformans F-10	>100	50	6.25	25	>50
Aspergillus niger F-16	>100	>100	100	>100	>100
Trichophyton asteroides 429	>100	>100	>100	>100	>50
T. mentagrophytes F-15 (833)	>100	>100	>100	>100	>50

inactive alkyl amino analog **9**. Methyl imino ether **4**, which has a D-alanine residue similar to **9**, shows no antifungal activity.

We have synthesized a number of benanomicin analogs through an important intermediate **6** and evaluated their antifungal activities *in vitro*. The present study provides the first chemical transformation of the amino acid moiety of **1**, which can be used in further derivatization. The antifungal activity of benanomicins entirely depends on the structure of the amino acid side chain. The structural requirements of the side chain at the C-15 position for the antifungal activity are as follows: 1) An amido bond at C-15, 2) α-amino acid, 3) *R*-configuration of the amino acid where the asymmetric carbon exists and 4) sterically unhindered substituent in the side chain. Antibiotic **1** binds to *Candida albicans* cells in the presence of calcium ion[9] and biologically inactive analogs show the negligible binding.[7] These structural features necessary for the amino acid might control the binding to fungal cell surface.

Fig. 2. Presumed conformation of the amino acid moiety which is active against fungi.



From results mentioned above, we presume that the steric and electrostatic interactions among the phenolic hydroxyl at C-1 position, carbonyl in amido and terminal carboxyl group fix the conformation of the amino acid residue and the conformation plays an important role for the binding to fungi and the occurrence of the antifungal activity of **1**. (Fig. 2)

Experimental

General

Mass spectra (FAB-MS) were measured on a Jeol JMX-SX102 mass spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Jeol JNM-GX400 spectrometer at 50°C unless otherwise noted.

Nona-*O*-acetylbenanomicin A Diphenylmethyl Ester (**2**)

To a solution of **1** (3.0 g) in a mixture of DMF (50 ml) and MeOH (50 ml) was added diazodiphenylmethane (1.1 g). After stirring overnight, the solution was concentrated to half volume. To this was added water (200 ml). The dark red precipitates were collected by filtration and washed with ethyl acetate (200 ml) and water (200 ml). Diphenylmethyl ester of **1** (3.0 g) was obtained in 83% yield. Negative FAB-MS *m/z* 993 (M^-). This ester (3.0 g) was acetylated with acetic anhydride (6 ml) in pyridine (120 ml) at 50°C for 3 hours. After concentration, the obtained syrup was treated with water (100 ml) to give yellow solid, which was washed with water. Compound **2** (4.1 g) was obtained as an yellow powder quantitatively. Negative FAB-MS *m/z* 1,370 ($(M-H)^-$).

Hepta-*O*-acetylbenanomicin Methyl Imino Ethers (**3a**～**3c**)

To a solution of **2** (4.2 g) in anhydr dichloromethane (80 ml) was added trimethyloxonium tetrafluoroborate (2.3 g). After stirring overnight at room temperature, a satd aq solution of $NaHCO_3$ (100 ml) was added. After 30 minutes, the organic layer was washed by 5% aq citric acid solution and water, dried over anhydr $MgSO_4$ and concentrated under reduced pressure to give a mixture of hepta-*O*-acetyl-imino ethers (**3a**～**3c**). For the chemical and biological analysis, a part of the mixture was chromatographed on silica gel with hexane-ethyl acetate (2:3) to give diphenylmethyl ester **3a**, methyl ester **3b** and carboxylic acid **3c** in 3:1:5 ratio. **3a**: Negative FAB-MS *m/z* 1,300 ($(M-H)^-$). ^1H NMR ($CDCl_3$, ambient temperature) δ 3.87 and 3.91 (OCH_3), 3.94 and 3.95 (OCH_3), 6.73 ($CH(C_6H_5)_2$) and 7.30～7.33 ($CH(C_6H_5)_2$).

3b: Negative FAB-MS *m/z* 1,148 ($(M-H)^-$). ^1H NMR ($CDCl_3$, ambient temperature) δ 3.70 (CO_2CH_3), 3.85 and 3.93 (OCH_3) and 3.95 (OCH_3). **3c**: Negative FAB-MS *m/z* 1,134 ($(M-H)^-$). ^1H NMR ($CDCl_3$, ambient temperature) δ 3.79 (OCH_3) and 3.98 (OCH_3). These NMR spectra showed that **3a**~**3c** contained rotamers.

Benanomicin A Methyl Imino Ether (**4**)

The mixture of acetyl-imino ethers (**3a**~**3b**) (3.9 g) was treated with 1 M NaOH (40 ml) in MeOH (40 ml) at room temperature overnight. After evaporation of MeOH, 1 M HCl was added to the solution to pH 1.6. An orange solid was precipitated, which was collected by centrifugation and washed with water and dried. Imino ether **4** (2.1 g) was obtained. This compound was used in the next reaction without further purification. Compound **4** was purified on silica gel column for preparing an analytical sample. Negative FAB-MS *m/z* 841 (M^-).

Desalaninebenanomicin A Methyl Ester (**5**)

A solution of **4** (2.0 g) in a mixture of acetone (60 ml) and water (30 ml) was refluxed for 3 hours. After evaporation of acetone, a red solid was precipitated. The solid was collected by centrifugation, dried and chromatographed on silica gel with chloroform - butanol - pyridine - water (40 : 20 : 25 : 3) to give desalanine-methyl ester **5** (1.36 g). Negative FAB-MS *m/z* 770 (M^-). ^1H NMR (DMSO-d_6) δ 3.82 (CO_2CH_3) and 3.94 (11-OCH_3). ^{13}C NMR δ 168.8 (CO_2CH_3) and 52.0 (CO_2CH_3).

Desalaninebenanomicin A (**6**)

Desalanine-methyl ester **5** (640 mg) was treated with 1.6 M NaOH in aq MeOH (64 ml) at 70°C for 24 hours. After acidification with 3 M HCl to pH 1.6 in an ice bath, the precipitates were collected by filtration. The obtained solid was chromatographed on silica gel with chloroform - butanol - pyridine - water (4 : 3 : 5 : 1) to give **6** as a pyridinium salt. The product was dissolved in water (50 ml), acidified with 1 M HCl to pH 1.6. The precipitates were collected by centrifugation to give **6** (430 mg). Negative FAB-MS *m/z* 756 (M^-). ^1H NMR (DMSO-d_6) δ 1.13 (d, J=6.4 Hz, 6″-H), 2.47 (16-H), 3.95 (11-OCH_3), 4.42 (d, J=7 Hz, 1‴-H), 4.53 (d, J=9 Hz, 5-H), 4.62 (d, J=9 Hz, 1′-H), 4.62 (d, J=9 Hz, 6-H), 6.90 (d, J=2 Hz, 10-H), 7.20 (4-H), 7.28 (d, J=2 Hz, 12-H), 8.05 (7-H) and 12.81 (9-OH). ^{13}C NMR δ 187.0 (C-13), 185.1 (C-8), 171.1 (C-15), 165.9 (C-11), 164.6 (C-9), 157.6 (C-14), 155.5 (C-1), 147.4 (C-6a), 140.6 (C-4a), 139.4 (C-3), 134.5 (C-12a), 131.4 (C-7a), 125.3 (C-2 and C-14a), 118.2 (C-4), 115.4 (C-13a), 114.8 (C-7), 114.2 (C-14b), 110.0 (C-8a), 107.3 (C-12), 106.6 (C-10), 105.0 (C-1‴), 104.2 (C-1′), 82.8 (C-3″), 81.3 (C-5), 75.9 (C-3‴), 73.5 (C-2‴), 71.6 (C-6), 70.3 (C-4″), 70.0 (C-5″), 69.9 (C-2″), 69.3 (C-4‴) 65.5 (C-5‴), 56.2 (11-OCH_3), 21.5 (C-16) and 16.2 (C-6″).

Nona-*O*-acetyldesalaninebenanomicin A (**7**)

Compound **6** (640 mg) was acetylated with acetic anhydride (5 ml) in pyridine (10 ml). After 3 hours at 70°C, the solution was concentrated to solid which was chromatographed on silica gel with chloroform - MeOH - water (1000 : 20 : 1) to give nonaacetate **7** (643 mg). Negative FAB-MS *m/z* 1,134 (M^-).

(2′*S*,3′*R*)-3′-Hydroxy-3′-methylbenanomicin A (**8d**) (L-Thr Analog)

To a solution of **7** (100 mg) and HOBT (74 mg) in acetonitrile (4 ml) were added a solution of L-threonine diphenylmethyl ester *p*-toluenesulfonate (168 mg) and *N*-methylmorpholine (60 μl) in acetonitrile (3 ml) and DCC (57 mg) at 4°C. After 4 hours at room temperature, acetic acid (0.2 ml) was added. Precipitates were filtered and the filtrate was concentrated to solid. The solid was dissolved in ethyl acetate (20 ml) and the solution was washed with 10% citric acid solution, satd $NaHCO_3$ solution and water, dried over anhydr $MgSO_4$ and evaporated to give the product (130 mg). The product was treated with 0.2 M aq K_2CO_3 in a mixture of MeOH (5 ml) and pyridine (3 ml) at 70°C overnight. After acidifying with 1 M HCl to pH 2.0, precipitates were collected by centrifugation and chromatographed on silica gel with chloroform - butanol - pyridine - water (40 : 20 : 25 : 3). The obtained pyridinium salt was dissolved in water and the pH of the solution was adjusted to pH 2.0 with 2 M HCl. Precipitates were collected by

centrifugation, washed with water and dried to give **8d** (12 mg). Negative FAB-MS *m/z* 857 (M^-). ^1H NMR (DMSO-d_6) δ 1.22 (3'-CH_3), 4.19 (3'-H), 4.44 (2'-H) and 7.76 (NH). ^{13}C NMR δ 171.7 (C-1'), 167.3 (C-15), 66.4 (C-3'), 58.0 (C-2') and 20.2 (3'-CH_3).

Benanomicin A and Amino Acid Analogs of Benanomicin A (**1**, **8a**~**8c**, **8e**~**8h**, **8j**~**8l**, **8n** and **8o**)

These compounds were prepared by a similar procedure used for **8d**.

Benanomicin A (**1**) (40 mg) was prepared from **7** (100 mg) and D-alanine benzyl ester *p*-toluenesulfonate (128 mg). The properties of synthetic **1** were identical with the natural antibiotic in all respects.[1,4]

2'-*Epi*-benanomicin A (**8a**, L-Ala analog) (41 mg) was prepared from **7** (100 mg) and L-alanine benzyl ester *p*-toluenesulfonate (129 mg). Negative FAB-MS *m/z* 827 (M^-). ^1H NMR (DMSO-d_6) δ 1.38 (3'-H), 4.46 (2'-H) and 8.42 (NH). ^{13}C NMR δ 173.8 (C-1'), 166.9 (C-15), 47.6 (C-2') and 16.8 (C-3').

(2'*R*,3'*S*)-3'-Hydroxy-3'-methylbenanomicin A (**8b**, D-Thr analog) (30 mg) was prepared from **7** (100 mg) and D-threonine diphenylmethyl ester *p*-toluenesulfonate (168 mg). Negative FAB-MS *m/z* 857 (M^-). ^1H NMR (DMSO-d_6) δ 1.20 (3'-CH_3), 4.18 (3'-H), 4.42 (2'-H) and 7.74 (NH). ^{13}C NMR δ 171.7 (C-1'), 167.2 (C-15), 66.4 (C-3'), 57.9 (C-2') and 20.2 (3'-CH_3).

(2'*R*,3'*R*)-3'-Hydroxy-3'-methylbenanomicin A (**8c**, D-aThr analog) (11 mg) was prepared from **7** (100 mg) and D-allothreonine diphenylmethyl ester *p*-toluenesulfonate (168 mg). Negative FAB-MS *m/z* 857 (M^-). ^1H NMR (DMSO-d_6) δ 1.20 (3'-CH_3), 3.96 (3'-H), 4.42 (2'-H) and 8.18 (NH). ^{13}C NMR δ 171.6 (C-1'), 167.2 (C-15), 66.6 (C-3'), 58.5 (C-2') and 19.9 (3'-CH_3).

(2'*S*,3'*S*)-3'-Hydroxy-3'-methylbenanomicin A (**8e**, L-aThr analog) (6.4 mg) was prepared from **7** (100 mg) and L-allothreonine diphenylmethyl ester *p*-toluenesulfonate (168 mg). Negative FAB-MS *m/z* 857 (M^-). ^1H NMR (DMSO-d_6) δ 1.22 (3'-CH_3), 4.00 (3'-H), 4.41 (2'-H) and 8.15 (NH). ^{13}C NMR δ 171.5 (C-1'), 167.0 (C-15), 66.6 (C-3'), 58.5 (C-2') and 19.2 (3'-CH_3).

3'-Carboxybenanomicin A (**8f**, D-Asp analog) (38 mg) was prepared from **7** (80 mg) and D-aspartic acid dibenzyl ester *p*-toluenesulfonate (142 mg). Negative FAB-MS *m/z* 871 (M^-). ^1H NMR (DMSO-d_6) δ 2.67 and 2.81 (3'-Ha and 3'-Hb), 4.78 (2'-H) and 8.39 (NH). ^{13}C NMR δ 172.0 and 171.4 (C-1' and 3'-CO_2H), 166.8 (C-15), 48.7 (C-2') and 35.8 (C-3').

(2'*R*)-3'-Methylbenanomicin A (**8g**, D-butyrine analog) (15 mg) was prepared from **7** (100 mg) and D-butyrine diphenylmethyl ester *p*-toluenesulfonate (168 mg). Negative FAB-MS *m/z* 841 (M^-). ^1H NMR (DMSO-d_6) δ 0.97 (3'-CH_3), 1.76 (3'-Ha and 3'-Hb), 4.34 (2'-H) and 8.30 (NH). ^{13}C NMR δ 173.2 (C-1'), 167.1 (C-15), 53.4 (C-2'), 24.0 (C-3') and 10.3 (3'-CH_3).

3'-Phenylbenanomicin A (**8h**, D-Phe analog) (19 mg) was prepared from **7** (80 mg) and D-phenylalanine benzyl ester *p*-toluenesulfonate (125 mg). Negative FAB-MS *m/z* 903 (M^-). ^1H NMR (DMSO-d_6) δ 3.2 and 3.8 (3'-Ha and 3'-Hb), 4.8 (2'-H), 7.25~7.45 (C_6H_5) and 8.57 (NH). ^{13}C NMR δ 172.7 (C-1'), 167.1 (C-15), 53.6 (C-2') and 36.3 (C-3').

2'-Carboxy-2'-demethylbenanomicin A (**8j**, aminomalonic acid analog) disodium salt (38 mg) was prepared from **7** (100 mg) and aminomalonic acid diethyl ester hydrochloride (77 mg). Negative FAB-MS *m/z* 901 ($(M+2Na-2H)^-$, disodium salt). ^1H NMR (D_2O, 70°C) δ 3.81 (2'-H). ^{13}C NMR δ 175.4 and 172.0 (each CO_2H) and 72.4 (C-2').

2'-Demethylbenanomicin A (**8k**, Gly analog) (46 mg) was prepared from **7** (100 mg) and glycine ethyl ester hydrochloride (66 mg). The properties of **8k** were identical with natural product in all respects.[6]

N-(2-Carboxyethyl)desalaninebenanomicin A-amide (**8l**, β-Ala analog) (41 mg) was prepared from **7** (80 mg) and β-alanine methyl ester trifluoroacetate (57 mg). Negative FAB-MS *m/z* 827 (M^-). ^1H NMR (DMSO-d_6) δ 2.52 (3'-H), 3.46 (3'-H) and 8.11 (NH). ^{13}C NMR δ 172.5 (C-1'), 167.1 (C-15), 35.0 (C-3') and 33.6 (C-2').

2'-Demethyl-*N*-methylbenanomicin A (**8n**, Sar analog) (19 mg) was prepared from **7** (80 mg) and sarcosine ethyl ester hydrochloride (45 mg). Negative FAB-MS *m/z* 827 (M^-). ^1H NMR (DMSO-d_6) δ 2.80 and 2.92 (N–CH_3, rotamer) and 3.81 and 3.87 (2'-H, rotamer). ^{13}C NMR δ 170.2 (C-1'), 168.3 (C-15), 47.9 and 47.7 (C-2', rotamer) and 36.0 and 35.9 (N–CH_3, rotamer).

N-Carboxymethyl-2'-demethylbenanomicin A (**8o**, iminodiacetic acid analog) (7 mg) was prepared from **7** (100 mg) and iminodiacetic acid bis(diphenylmethyl)ester *p*-toluenesulfonate (240 mg). Negative FAB-MS *m/z* 871 (M^-). ^1H NMR (disodium salt, D_2O, 70°C) δ 3.95 and 3.98 (3'-H, rotamer) and 4.22 and 4.23 (2'-H, rotamer). ^{13}C NMR δ 181.2, 177.2 and 176.9 (C-1', C-4' and C-15), 54.2 (C-2') and 50.9 (C-3').

2'-Hydroxybenanomicin A (**8i**, D-Ser Analog)

To a solution of **7** (104 mg) in acetonitrile (3 ml) were added *p*-hydroxyphenyldimethylsulfonium methylsulfate (25 mg) and DCC (19 mg) at 4°C. After stirring at room temperature overnight, precipitates were removed. The filtrate was concentrated to give a water soluble active ester. To a solution of the active ester in a mixture of acetonitrile (0.5 ml) and H_2O (1 ml) was added a solution of D-serine *N*,*N*-dicyclohexylamine salt (200 mg) in a mixture of acetonitrile (0.27 ml) and water (0.45 ml). After stirring for 3 days at room temperature, 1 M aq K_2CO_3 (4 ml) and MeOH (4 ml) were added to the reaction solution. Acidification of the solution with 1 M HCl to pH 1.6, followed by centrifugation afforded a red solid. The solid was chromatographed on silica gel with chloroform - butanol - pyridine - water (4 : 3 : 5 : 1) to give a product. The product was reprecipitated by the similar procedure to **8d** to give D-serine analog **8i** (15 mg). The properties of **8i** were identical with the natural antibiotic in all respects.[7]

N-(3-Carboxypropyl)desalaninebenanomicin A-amide (**8m**, GABA Analog)

γ-Aminobutyric acid analog **8m** (7 mg) was prepared from **7** (65 mg) and γ-aminobutyric acid tetra-butylammonium salt (70 mg) by the similar procedure to **8i**. Negative FAB-MS *m/z* 841 (M^-). ^1H NMR (DMSO-d_6) δ 1.76 (3'-H), 2.32 (2'-H), 3.25 (4'-H) and 8.10 (NH). ^{13}C NMR δ 173.9 (C-1'), 167.0 (C-15), 38.1 (C-4'), 31.0 (C-2') and 24.4 (C-3').

N-(Sulfomethyl)desalaninebenanomicin A-amide (**8p**, Aminomethanesulfonic Acid Analog)

Compound **8p** (10 mg) was prepared from **7** (100 mg) and aminomethanesulfonic acid tetra-butylammonium salt (129 mg) with HOBT (74 mg) and water soluble 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (53 mg) in DMF by the similar work up to **8i**. Negative FAB-MS *m/z* 849 (M^-). ^1H NMR (sodium salt, D_2O, 70°C) δ 4.63 (1'-H). ^{13}C NMR δ 173.2 (C-15) and 57.1 (C-1').

15-Deoxobenanomicin A (**9**)

To a solution of **4** sodium salt (73 mg) in aq EtOH (6 ml) was added a solution of $NaBH_4$ (21 mg) in EtOH (0.5 ml) at −30°C. After stirring for 4 hours at this temperature, the solution was concentrated to solid. The residue was chromatographed on silica gel with chloroform - butanol - pyridine - water (4 : 4 : 7 : 2) to give a product. The product was dissolved in water (10 ml). The solution was acidified with 2 M HCl to pH 2.0. Precipitates were collected by centrifugation to give **9** hydrochloride (8 mg). Negative FAB-MS *m/z* 813 (M^-). ^1H NMR (DMSO-d_6) δ 1.53 (3'-H), 4.00 (2'-H) and 4.22 (15-H). ^{13}C NMR δ 170.8 (C-1'), 55.9 (C-2'), 43.3 (C-15) and 16.2 (C-3').

Antifungal Activity

MICs on a glucose - nutrient agar were determined by the 2-fold agar dilution method after incubation at 27°C for 42 hours according to the method described in a preceding paper.[3]

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