NEW ANTIFUNGAL ANTIBIOTICS PRADIMICINS FA-1 AND FA-2: D-SERINE ANALOGS OF PRADIMICINS A AND C

YOSUKE SAWADA, MASAMI HATORI, HARUAKI YAMAMOTO, MAKI NISHIO, TAKEO MIYAKI and TOSHIKAZU OKI

Bristol-Myers Research Institute, Ltd., Tokyo Research Center, 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

(Received for publication May 31, 1990)

Pradimicin FA-1 was produced *via* directed biosynthesis with substitution of D-serine for D-alanine in the 15-position of pradimicin A. This substitution was achieved by the addition of D-serine to the culture medium of *Actinomadura hibisca* P157-2. Likewise, pradimicin FA-2 was co-produced along with pradimicin FA-1 when the pradimicins A and C producing strain, *A. hibisca* A2493 was grown in D-serine-supplemented medium. The new pradimicin analogs share a common core structure of 5,6-dihydrobenzo[*a*]naphthacenequinone substituted by D-serine at C-15, but differ in the disaccharide moiety at C-5. Pradimicin FA-1 has an *N*-methylamino sugar and D-xylose. Pradimicin FA-2 is the des-*N*-methyl analog of pradimicin FA-1. The *in vitro* and *in vivo* antifungal activity of the analogs was comparable to that of pradimicin A.

Pradimicins A, B and C[1~4] and benanomicins A and B[5,6] belong to a novel group of antifungal antibiotics possessing a core structure of a glycosylated dihydrobenzo[*a*]naphthacenequinone substituted by D-alanine at C-15. They are produced by *Actinomadura hibisca* P157-2 (ATCC 53557) and an actinomycete designated MH193-16F4, respectively. Recently, we reported that a mutant strain A2660 (ATCC 53762) of *A. hibisca* P157-2 produces pradimicins D and E, glycine analogs of pradimicins A and C, which showed *in vitro* and *in vivo* antifungal activity similar to pradimicin A.[7] However, the L-alanine isomer of pradimicin A was inactive against various yeasts and fungi.[8] Since the amino acid substitution in pradimicin appears to play an important role in expression of antifungal activity, we attempted the controlled, directed biosynthesis of new pradimicin analogs containing different amino acids.

This paper describes the production of pradimicins FA-1 and FA-2, D-serine analogs of pradimicins A and C, by the parent or a mutant strain of *A. hibisca* P157-2 in D-serine-supplemented medium and their *in vitro* and *in vivo* antifungal activities.

Materials and Methods

Producing Strains

A. hibisca P157-2 and mutant strain A2493 (ATCC 53815) obtained by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) treatment[7] were grown on modified BENNETT's agar medium composed of soluble starch 0.5%, glucose 0.5%, fish meat extract 0.1%, yeast extract 0.1%, NZ-case 0.2%, NaCl 0.2%, $CaCO_3$ 0.1% and agar 1.6%, pH 7.0, for 10 days at 28°C and maintained at 4°C.

Identification of the Products

For silica gel TLC, fermentation broth was adjusted to pH 2.0 with 6 N HCl and mixed with an equal volume of butanol. After centrifugation at 10,000 rpm for 10 minutes, 10 μl of the butanol extract was spotted on a silica gel TLC plate ($60F_{254}$, E. Merck, 0.25 mm thick) and developed in a solvent system of methyl acetate-propanol-28% ammonia (3:7:4). Rf: 0.45 for pradimicin A; 0.40 for pradimicin C,

0.33 for pradimicin FA-1; 0.29 for pradimicin FA-2. For HPLC, fermentation broth was acidified to pH 2.0 and centrifuged. The supernate was adjusted to pH 5.0 with 6 N NaOH and recentrifuged. The precipitate was dissolved in water at pH 3.5 and loaded on a Sep-Pak C18 cartridge filter (Millipore-Waters). Pradimicins were eluted with a 1:1 mixture of CH_3CN and 0.15% KH_2PO_4, pH 3.5. The filtrate was mixed with an equal volume of DMSO and passed through an HV filter (Millipore-Waters, 0.45 μm). HPLC analysis conditions were as follows: Waters M600; column YMC A-301-3, 4.6 mm i.d. × 100 mm, 3 μm, Yamamura Chemical Lab.; eluant CH_3CN - 0.15% KH_2PO_4, pH 3.5 (1:3); flow rate 0.8 ml/minute; UV detection at 254 nm. Rt (minutes): 19.11 for pradimicin A; 16.73 for pradimicin C, 8.61 for pradimicin FA-1; 7.65 for pradimicin FA-2.

Fermentation

Parent strain P157-2 or mutant strain A2493 was grown in a 500-ml Erlenmeyer flask in medium A, consisting of glucose 3%, soybean meal 3%, Pharmamedia 0.5%, yeast extract 0.1% and $CaCO_3$ 0.3% (pH 7.0), at 28°C for 6 days at 200 rpm on a rotary shaker. The seed culture, 5 ml, was transferred into 100 ml of fresh medium A in a 500-ml Erlenmeyer flask. The amino acid supplement (250 mg for 0.25%) was autoclaved separately in the solid state, dissolved in 1.0 ml of sterile water, and added to the fermentation. After rotary shaking at 28°C for 7 days at 200 rpm, the supernate was diluted with a 1:1 mixture of 0.1 N NaOH and MeOH. The total concentration of pradimicins was measured spectrophotometrically at 500 nm and is expressed in μg/ml as the equivalent amount of pradimicin A (zwitterionic form, $E^{1\%}_{1cm}$ at 500 nm = 180 at pH $\geqq$11).

For isolation and characterization of pradimicins FA-1 and FA-2, 10 liters of broth from strain A2493 was obtained from 120 flasks. D-Serine at 0.25% was added to the medium A just before inoculation of the seed culture. The fermentation was carried out at 28°C for 7 days on a rotary shaker. The composition of pradimicins in the fermentation broth of strain A2493 was as follows: Total concentration 480 μg/ml as pradimicin A; percent composition: pradimicin FA-1 29.8%, pradimicin FA-2 28.9%, pradimicin A 21.8% and pradimicin C 19.5%.

Isolation and Purification

The fermentation broth (10 liters) was centrifuged and the supernate was acidified to pH 2.0 with 6 N HCl and then filtered. The filtrate was adjusted to pH 5.0 with 6 N NaOH and the dark red precipitate deposited was recovered by filtration and then dissolved in 4 liters of water adjusted to pH 9.0 with 6 N NaOH. Insoluble materials were removed by filtration. The filtrate was adjusted pH to 2.0, applied to a column of Diaion HP-20 (2 liters), and eluted with water - acetone (2:3, pH 3.0 with 6 N HCl). Concentration of the eluate *in vacuo* produced a crude solid of pradimicins (3.1 g, hydrochloride). The solid (3.0 g) was dissolved in 120 ml of methanol and filtered. To the stirred filtrate, 720 ml of ethyl acetate was added dropwise. The solution was allowed to stand at 5°C for 15 hours. The precipitate was collected by filtration and dried *in vacuo*. The solid (1.28 g) was chromatographed on a 10-liter column of YMC gel ODS A60 (Yamamura Chemical Lab.) eluted with a mixture of CH_3CN - 0.15% KH_2PO_4, pH 3.5 (21:79). Monitoring of the eluate by HPLC indicated that pure pradimicin FA-2 was eluted first followed by pure pradimicin FA-1. The relevant fractions were concentrated, desalted by Diaion HP-20, and then concentrated to dryness to give 50 mg of pradimicin FA-1 hydrochloride and 75 mg of pradimicin FA-2 hydrochloride. For preparation of the zwitterionic form, and aqueous solution of pradimicins FA-1 or FA-2 was adjusted to pH 5.5 with 0.1 N NaOH and allowed to stand until a precipitate was formed, yielding 11 mg of FA-1 or 48 mg of FA-2.

Determination of the Configuration of C-17 Position in Pradimicins FA-1 and FA-2

A solution of 12 mg of pradimicin FA-1 or FA-2 in 5 ml of 6 N HCl was heated at 115°C for 14 hours in a sealed tube. After cooling and filtering, the filtrate was passed through a 30-ml column of Diaion HP-20. The ninhydrin-positive effluent was collected and concentrated to dryness *in vacuo* to afford a white solid containing serine (3 mg). The stereochemistry of the serine was determined by HPLC (HPLC column: MCI gel ODS 1HU, 4.6 mm i.d. × 150 mm, 5 μm in size, Mitsubishi Chemical Industries Limited; elution solvent 2 mM *N*,*N*-dipropyl-L-alanine and 1 mM copper acetate, pH 5.7; flow rate 0.8 ml; detection UV 254 nm; Rt (minutes) D-serine 5.3 and L-serine 6.1).

In Vitro and *In Vivo* Antifungal Activity

MICs of pradimicins against various fungi were determined by serial agar dilution using Sabouraud dextrose agar, pH 7.0. The test organism (5 μl, 1×10^6 cfu/ml) was inoculated on the agar plate containing the test antibiotic and incubated at 28°C for 40 hours.

In vivo antifungal activity was examined by systemic infection of *Candida albicans* A9540 in male ICR mice (20~24 g body weight). The pathogen was cultivated for 18 hours at 28°C in YGP medium (yeast extract 0.2%, glucose 1.5%, peptone 0.5%, K_2HPO_4 0.05%, $MgSO_4$ 0.05%) and resuspended in saline. The intravenous inoculum was ten times the median lethal dose. Test compounds were intravenously administered to groups of 5 mice once immediately after fungal challenge. Doses protecting 50% of the mice (PD_{50}'s) were calculated from survival rates recorded on the 20th day after fungal infection.

General

MP's were measured with a Yanagimoto micro melting point apparatus (Type MP-3S) and are uncorrected. IR absorption spectra (in KBr pellets) were obtained with a Jasco IR-810 spectrometer and UV and visible absorption spectra with a Jasco UVIDEC-610C spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Jeol JMN-GX 400 spectrometer using DMSO-d_6 as internal standard. SI-MS and HRFAB-MS were obtained with a Hitachi M80B mass spectrometer and a Jeol JMS-AX 505H spectrometer, respectively. Optical rotations were measured with a Jasco (Model DIP-140) polarimeter. CD spectra were recorded on a Jasco J-500A spectrometer.

Results

Controlled Biosynthesis of New Pradimicin Analogs with Various Amino Acids

Pradimicin A biosynthesis studies[9] indicated that this group of antibiotics is produced *via* chain reactions similar to lipid biosynthesis or the nonribosomal RNA system of protein synthesis. This suggested a high probability of directed biosynthesis of new pradimicins by *A. hibisca* P157-2 with supplemental amino acids. Using the parent strain and medium A, the production of new pradimicin analogs in the presence of 0.25% of the following amino acids was examined: DL-Mixtures of α-aminoadipic acid, α-aminobutyric acid, aspartic acid, cysteine, cystine, α,β-diaminopropionic acid, isoleucine, leucine, lysine, norleucine, norvaline, ornithine, phenylalanine, proline, serine, threonine and valine; D-forms of tyrosine; and β-alanine, anthranilic acid, γ-aminobutyric acid, ε-aminocapronic acid, glycine, phenylglycine and sarcosine. Among the amino acids tested, only DL-serine generated a new analog designated pradimicin FA-1. The comparative effects of DL-serine, D-serine and L-serine at a concentration of 0.25% on the formation of pradimicin FA-1 by the parent strain were examined in medium A. DL-Serine and D-serine effectively induced the production of FA-1, indicating that D-serine was incorporated into the molecule. In order to look for a second D-serine analog, a pradimicins A and C producing mutant A2493 was utilized for directed biosynthesis. Table 1 shows the comparative production of pradimicins FA-1 and FA-2 by the

Table 1. Production of pradimicins FA-1 and FA-2 on day 7 by strains P157-2 (parent) and A2493 (mutant) in the presence or absence of D-serine.

Strain	0.25% D-serine	Total production (μg/ml)	Ratio (%)			
			Pradimicins			
			A	C	FA-1	FA-2
P157-2	−	600	98.8	1.2	0	0
	+	440	25.9	1.1	72.9	Trace
A2493	−	830	37.4	62.6	0	0
	+	480	21.8	19.5	29.8	28.9

parent and the mutant strains of *A. hibisca* in the presence and absence of 0.25% D-serine. Total antibiotic production was depressed to some extent with 0.25% D-serine in both strains. It is apparent that the parent strain produced a significant amount of pradimicin FA-1 and a trace amount of pradimicin FA-2 in the presence of D-serine, while the mutant strain produced substantial amount of pradimicins FA-1 and FA-2 together with pradimicins A and C. No production of FA-1 and FA-2 was observed in either strain grown in the absence of D-serine.

Physico-chemical Properties and Structures of Pradimicins FA-1 and FA-2

The physico-chemical properties of pradimicins FA-1 and FA-2 are described in Table 2. These components are distinguished from each other and from the other pradimicin components by their HPLC and TLC mobilities (see Materials and Methods). They are soluble in DMSO, DMF, acidic and alkaline water, slightly soluble in ethanol, methanol and neutral water, but practically insoluble in other common organic solvents. The UV and visible spectra of pradimicins FA-1 and FA-2 are superimposable on those of pradimicin A at various pHs, demonstrating that they share the same chromophore as pradimicin A. The IR spectra of pradimicins FA-1 and FA-2 are very similar to that of pradimicin A. The molecular formulae of pradimicins FA-1 and FA-2 were determined to be $C_{40}H_{44}N_2O_{19}$ and $C_{39}H_{42}N_2O_{19}$, respectively, based on their HRFAB-MS and ^{13}C NMR spectra.

The structures of pradimicins FA-1 and FA-2 were determined by analyzing their ^1H and ^{13}C NMR spectral data (Tables 3 and 4) as well as their chemical degradation products. The ^1H and ^{13}C signals of pradimicin FA-1 closely resembled those of pradimicin A except for the signals assignable to the D-alanine moiety. The 17-CH_3 signal (δ_H 1.35 and δ_C 17.6) in pradimicin A was missing, while a new methylene signal (δ_H 3.74 and δ_C 61.6) was observed in pradimicin FA-1 with concomitant shifts of the C-17 and C-18 signals. Vigorous acid hydrolysis of pradimicin FA-1 or FA-2 afforded a ninhydrin positive substance which was identified as serine by silica gel TLC and amino acid analysis, along with several aglycones. The configuration of the serine was determined to be *R* (D-serine) by analyzing the serine on HPLC. These data clearly indicated that the D-alanine of pradimicins A and C was replaced by a D-serine in pradimicins FA-1 and FA-2. The CD spectrum of pradimicin FA-1 ($\lambda_{extreme}^{0.01\,N\text{-}HCl}$ nm ($\Delta\varepsilon$) 216 (+1.4), 247 (−9.3), 289 (+9.8), 337 (−3.3), 512 (+3.5)) showed that pradimicin FA-1 retained the 5*S*, 6*S* absolute configuration

Table 2. Physico-chemical properties of pradimicins FA-1 and FA-2.

	Pradimicin FA-1	Pradimicin FA-2
Nature	Dark red amorphous powder	Dark red amorphous powder
MP (°C, dec)	215~220	186~190
$[\alpha]_D^{24}$ (*c* 0.1, 0.1 N HCl)	+919°	+79°
SI-MS ($(M+H)^+$ *m/z*)	857	843
HRFAB-MS	Obsd: 857.2637 Calcd for $C_{40}H_{45}N_2O_{19}$: 857.2617	Obsd: 843.2474 Calcd for $C_{39}H_{43}N_2O_{19}$: 843.2460
UV λ_{max} nm (ε)		
in 50% MeOH	221 (32,100), 276 (27,400), 499 (12,900)	223 (27,700), 277 (25,400), 499 (12,200)
in 0.02 N HCl-MeOH (1:1)	234 (37,400), 299 (31,100), 460 (12,900)	234 (34,500), 296 (28,900), 460 (12,000)
in 0.02 N NaOH-MeOH (1:1)	244 (34,100), 320 (15,700), 498 (14,900)	233 (37,200), 319 (16,600), 498 (15,100)
IR (KBr) cm^{-1}	3400, 2920, 1605, 1385, 1295, 1260, 1160, 1040	3400, 2920, 1600, 1385, 1295, 1255, 1160, 1040

Table 3. ^{1}H NMR spectra of pradimicins A, FA-1 and FA-2 (400 MHz, DMSO-d_6).

Proton	Pradimicin A	Pradimicin FA-1	Pradimicin FA-2
5′-CH_3	1.25 (3H, d, J=6.2)	1.26 (3H, d, J=6.4)	1.15 (3H, d, J=6.4)
17-CH_3	1.35 (3H, d, J=7.0)	—	—
3-CH_3	2.29 (3H, s)	2.31 (3H, s)	2.32 (3H, s)
4′-NCH_3	2.61 (3H, s)	2.61 (3H, s)	—
4′ 2″～5″-H	3.1～3.2 (5H, m)	3.1～3.2 (5H, m)	3.1～3.2 (5H, m)
2′-H	3.53 (1H, m)	3.53 (1H, m)	3.54 (1H, m)
5″-H	3.74 (1H, dd, J=5.3, 11.2)	3.73 (1H, dd, J=5.3, 11.2)	3.72 (1H, dd, J=5.3, 11.1)
17-CH_2	—	3.74 (2H, dd, J=4.9, 10.9)	3.74 (2H, dd, J=4.9, 10.9)
5′-H 3′-H	3.84 (1H, q, J=6.2) 3.91 (1H, m)	3.86 (2H, m)	3.89 (2H, m)
11-OCH_3	3.91 (3H, s)	3.90 (3H, s)	3.91 (3H, s)
17-H	4.39 (1H, qui, J=7.0)	4.45 (1H, m)	4.49 (1H, m)
1″-H	4.44 (1H, d, J=7.3)	4.48 (1H, d, J=7.3)	4.45 (1H, d, J=7.3)
5-H	4.46 (1H, d, J=10.3)	4.45 (1H, d, J=10.4)	4.46 (1H, d, J=10.5)
6-H	4.49 (1H, br d, J=10.3)	4.50 (1H, d, J=10.4)	4.52 (1H, dd, J=2.1, 10.5)
1′-H	4.73 (1H, d, J=7.7), 5.02 (2H, br s)[a], 5.08 (1H, s)[a], 5.68 (1H, s)[a], 5.91 (1H, s)[a]	4.75 (1H, d, J=7.7), 4.90 (1H, br s)[a], 5.03 (1H, br s)[a], 5.11 (1H, s)[a], 5.71 (1H, s)[a], 5.94 (1H, s)[a]	4.74 (1H, d, J=7.7), 4.91 (1H, br s)[a], 5.02 (1H, br s)[a], 5.10 (1H, s)[a], 5.71 (1H, s)[a], 5.93 (1H, s)[a]
10-H	6.71 (1H, d, J=2.4)	6.72 (1H, d, J=2.1)	6.72 (1H, d, J=2.4)
4-H	6.85 (1H, s)	6.86 (1H, s)	6.90 (1H, s)
12-H	7.11 (1H, d, J=2.4)	7.10 (1H, d, J=2.1)	7.11 (1H, d, J=2.4)
7-H	7.69 (1H, s)	7.70 (1H, s)	7.70 (1H, s)
16-NH	8.78 (1H, d, J=7.0)[a], 13.16 (1H, s)[a]	8.62 (1H, d)[a], 13.15 (1H, s)[a], 15.45 (1H, br s)[a]	8.72 (1H, d)[a], 13.16 (1H, s)[a], 15.64 (1H, br s)[a]

[a] Disappeared upon D_2O addition. J in Hz.

Table 4. ^{13}C NMR spectra of pradimicins A, FA-1 and FA-2 (100 MHz, DMSO-d_6).

Carbon	Pradimicin A	Pradimicin FA-1	Pradimicin FA-2	Carbon	Pradimicin A	Pradimicin FA-1	Pradimicin FA-2
5′-CH_3	16.4 q[a]	16.3 q	16.4 q	8a	110.5 s	110.3 s	110.4 s
17-CH_3	17.6 q	—	—	7	111.6 d	111.4 d	111.5 d
3-CH_3	20.0 q	20.3 q	20.4 q	4	116.9 d	117.1 d	117.2 d
4′-NCH_3	36.6 q	36.4 q	—	14b	119.0 s	119.0 s	119.1 s
17	48.2 d	54.9 d	55.0 d	13a	119.3 s	119.2 s	119.3 s
11-OCH_3	56.2 q	56.1 q	56.2 q	2	126.9 s	126.3 s	126.3 s
17-CH_2	—	61.6 t	61.7 t	7a	132.2 s	132.1 s	132.1 s
4′	63.4 d	63.1 d	54.3 d	14a	133.1 s	133.0 s	133.1 s
5″	66.0 t	65.9 t	65.9 t	3	136.5 s	136.8 s	136.8 s
5′	67.9 d	67.8 d	67.3 d	4a	137.7 s	137.6 s	137.8 s
4″	69.6 d	69.3 d	69.4 d	12a	138.0 s	137.9 s	138.0 s
2′	70.2 d	69.9 d	69.7 q	6a	143.7 s	143.5 s	143.5 s
6	71.9 d	71.7 d	71.8 d	1	157.6 s	158.0 s	158.2 s
2″	73.8 d	73.6 d	73.5 d	9	164.1 s	163.6 s	163.7 s
3″	76.1 d	75.8 d	75.9 d	11	166.0 s	165.8 s	165.8 s
3′	80.4 d	80.5 d	79.1 d	14	166.4 s	166.2 s	166.2 s
5	82.7 d	82.1 d	82.5 d	15	168.9 s	168.5 s	168.6 s
10	104.4 d	104.1 d	104.2 d	18	174.6 s	172.2 s	172.3 s
1′	104.5 d	104.2 d	104.3 d	13	180.6 s	180.2 s	180.1 s
1″	105.3 d	105.2 d	105.1 d	8	187.5 s	187.3 s	187.4 s
12	106.3 d	106.0 d	106.1 d				

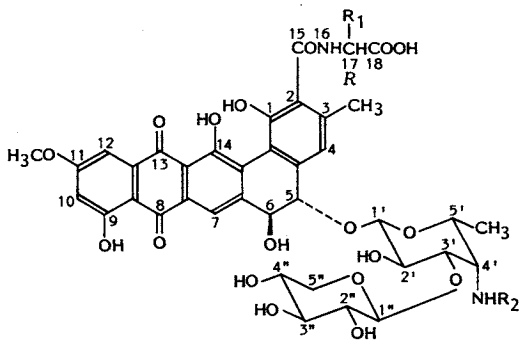
[a] Multiplicities determined from DEPT spectra.

Table 5. *In vitro* antifungal activity.

Test organism	MIC (μg/ml)				
	Pradimicins			AMPH-B	KCZ
	A	FA-1	FA-2		
Candida albicans IAM 4888	6.3	6.3	6.3	1.6	50
C. albicans A9540	50	12.5	6.3	1.6	50
Cryptococcus neoformans D49	0.8	0.8	0.8	0.8	0.4
C. neoformans IAM 4514	0.8	0.8	0.8	0.8	0.4
Aspergillus fumigatus IAM 2530	3.1	3.1	3.1	3.1	3.1
A. fumigatus IAM 2034	3.1	6.3	6.3	3.1	6.3
A. flavus FA21436 NRRL 484	6.3	6.3	6.3	1.6	0.4
Fusarium moniliforme A2284	3.1	6.3	6.3	3.1	6.3
Trichophyton mentagrophytes D155	6.3	6.3	6.3	6.3	1.6
T. mentagrophytes No. 4329	3.1	6.3	6.3	6.3	1.6
Blastomyces dermatitidis IFO 8144	3.1	3.1	3.1	12.5	0.4
Sporothrix schenckii IFO 8158	1.6	1.6	1.6	>100	6.3
Petriellidium boydii IFO 8078	12.5	12.5	25	>100	1.6
Mucor spinosus IFO 5317	>100	>100	>100	6.3	100

AMPH-B: Amphotericin B, KCZ: ketoconazole.

Fig. 1. Structures of pradimicins A, C, FA-1 and FA-2.



	R_1	R_2
Pradimicin A	$-CH_3$	$-CH_3$
Pradimicin C	$-CH_3$	$-H$
Pradimicin FA-1	$-CH_2OH$	$-CH_3$
Pradimicin FA-2	$-CH_2OH$	$-H$

Table 6. *In vivo* activity against *Candida albicans* A9540 systemic infection in ICR mice.

Compound	PD_{50}
	mg/kg (iv)
Pradimicin FA-1	18
Pradimicin FA-2	7.4
Pradimicin A	8.9

of pradimicin A.[3] The NMR spectral data of pradimicin FA-2 corresponded well with those of pradimicin FA-1, differing only in that the *N*-CH_3 was missing and the C-4′ signal shifted upfield by 8.8 ppm in FA-2. Therefore the des-*N*-methyl structure was assigned to pradimicin FA-2. The structures are shown in Fig. 1.

Antifungal Activity

The *in vitro* antifungal activity of pradimicins FA-1 and FA-2 was determined using various yeasts and fungi. As shown in Table 5 both pradimicins FA-1 and FA-2 were active against all organisms tested with potency comparable to that of pradimicin A. The *in vivo* efficacy of pradimicins FA-1 and FA-2 against *C. albicans* A9540 is shown in Table 6. Pradimicin FA-1 was less potent than pradimicins FA-2 and A in terms of the observed survival on the 20th day.

Discussion

Pradimicins[1~4] and benanomicins[5,6] are interesting antifungal antibiotics with potent *in vitro* and *in vivo* activities and relatively low toxicity. In an attempt to improve the antifungal activity of pradimicins,

substitution of the D-alanine moiety with other amino acids was investigated by means of directed biosynthesis. In our biosynthetic study of pradimicin A,[9] D-alanine seemed to be a direct precursor of the skeleton biosynthesis. If so, D-alanine biomimetic compounds should be incorporated into the molecule to produce novel analogs. However, only D-serine among 25 amino acids tested could replace D-alanine. No other amino acid served as substrate for the D-alanine-carrying enzyme. Production of both D-alanine- and D-serine-containing antibiotics in the same culture indicated that the D-alanine-carrying enzyme recognized D-serine if the normal D-alanine pool was changed by exogenous D-serine, resulting in competitive incorporation of D-alanine and D-serine into the molecule. The parent or mutant strain produced insignificant amounts of the glycine-containing antibiotics pradimicins D and E even in the presence of exogenous glycine (data not shown). On the other hand, mutant A2660 produced substantial amounts of pradimicins D and E in medium A which is free from exogeneous glycine and showed depressed antibiotic production when glycine was added.[7] These results suggest that the mode of incorporation of D-serine by the parent strain or mutant A2493 and of glycine by A2660 into pradimicin may be different.

We found that D-serine analogs FA-1 and FA-2 have virtually the same antifungal activity and spectrum as those of original pradimicins A and C. However, such analogs may reveal some advantageous characteristics such as improved water solubility after further modification by chemical or microbial means.[10]

Acknowledgments

The authors wish to thank Dr. M. OHASHI, Professor of the University of Electrocommunication, for mass spectroscopic analysis and valuable discussions, and also to Dr. T. TSUNO for spectral data, Mr. M. HIRANO and Miss H. FUJIMURA and Ms. M. SHINODA for determination of *in vitro* and *in vivo* antifungal activities.

References

1) 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
2) TSUNAKAWA, M.; M. NISHIO, H. OHKUMA, T. TSUNO, M. KONISHI, T. NAITO, T. OKI & H. KAWAGUCHI: The structures of pradimicins A, B and C: A novel family of antifungal antibiotics. J. Org. Chem. 54: 2532~2536, 1989
3) TOMITA, K.; M. NISHIO, K. SAITOH, H. YAMAMOTO, Y. HOSHINO, H. OHKUMA, M. KONISHI, T. MIYAKI & T. OKI: Pradimicins A, B and C: New antifungal antibiotics. I. Taxonomy, production, isolation and physico-chemical properties. J. Antibiotics 43: 755~762, 1990
4) OKI, T.; O. TENMYO, M. HIRANO, K. TOMATSU & H. KAMEI: Pradimicins A, B and C: New antifungal antibiotics. II. *In vitro* and *in vivo* biological activities. J. Antibiotics 43: 763~770, 1990
5) 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
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) SAWADA, Y.; M. NISHIO, H. YAMAMOTO, M. HATORI, T. MIYAKI, M. KONISHI & T. OKI: New antifungal antibiotics, pradimicins D and E. Glycine analogs of pradimicins A and C. J. Antibiotics 43: 771~777, 1990
8) KAKUSHIMA, M.; M. NISHIO, K. NUMATA, M. KONISHI & T. OKI: Effect of stereochemistry at the C-17 position on the antifungal activity of pradimicin A. J. Antibiotics 43: 1028~1030, 1990
9) KAKUSHIMA, M.; Y. SAWADA, M. NISHIO, T. TSUNO & T. OKI: Biosynthesis of pradimicin A. J. Org. Chem. 54: 2536~2539, 1989
10) OKI, T.; M. KAKUSHIMA, M. NISHIO, H. KAMEI, M. HIRANO, Y. SAWADA & M. KONISHI: Water-soluble pradimicin derivatives, synthesis and antifungal evaluation of *N,N*-dimethyl pradimicins. J. Antibiotics 43: 1230~1235, 1990