MICROBIAL MODIFICATION OF PRADIMICINS AT C-11 LEADING TO 11-*O*-DEMETHYL- AND 11-*O*-L-XYLOSYLPRADIMICINS A AND FA-1

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In a screen of pradimicin-nonproducing mutants derived from *Actinomadura verrucosospora* subsp. *neohibisca* R103-3, we found a strain capable of producing 11-hydroxyl analogs of pradimicins A and FA-1, designated pradimicins H and FH, respectively. Feeding of pradimicins H and FH to growing cultures of an actinomycete strain AA3798 produced 11-*O*-L-xylosylpradimicins H and FH, respectively. These 11-*O*-L-xylosylpradimicins had a broad spectrum of antifungal activity and demonstrated *in vivo* efficacies against *Candida albicans* in mice.

The pradimicin and benanomicin are important microbial products possessing potent antifungal and anti-HIV activities[1~11]. They belong to novel family of antibiotics having a dihydrobenzo[*a*]naphthacenequinone nucleus substituted with one or two sugars at C-5, a methoxyl or L-xylosyl group at C-11, and an amino acid side chain at C-15. In a series of papers, we have presented the results of our biosynthetic studies on pradimicin A, benanomicin A and pradimicin T1 using blocked mutants of *Actinomadura verrucosospora* subsp. *neohibisca* E-40 (a high pradimicin-producer), *Actinomadura* sp. AB1236 and an actinomycete strain AA3798 (pradimicin T1-producer), and proposed plausible biosynthetic pathways leading to these antibiotics[10,12~14].

Of particular interest is the finding that 2 types of methyltransferases are involved in the biosynthesis of this family[14]. This suggested a possibility that a mutant blocked at 11-*O*-methylation specifically should be able to produce 11-hydroxyl analogs of pradimicins A and FA-1, designated pradimicins H and FH, respectively. We have screened culture broths of pradimicin-nonproducers derived from *A. verrucosospora* subsp. *neohibisca* R103-3, and isolated mutant JN-380 among them. We now wish to report, in detail, on the production of pradimicins H and FH by this strain and on the use of these intermediates for the production of 11-*O*-L-xylosylpradimicins H and FH, 11-*O*-L-xylosyl analogs of pradimicins A and FA-1, respectively, by an actinomycete strain AA3798. Structures of pradimicins H and FH, and 11-*O*-L-xylosylpradimicins H and FH are shown in Fig. 1.

Materials and Methods

Strain

A. verrucosospora subsp. *neohibisca* R103-3 is a producer of pradimicins A and L, and its cultural, morphological and chemotaxonomical characteristics have been described previously[5]. Strain JN-380

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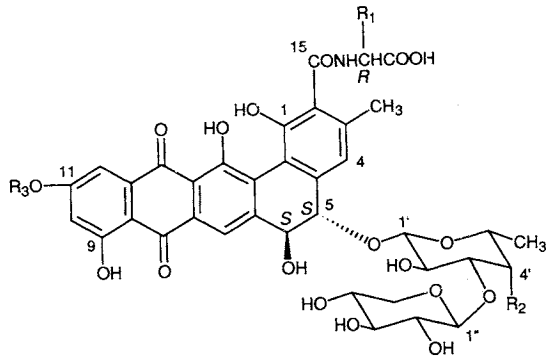
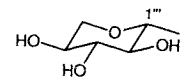
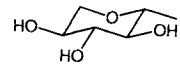
Fig. 1. Structures of pradimicins H and FH, and 11-*O*-L-xylosylpradimicins H and FH.



	R_1	R_2	R_3
Pradimicin H	CH_3	$NHCH_3$	H
Pradimicin FH	CH_2OH	$NHCH_3$	H
11-*O*-L-Xylosyl-pradimicin H	CH_3	$NHCH_3$	
11-*O*-L-Xylosyl-pradimicin FH	CH_2OH	$NHCH_3$	

which produces pradimicins H and FH was derived from *A. verrucosospora* subsp. *neohibisca* R103-3 by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) treatment. An actinomycete strain AA3798, a producer of pradimicins T1 and T2, has been characterized previously[9] and used for the production of 11-*O*-L-xylosylpradimicins H and FH.

Assay of Antibiotics

Two-ml aliquots were taken from the fermentation broths and total antibiotics production was monitored by measuring visible absorption at 500 nm in 0.02 N NaOH-MeOH (1:1) solution. For quantitative analysis, samples were diluted ten-fold with dimethylsulfoxide followed by filtration through Ekicro disc 13CR (Pore size: 0.45 μm, Gelman Science Japan, Ltd.). The filtrates was analyzed by an LC-100 HPLC system (Yokogawa Electronic Co., Ltd.) equipped with YMC-ODS A-301-5 column (YMC Co., 10 cm × 4.6 mm i.d.) using acetonitrile-0.15% phosphate buffer, pH 3.5 (17:83) at a flow rate of 1 ml/minute with 460 nm detection. The retention times of pradimicins H and FH, and 11-*O*-L-xylosylpradimicins H and FH were 25.7, 11.2, 5.6 and 2.7 minutes, respectively.

Production of Pradimicin H

Strain JN-380 grown on yeast starch (YS) agar composed of yeast extract (Difco Laboratories Inc.) 0.2%, soluble starch 1% and agar 1.8% was inoculated into five 500-ml Erlenmeyer flasks containing 100 ml of V-15 medium composed of glucose 1.0%, peptone (Daigo Eiyo Co.) 0.2%, NZ-Case (Sheffield Products) 0.2%, meat extract (Mikuni Kogyo Co.) 1.0%, $NaNO_3$ 0.2%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and $CaCl_2 \cdot 2H_2O$ 0.05%, and the flasks were incubated for 6 days at 32°C on a rotary shaker (200 rpm). The resulting culture (100 ml) was inoculated into 1 liter of production medium (FR-18) composed of glucose 3%, Pharmamedia (Traders Protein, The Procter and Gamble Oilseed Productions Company) 3% and $CaCO_3$ 0.3%, pH 8.0 before autoclaving, in five 5-liter Erlenmeyer flasks, and the flasks were cultured for 5 days at 28°C on a rotary shaker (150 rpm). The seed culture (5 liters) of strain JN-380 was transferred into a 200-liter tank fermentor containing 120 liters of production medium composed of glucose 3%, Pharmamedia 3%, $CaCO_3$ 0.3%, $CoCl_2 \cdot 6H_2O$ 0.001% and DL-alanine 0.2%. The pH was adjusted to 8.0 before autoclaving. The tank fermentor was stirred for 10 days at 28°C and 150 rpm with aeration

at 0.5 v/v/minute. The total titer of pradimicin H (retention time, 25.7 minutes) reached a level of 200 μg/ml after 10 days of fermentation.

Production of Pradimicin FH

Five-ml aliquots of the seed culture of strain JN-380 were transferred into two hundred 500-ml Erlenmeyer flasks containing 100 ml of FR-18 supplemented with $CoCl_2 \cdot 6H_2O$ 0.001% and D-serine 0.2%, and the flasks were incubated at 28°C on a rotary shaker (200 rpm) for 7 days, during which the production of pradimicin FH (retention time, 11.2 minutes) reached a level of 300 μg/ml.

Production of 11-*O*-L-Xylosylpradimicin H

Strain AA3798 grown on YSM agar prepared with yeast extract 0.1%, Bact soytone (Difco Laboratories Inc.) 0.1%, soluble starch 1%, $CaCl_2 \cdot 2H_2O$ 0.05% and agar 1.8% was inoculated into five 500-ml Erlenmeyer flasks containing 100 ml of V-21 medium composed of glucose 2%, defatted soybean meal (Esusan mi-to, Ajinomoto Co., Inc.) 1.5%, Bact soytone (Difco Laboratories Inc.) 0.1%, yeast extract (Oriental Yeast Co.) 0.1%, $CaCO_3$ 0.1% and $CoCl_2 \cdot 6H_2O$ 0.0005%, and the flasks were incubated for 5 days at 32°C on a rotary shaker (200 rpm). Five-ml aliquots of the seed culture were transferred into eighty 500-ml Erlenmeyer flasks containing 100 ml of production medium (FR-25) composed of glucose 3%, defatted soybean meal 3% and $CaCO_3$ 0.3%, and the flasks were preincubated for 48 hours at 28°C on a rotary shaker (200 rpm), and pradimicin H at a final concentration of 100 μg/ml was added. Incubation was continued for additional 24 hours, during which pradimicin H was consumed and incorporated into 11-*O*-L-xylosylpradimicin H (retention time, 5.6 minutes; bioconversion rate: 98.5%).

Production of 11-*O*-L-Xylosylpradimicin FH

Five-ml aliquots of the seed culture of strain AA3798 was transferred into one hundred 500-ml Erlenmeyer flasks each containing 100 ml of FR-25 medium. The flasks were preincubated for 48 hours at 28°C on a rotary shaker (200 rpm) and pradimicin FH was added at a concentration of 75 μg/ml. Incubation was continued for additional 9 hours, during which pradimicin FH was consumed and converted to 11-*O*-L-xylosylpradimicin FH (retention time, 2.7 minutes; bioconversion rate: 95%).

In Vitro Antifungal Evaluation

The MICs against 12 fungi were determined by the agar dilution method on yeast morphology agar containing 1/15 M phosphate buffer (pH 7.0) after 40 hours of incubation at 28°C. The MICs were defined as the lowest antibiotic concentrations showing no growth or less than five discrete colonies per spot.

In Vivo Antifungal Evaluation

The *in vivo* efficacies were determined in a systemic *Candida albicans* infection in model in male ICR mice as described by OKI *et al.*[15]. Groups of 5 mice weighing 20~24 g at each dose level were infected intravenously with 10 LD_{50} of *C. albicans* A9540, and antibiotics given once daily for 2 consecutive days beginning immediately after the fungal infection. The 50% protective dose (PD_{50}) was calculated from the survival rate 20 days after the fungal infection.

Acute Toxicity Assessment in Mice

Test compounds were dissolved in 10% DMSO at pH 7.5 and 2-fold dilutions were made with distilled water and filter sterilized. Groups of 3 male ICR mice weighing 20~24 g at each dose level were treated intravenously with test solutions (0.2 ml per 10 g of body weight) and observed for 10 days.

Results

Isolation of Pradimicin H-Producing Mutant

Strain JN-380 was selected among the non-pradimicin-producing mutants of *A. verrucosospora* subsp. *neohibisca* R103-3 in a screen for a pradimicin H-producer. Thus, spores of strain R103-3 treated with NTG as described previously[12] were spread on the YS agar plates and incubated for 7 days at

30°C. Colonies producing pale pink and pink diffusible pigments were cultivated in FR-18 medium. When the culture broths were analyzed by HPLC, strain JN-380 was found to produce pradimicin H.

Production and Isolation of Pradimicin H

When strain JN-380 was incubated in a 200-liter tank containing 120 liters of FR-18 medium supplemented with $CoCl_2 \cdot 6H_2O$ 0.001% and DL-alanine 0.2% for 10 days at 28°C, pradimicin H was produced at a level of 200 μg/ml. The fermentation broth filtered by Sharples-type centrifuge (Kokusan Seiko Co.), and the supernatant (90 liters) was mixed with Diaion HP-20 (6 liters). The resin was washed with 0.01 N HCl (40 liters), and the product was eluted with a mixture of 35% acetone - 0.01 N HCl (30 liters). The eluate was concentrated *in vacuo*, and the resulting aqueous residue (12 liters) was extracted twice with a mixture of *n*-butanol (6 liters) and methanol (0.6 liter) at pH 2.0. The organic layer (13 liters) was washed with water (6 liters) and the product was extracted into alkaline water (6 liters, pH 8). The aqueous layer was concentrated *in vacuo* and the residue (2 liters) was mixed with Diaion HP-20 (2 liters). The resin was washed with 0.01 N HCl (10 liters), and the product was eluted with a mixture of 60% aq acetone - 0.001 N HCl (3 liters). The eluate was concentrated *in vacuo*, and the residue was lyophilized to yield a crude solid (5.17 g) which contained 2.48 g of pradimicin H by HPLC analysis. Part (1.1 g) of this crude material was dissolved in a mixture of acetonitrile - 0.15% phosphate buffer, pH 3.5 (15 : 85, 100 ml) and chromatographed on a column of YMC-gel, ODS-A60 (1 liter) using the same solvent as eluent. Each fraction was monitored by HPLC, and the fractions (35 liters) containing pradimicin H were pooled and concentrated *in vacuo*. The concentrate was mixed with Diaion HP-20 (300 ml) and the product was eluted with a mixture of 60% acetone - 0.001 N HCl (300 ml). The solvent was removed *in vacuo* and the aqueous residue was lyophilized to give 330 mg of pradimicin H.

Production and Isolation of Pradimicin FH

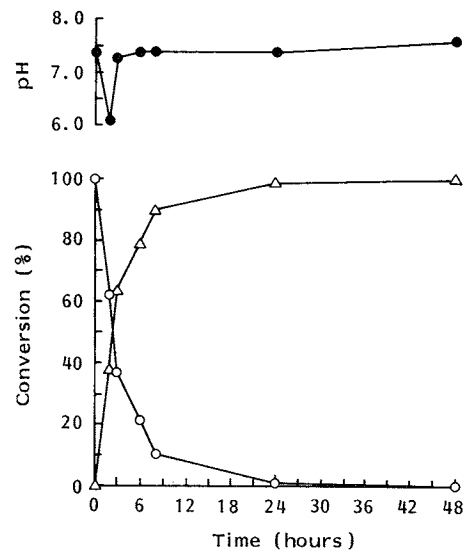
When strain JN-380 was inoculated in two hundred 500-ml flasks containing 100 ml of the FR-18 medium supplemented with $CoCl_2 \cdot 6H_2O$ 0.001% and D-serine 0.2%, and the flasks were incubated for 7 days at 28°C, pradimicin FH was produced at a level of 300 μg/ml. Isolation of the product from the culture broth (17 liters) by a procedure similar to that used for the isolation of pradimicin H gave 2.4 g of pradimicin FH.

Production and Isolation of 11-*O*-L-Xylosylpradimicin H

A small-scale experiment indicated that feeding of pradimicin H to growing cultures of strain AA3798 in FR-25 medium resulted in a rapid conversion of pradimicin H into 11-*O*-L-xylosyl-pradimicin H as shown in Fig. 2. Thus, pradimicin H at a final concentration of 100 μg/ml was fed to growing cultures of strain AA3798 in eighty 500-ml

Fig. 2. Time course of pradimicin H conversion by an actinomycete strain AA3798.

(○) Pradimicin H, (△) 11-*O*-L-xylosylpradimicin H, (●) pH.



Erlenmeyer flasks each containing 100 ml of the FR-25 medium, and fermentation was continued for additional 24 hours. The fermentation broths were combined and centrifuged by Sharples-type centrifuge, and the supernatant (7.5 liters) was mixed with Diaion HP-20 (1.3 liters). The resin was washed with 0.01 N HCl (15 liters), and the product was eluted with a mixture of 60% acetone - 0.01 N HCl (11 liters). The eluate was concentrated *in vacuo*, and the residue was lyophilized to yield a crude solid (27.3 g). The solid was dissolved in a mixture of acetonitrile - 0.15% phosphate buffer, pH 3.5 (15.5 : 84.5, 1.5 liters) and centrifuged at 3,000 rpm for 15 minutes. The aqueous solution was chromatographed on a column of YMC-gel, ODS-A60 (10 liters) using the same solvent as eluent. The fractions (46.8 liters) containing the product were pooled, concentrated *in vacuo* and then mixed with Diaion HP-20 (500 ml). The resin was washed with 0.001 N HCl (10 liters), and the product was eluted with 60% aq acetone (600 ml). The eluate was concentrated *in vacuo* and lyophilized to afford 1.58 g of semi-pure material. A portion (600 mg) of this material was chromatographed on a column of YMC-gel, ODS-A60 (5 liters) to give, after lyophilization from water, 260 mg of 11-*O*-L-xylosylpradimicin H. The bioconversion rate based on the precursor used was 98.5%.

Production and Isolation of 11-*O*-L-Xylosylpradimicin FH

When pradimicin FH at a concentration of 75 μg/ml was fed to growing cultures of strain AA3798 in one hundred 500-ml Erlenmeyer flasks each containing 100 ml of FR-25 medium and incubation was continued for additional 9 hours, pradimicin FH was consumed and converted into 11-*O*-L-

Table 1. Physico-chemical properties of compounds.

	Pradimicin H	Pradimicin FH	11-*O*-L-Xylosyl-pradimicin H	11-*O*-L-Xylosyl-pradimicin FH
Appearance	Red amorphous powder	Red amorphous powder	Red amorphous powder	Red amorphous powder
MP (°C, dec)	210~220	200~210	210~220	210~220
FAB-MS (*m/z*)	827 $(M+H)^+$	843 $(M+H)^+$	959 $(M+H)^+$	975 $(M+H)^+$
HRFAB-MS Obsd:	827.2498	843.2457	959.2974	975.2866
Calcd:	827.2512	843.2460	959.2933	975.3090
Molecular formula	$C_{39}H_{42}N_2O_{18}$	$C_{39}H_{42}N_2O_{19}$	$C_{44}H_{50}N_2O_{22}$	$C_{44}H_{50}N_2O_{23}$
UV λ_{max} nm (ε)				
in 0.02 N NaOH - MeOH (1 : 1)	247 (27,700), 308 (19,800), 504 (15,000)	245 (27,400), 309 (19,400), 502 (13,760)	242 (29,400), 316 (13,000), 501 (13,600)	243 (33,700), 315 (14,500), 501 (13,800)
in 0.02 N HCl - MeOH (1 : 1)	234 (24,600), 301 (23,400), 461 (9,000)	231 (28,300), 296 (23,200), 458 (9,500)	232 (31,400), 289 (23,500), 457 (10,500)	233 (74,000), 290 (24,900), 457 (11,100)
IR ν_{max} (KBr) cm^{-1}	3370, 1725, 1630, 1610, 1405, 1360, 1060	3400, 1620, 1610, 1390, 1340, 1296, 1050	3400, 1730, 1630, 1610, 1380, 1295, 1258, 1055	3400, 1630, 1610, 1390, 1295, 1260, 1170, 1130, 1050, 1000
TLC (SiO_2), Rf				
(*n*-BuOH - AcOH - H_2O, 2 : 1 : 1)	0.43	0.31	0.24	0.1
(*n*-BuOH - AcOH - pyridine - H_2O, 6 : 1 : 4 : 3)	0.30	0.31	0.10	0.17
HPLC, Rt (minutes)				
(ODS, CH_3CN - 0.15% KH_2PO_4, pH 3.5, 17 : 83 v/v)	25.7	8.7	5.7	2.4

xylosylpradimicin FH. Isolation of the product by a procedure similar to that used for 11-*O*-L-xylosylpradimicin H gave 147 mg of 11-*O*-L-xylosylpradimicin FH. The bioconversion rate based on the precursor used was 95%.

Physico-chemical Properties

Table 1 summarizes physico-chemical properties of pradimicins H and FH, and 11-*O*-L-xylosylpradimicins H and FH. They are amphoteric antibiotics, readily soluble in acidic and alkaline aqueous media, dimethylsulfoxide and dimethylformamide, slightly soluble in ethanol, methanol and acetone, but practically insoluble in other common organic solvents such as benzene and hexane.

Structures of Pradimicins H and FH

The structures of pradimicins H and FH were confirmed by direct comparison with authentic samples prepared from pradimicins A and FA-1, respectively[16]. The structures of 11-*O*-L-xylosylpradimicins H and FH were confirmed by FAB-MS spectra which showed an increase of 132 mass units over the spectra of the parents, pradimicins H and FH, as a result of an additional xylopyranosyl moiety and ^{1}H NMR spectra which showed a new doublet peak at 5.08 ppm (J=7.3 Hz) assignable to the anomeric proton of the newly introduced xylopyranosyl moiety with the β-configuration. The glycosidation site was shown to be C-11 by the strong NOE observed between the anomeric (1‴-H) and 10-/12-protons in the ^{1}H-^{1}H NOESY spectra of 11-*O*-L-xylosylpradimicins H and FH. Acidic methanolyses of both compounds afforded

Table 2. ^{1}H NMR data.

	Pradimicin H	Pradimicin FH	11-*O*-L-Xylosyl-pradimicin H	11-*O*-L-Xylosyl-pradimicin FH
3-CH_3	2.30 (s)	2.32 (s)	2.28 (s)	2.30 (s)
4-H	6.95 (s)	6.90 (s)	6.86 (s)	6.89 (s)
5	4.53 (br s)	4.43 (d, 9.8)	4.49 (br s)	4.43 (d, 10.3)
6	4.53 (br s)	4.48 (d, 9.8)	4.49 (br s)	4.48 (d, 10.3)
7	7.79 (s)	7.72 (s)	7.67 (s)	7.73 (s)
10	6.55 (br d)	6.46 (d, 2.6)	6.77 (d, 2.1)	6.77 (d, 2.6)
12	7.14 (br d)	7.08 (d, 2.6)	7.18 (d, 2.1)	7.22 (d, 2.6)
17	4.40 (q, 7.3)	4.45 (m)	4.38 (q, 7.3)	4.38 (m)
19	1.34 (d, 7.3)	3.78 (m)	1.34 (d, 7.3)	3.69 (m)
1′	4.79 (d, 8.1)	4.68 (d, 7.7)	4.75 (d, 7.7)	4.67 (d, 7.7)
2′	3.39 (m)	3.59 (dd, 7.7, 8.8)	3.51 (dd, 7.7, 9.4)	3.56 (dd, 7.7, 8.5)
3′	3.97 (m)	3.70 (m)	3.91 (m)	3.79 (m)
4′	3.43 (m)	3.33 (m)	3.35 (m)	3.33 (m)
4′-NCH_3	2.65 (s)	2.54 (s)	2.61 (s)	2.58 (s)
5′	3.90 (q, 6.8)	3.72 (m)	3.86 (q, 6.8)	3.76 (m)
5′-CH_3	1.27 (d, 6.8)	1.21 (d, 6.4)	1.25 (d, 6.8)	1.20 (d, 6.4)
1″	4.46 (d, 6.8)	4.40 (d, 7.7)	4.44 (d, 7.3)	4.40 (d, 7.3)
2″	3.14 (dd, 6.8, 8.1)	3.08 (dd, 7.7, 8.1)	3.13 (dd, 7.3, 8.6)	3.08 (dd, 7.3, 9.0)
3″	3.16 (dd, 8.1, 9.0)	3.16 (dd, 8.1, 9.4)	3.16 (t, 8.6)	3.15 (t, 9.0)
4″	3.32 (m)	3.29 (m)	3.29 (m)	3.28 (m)
5″ax	3.12 (t, 11.1)	3.11 (t, 10.7)	3.12 (t, 11.1)	3.10 (t, 11.1)
5″eq	3.75 (dd, 5.1, 11.1)	3.77 (dd, 5.1, 11.1)	3.74 (dd, 5.1, 11.1)	3.77 (dd, 5.1, 11.1)
1‴	—	—	5.08 (d, 7.3)	5.08 (d, 7.3)
2‴	—	—	3.35 (m)	3.34 (m)
3‴	—	—	3.34 (m)	3.33 (m)
4‴	—	—	3.44 (m)	3.44 (m)
5‴ax	—	—	3.43 (m)	3.43 (m)
5‴eq	—	—	3.79 (m)	3.76 (m)

Table 3. ^{13}C NMR data.

		Pradimicin H	Pradimicin FH	11-*O*-L-Xylosyl-pradimicin H	11-*O*-L-Xylosyl-pradimicin FH
C-1	(s)	152.9	153.2	151.1	152.4
2	(s)	127.2	131.8	127.4	131.6
3	(s)	137.0	137.6	137.3	137.6
3-CH_3	(q)	19.3	20.2	19.0	20.2
4	(d)	118.8	116.2	119.5	116.6
4a	(s)	137.4	138.0	137.6	137.8
5	(d)	81.0	81.9	80.7	81.8
6	(d)	71.3	71.6	71.3	71.5
6a	(s)	146.5	143.1	147.8	143.2
7	(d)	114.8	111.0	116.0	111.4
7a	(s)	131.5	132.4	131.2	133.0
8	(s)	185.1	180.1	185.1	179.6
8a	(s)	109.2	109.3	111.1	111.1
9	(s)	165.5	165.2	164.1	165.5
10	(d)	108.2	107.0	109.5	107.1
11	(s)	164.4	164.8	163.5	163.2
12	(d)	107.5	105.3	108.5	106.4
12a	(s)	135.2	136.8	134.4	136.9
13	(s)	186.2	186.8	187.2	187.4
13a	(s)	116.4	119.0	115.6	119.0
14	(s)	158.7	158.4	156.7	158.7
14a	(s)	127.0	125.4	126.0	125.6
14b	(s)	115.2	118.0	113.9	118.9
15	(s)	167.1	168.4	166.8	168.3
17	(d)	47.5	54.8	47.6	54.8
18	(s)	173.7	171.9	173.6	171.9
19		16.9 (q)	61.6 (t)	16.8 (q)	61.5 (t)
1′	(d)	103.9	104.1	104.0	104.0
2′	(d)	69.8	70.2	69.9	70.0
3′	(d)	78.9	81.7	78.5	81.0
4′	(d)	63.1	63.0	63.1	63.0
4′-NMe	(q)	36.1	36.9	36.1	36.6
5′	(d)	67.3	68.9	67.3	68.3
5′-CH_3	(q)	15.9	16.4	16.0	16.2
1″	(d)	104.7	105.0	104.5	105.0
2″	(d)	73.4	73.6	73.4	73.5
3″	(d)	75.8	75.8	75.8	75.9
4″	(d)	69.3	69.3	69.3	69.2
5″	(t)	65.6	65.6	65.6	65.6
1‴	(d)	—	—	100.5	100.4
2‴	(d)	—	—	72.7	72.7
3‴	(d)	—	—	75.8	75.8
4‴	(d)	—	—	69.1	69.1
5‴	(t)	—	—	65.3	65.6

a 1 : 1 mixture of methyl β-D- and L-xylopyranoside ($[\alpha]_D = 0°$), which indicated the newly incorporated xylosyl moiety at C-11 to have the L-configuration as in the case of pradimicin T1.

Biological Properties

Table 4 lists MICs of 11-*O*-L-xylosylpradimicins H and FH along with those of amphotericin B and ketoconazole. There is no cross resistance between the pradimicins and amphotericin B or ketoconazole. Table 5 summarizes *in vivo* efficacies against a systemic candida infection and tolerance in mice. No acute toxicity was observed with either of the two pradimicins at 300 mg/kg in mice after an iv administration.

Table 4. *In vitro* antifungal activity.

Test organisms	MIC (μg/ml)			
	11-*O*-L-Xylosyl-pradimicin H	11-*O*-L-Xylosyl-pradimicin FH	Amphotericin B	Ketoconazole
Saccharomyces cerevisiae ATCC 9763	3.1	3.1	0.4	50
Candida albicans IAM 4886	6.3	6.3	0.4	50
C. albicans A9540	6.3	12.5	0.4	50
C. albicans ATCC 28247	1.6	3.1	12.5	12.5
C. albicans ATCC 32354	6.3	6.3	0.4	50
C. albicans 83-2-14	12.5	12.5	0.4	25
C. tropicalis 85-8	25	25	0.8	50
C. tropicalis IFO 10241	25	25	0.8	50
Cryptococcus neoformans D49	1.6	6.3	0.4	0.8
C. neoformans IAM 4514	1.6	3.1	0.4	0.4
Aspergillus fumigatus IAM 2034	6.3	12.5	0.8	6.3
Trichophyton mentagrophytes No. 4329	6.3	6.3	0.8	6.3

Medium: yeast morphology agar + 1/15 M phosphate buffer (pH 7.0). Inoculum: 10^6 cells/ml (except for *T. mentagrophytes*: 10^7 cells/ml). Incubation conditions: 28°C, 40 hours (60 hours *T. mentagrophytes*).

Table 5. *In vivo* efficacies against *C. albicans* A9540 systemic infection and tolerance in mice.

Compounds	PD_{50} (mg/kg)	LD_{50} (mg/kg)
11-*O*-L-Xylosylpradimicin H	18	>300
11-*O*-L-Xylosylpradimicin FH	20	>300

Discussion

The biosynthesis of microbial products continues to provide useful ground for testing new strategies. The method described here is based on the knowledge gained during the studies on the biosynthesis of the pradimicin family. It is our belief that this method should be applicable to the production of 11-*O*-L-xylosylbenanomicins. It is also important to note that easy access to pradimicins H and FH makes them valuable intermediates for chemical modification of ring E. The antifungal activities observed for 11-*O*-L-xylosylpradimicins H and FH may provide a new dimension in the structure-activity relationship studies.

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