PRADIMICINS T1 AND T2, NEW ANTIFUNGAL ANTIBIOTICS PRODUCED BY AN ACTINOMYCETE

II. STRUCTURES AND BIOSYNTHESIS

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Pradimicins T1 and T2 are new members of the pradimicin family of antibiotics produced by an actinomycete strain AA3798. Pradimicins T1 and T2 are dihydrobenzo[*a*]naphthacenequinones substituted with 3 and 2 sugar moieties, respectively. The salient feature in their structures is an L-xylose attached to the phenolic hydroxyl group at C-11. Bioconversion experiments using a blocked mutant B-54 of strain AA3798 not only explored a plausible biosynthetic pathway of pradimicins T1 and T2, but also provided evidence of 5*S*,6*S* configuration.

An actinomycete strain AA3798, which had been isolated from a soil sample collected at Nerima, Tokyo, Japan, was shown to produce a pair of new antifungal antibiotics. In the preceding paper[1], we have described the taxonomy of the producing strain, production, isolation, physico-chemical and biological properties of these antibiotics designated pradimicin T1 (PRM T1) and pradimicin T2 (PRM T2). In this paper, we describe the structures and biosynthesis of PRM T1 and PRM T2.

Structure of PRM T1

The molecular formula of PRM T1 determined as $C_{42}H_{45}NO_{23}$ from elemental analysis (*Anal.* Calcd for $C_{42}H_{45}NO_{23} \cdot 2H_2O$: C 52.12, H 5.10, N 1.45; Found: C 52.41, H 4.84, N 1.41), was confirmed by HRFAB-MS (Observed: 932.2453, Calcd for M+H: 932.2461) and ^{13}C NMR measurements.

The ^1H NMR spectrum of PRM T1 showed 2 singlets at δ 7.16 (4-H) and 8.09 ppm (7-H), 2 *meta*-coupled doublets at δ 6.98 (10-H) and 7.38 ppm (12-H), a pair of doublets at 4.52 (5-H) and 4.62 ppm (6-H), and a three-proton singlet at δ 2.35 ppm assignable to the aromatic methyl substituent at C-3, indicating a structure and the positions of substituents similar to those of PRM A[2,3]. However, the two-proton doublet at δ 3.95 ppm coupled to NH at δ 8.37 ppm, suggested a glycine side chain in place of the D-alanine in PRM A. This was supported by the prominent FAB-MS peak at *m*/*z* 857 (MH^+-glycine) and confirmed by the isolation of the aglycone **1** (FAB-MS *m*/*z* 522 $(M+H)^+$) from the methanolysis products of PRM T1.

The ^{13}C and DEPT NMR experiments showed the presence of 20 sp^3 carbons including 2 CH_3, 3 CH_2 and 15 CH, and 22 sp^2 carbons including 4 CH, 14 quaternary and 4 carbonyl carbons. The ^1H and ^{13}C NMR assignments (Table 1) were based on the ^1H-^1H COSY, ^1H-^1H NOESY and ^{13}C-^1H COSY spectra, and the full structure of PRM T1 (Fig. 1) was established by comparing these NMR data with those of PRM A[3]. The presence of 3 sugar moieties and the positions to which the fucose and one of the xylose moieties attached came from the ^1H-^{13}C long range couplings from 5-H (δ 4.52) to C-1′

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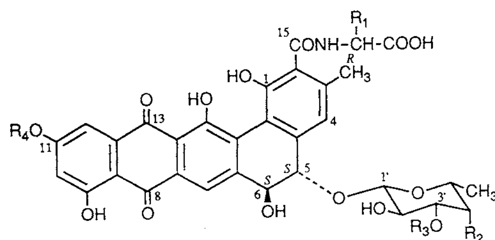
Table 1. ^{1}H and ^{13}C NMR assignments of PRM T1 in DMSO-d_6.

Position	δ_H	δ_C	Position	δ_H	δ_C
Chromophore			Glycine		
1		151.3 (s)	$N^{16}H$	8.37 (t, 5.55)	
2		126.8 (s)	17	3.95 (d, 5.55)	40.8 (t)
3		137.3 (s)	CO_2H		170.8 (s)
3-CH_3	2.35 (s)	19.1 (q)	5-*O*-Fucose		
4	7.16 (s)	118.9 (d)	1′	4.63 (d, 7.70)	104.2 (d)
4a		138.1 (s)	2′	3.73 (dd, 7.70, 9.40)	70.0 (d)
5	4.52 (d, 10.26)	81.2 (d)	3′	3.56 (dd, 9.40, 2.99)	82.8 (d)
6	4.62 (d, 10.26)	71.7 (d)	4′	3.63 (br d, 2.99)	70.3 (d)
6a		147.7 (s)	5′	3.61 (m)	70.0 (d)
7	8.09 (s)	115.5 (d)	5′-CH_3	1.14 (d, 6.39)	16.1 (q)
7a		131.2 (s)	3′-*O*-Xylose		
8		185.2 (s)	1″	4.45 (d, 6.84)	105.0 (d)
8a		111.1 (s)	2″	3.14 (dd, 6.84, 7.27)	73.5 (d)
9		164.1 (s)	3″	3.18 (dd, 8.55, 7.27)	75.9 (d)
10	6.98 (d, 2.56)	109.4 (d)	4″	3.32 (ddd, 8.55, 5.12, 10.26)	69.3 (d)
11		163.5 (s)			
12	7.38 (d, 2.56)	108.5 (d)	5″-H_{ax}	3.09 (dd, 10.26, 11.11)	65.4 (t)
12a		134.4 (s)	5″-H_{eq}	3.73 (dd, 5.12, 11.11)	
13		187.1 (s)	11-*O*-Xylose		
13a		115.5 (s)	1‴	5.17 (d, 6.84)	100.5 (d)
14		156.9 (s)	2‴	3.34 (m)	72.7 (d)
14a		125.8 (s)	3‴	3.34 (m)	75.8 (d)
14b		113.8 (s)	4‴	3.43 (m)	69.1 (d)
15		167.5 (s)	5‴-H_{ax}	3.43 (m)	65.7 (t)
			5‴-H_{eq}	3.75 (m)	

ppm (multiplicity, J=Hz).

Fig. 1. Structures of pradimicins.



Compound	R_1	R_2	R_3	R_4
PRM A	CH_3	$NHCH_3$	HO HO O OH	CH_3
PRM T1	H	OH	HO HO O OH	HO O OH HO
PRM T2	H	OH	H	HO O OH HO
2	H	OH	HO HO O OH	H
3	H	OH	H	H

(δ 104.2 ppm) and from 3'-H (δ 3.56 ppm) to C-1'' (δ 105.0 ppm). The attachment of the second xylose to the phenolic hydroxyl group at C-11 was ascertained by the observed NOEs between 1'''-H (δ 5.17 ppm) and 10-H (δ 6.98 ppm) and between 1'''-H and 12-H (δ 7.38 ppm) in the NOESY spectrum of PRM T1, and confirmed by converting PRM T1 into 11-*O*-dexylosylpradimicin T1 (**2**) (FAB-MS *m/z* 800 $(M+H)^+$; IR 1730 and 1620 cm^{-1}) by mild alkaline hydrolysis.

The stereochemical identities of the 3 sugars were proved by comparing the methylglycoside-2,3,4-triacetates isolated from the degradation (methanolysis/acetylation) products of PRM T1 and **2** with authentic samples. The methyl α-fucopyranoside-2,3,4-triacetate isolated from the degradation products of PRM T1 gave an $[\alpha]_D^{27}$ value of $+145°$ (*c* 2.42, $CHCl_3$), which corresponds to the D-isomer (Lit.[4] $[\alpha]_D^{22} = +146°$), indicating D-configuration of the fucose at C-5. Since PRM T1 apparently gave racemic methyl α-xylopyranoside-2,3,4-triacetate, **2** was subjected to the same degradation sequence. The methyl α-xylopyranoside-2,3,4-triacetate derived from **2** gave an $[\alpha]_D^{27}$ value of $+116°$ (*c* 1.30, $CHCl_3$), which corresponds to the D-isomer (Lit.[4] $[\alpha]_D^{22} = +121°$), indicating D-configuration of the xylose at C-3'. The L-configuration of the xylose at C-11 was ascertained by the ^1H NMR experiments on the methyl α-xylopyranoside-2,3,4-triacetate derived from PRM T1 using a chiral shift reagent, (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol. The ^1H NMR spectrum in $CDCl_3$ in the presence of 5 equiv of the chiral reagent showed a pair of OCH_3 signals at δ 3.38 and 3.39 ppm, where the signal at δ 3.39 ppm was enhanced by the addition of the authentic sample of methyl α-L-xylopyranoside-2,3,4-triacetate.

Structure of PRM T2

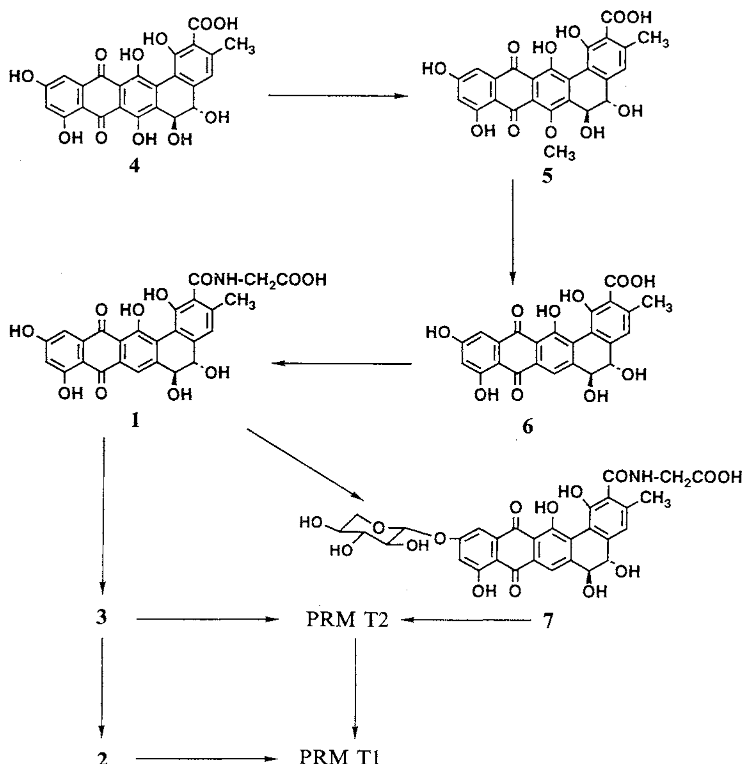
The molecular formula of PRM T2 determined as $C_{37}H_{37}NO_{19}$ from the FAB-MS (positive) peak

Table 2. ^1H and ^{13}C NMR assignments of PRM T2 in DMSO-d_6.

Position	δ_H	δ_C	Position	δ_H	δ_C
Chromophore			Glycine		
1		163.3 (s)	$N^{16}H$	8.32 (m)	
2		126.3 (s)	17	3.88 (d, 4.70)	41.5 (t)
3		136.3 (s)	CO_2H		171.3 (s)
3-CH_3	2.30 (s)	19.8 (q)	5-*O*-Fucose		
4	6.99 (s)	116.0 (d)	1'	4.55 (d, 7.69)	104.9 (d)
4a		137.9 (s)	2'	3.53 (dd, 7.69, 9.83)	71.3 (d)
5	4.38 (d, 10.68)	82.3 (d)	3'	3.42 (dd, 2.99, 9.83)	73.5 (d)
6	4.46 (d, 10.68)	72.0 (d)	4'	3.47 (br d, 2.99)	71.0 (d)
6a		143.8 (s)	5'	3.59 (m)	70.2 (d)
7	7.78 (s)	110.5 (d)	5'-CH_3	1.15 (d, 6.41)	16.3 (q)
7a		132.9 (s)	11-*O*-Xylose		
8		187.3 (s)	1'''	5.08 (d, 6.84)	100.4 (d)
8a		111.0 (s)	2'''	3.34 (m)	72.7 (d)
9		166.4 (s)	3'''	3.33 (m)	75.9 (d)
10	6.76 (d, 2.14)	106.5 (d)	4'''	3.44 (m)	69.1 (d)
11		163.2 (s)	5'''-H_{ax}	3.38 (m)	65.6 (t)
12	7.24 (d, 2.14)	107.3 (d)	5'''-H_{eq}	3.75 (m)	
12a		138.0 (s)			
13		180.0 (s)			
13a		118.7 (s)			
14		157.4 (s)			
14a		131.8 (s)			
14b		118.4 (s)			
15		168.6 (s)			

ppm (multiplicity, J = Hz).

Fig. 2. A plausible biosynthetic pathway of PRM T1 and PRM T2.



at m/z 800 (M+H) and elemental analysis (*Anal.* Calcd for $C_{37}H_{37}NO_{19} \cdot 2H_2O$: C 53.18, H 4.94, N 1.68; Found: C 52.75, H 5.00, N 1.95) was confirmed by the ^{13}C NMR measurement. Comparison of the spectral data of PRM T2 with those of PRM T1 allowed us to assign the ^1H and ^{13}C NMR signals (Table 2) and the structure of PRM T2 (Fig. 2). The attachment of the fucose moiety to C-5 was based on the ^1H-^{13}C long range coupling from 5-H (δ 4.38) to C-1′ (δ 104.9) and the attachment of the xylose moiety to C-11 came was confirmed by converting PRM T2 into 11-*O*-dexylosylpradimicin T2 (**3**) (FAB-MS m/z 668 $(M+H)^+$; IR 1730 and 1610 cm^{-1}).

The stereochemistry of the sugar moieties in PRM T2 was established by degradation experiments. The methyl α-fucopyranoside-2,3,4-triacetate and methyl α-xylopyranoside-2,3,4-triacetate isolated from the degradation products of PRM T2 gave $[\alpha]_D^{28}$ values of +119° (*c* 2.10, $CHCl_3$) and −108° (*c* 2.05, $CHCl_3$), respectively, indicating D-fucose at C-5 and L-xylose at C-11.

The presence of the L-xylose moiety in PRM T1 and PRM T2 is quite unusual, since no natural products containing such sugar have ever been reported in literature.

Biosynthesis of PRM T1 and PRM T2

During the biosynthetic studies on PRM A[5~7], we isolated blocked mutants JN-219 and JN-213 of *Actinomadura verrucosospora* subsp. *neohibisca* E-40, a high PRM A-producer. The mutant JN-219 produces 11-*O*-demethylpradinone II (**4**), 7-*O*-methyl-11-*O*-demethylpradinone II (**5**) and 11-*O*-demethylpradinone I (**6**), while the mutant JN-213 is able to convert these aglycones into PRM A, providing definite evidence for the biosynthetic pathway from **4** to PRM A through **5** and **6**. Since these aglycones

Table 3. Bioconversion of 11-*O*-demethylpradinone II (**4**) by growing cultures of blocked mutant B-54.

Hours	Bioconversion efficiencies (mol. %)						
	4	**5**	**1**	**3**	**2**	PRM T2	PRM T1
0	100.0	0	0	0	0	0	0
2	93.1	0	0	0	0	2.1	3.8
4	87.4	1.0	0	0.1	0.1	4.9	6.1
6	77.0	4.1	0	0.3	0.4	7.0	11.2
8	63.8	5.0	0	2.1	6.1	9.5	13.5
10	56.4	8.1	0	2.2	8.2	12.7	17.6
15	36.7	13.5	0	0	0	19.4	30.4
19	23.7	16.8	0	0	0	22.6	36.9
26	0	11.5	9.7	0	0	30.7	47.9
30	0	1.5	8.6	0	0	36.2	54.1

Method: Strain B-54 was preincubated at 28°C for 48 hours in 4 ml of medium composed of glucose 3%, Bact soytone 1.5% and $CaCl_2 \cdot 2H_2O$ 0.05%, and **4** at a final concentration of 0.8 μM was added. Incubation was continued for additional 30 hours and the fate of **4** was monitored by HPLC on YMC-ODS A 301-5 (4.6 i.d. × 100 mm) using acetonitrile - 0.1 M phosphate buffer (17~25 : 83~75, pH 3.5) as eluent at a flow rate of 1 ml/minute with detection at 460 nm.

contain a phenolic hydroxyl group at C-11, we became interested in the biosynthetic pathway of PRM T1 and PRM T2 in order to understand producer-metabolite relationships.

A blocked mutant B-54 selected for microbial conversion was generated from strain AA3798 by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment. The mutant B-54 was preincubated at 28°C for 48 hours in a medium composed of glucose 3%, soytone 1.5% and $CaCl_2 \cdot 2H_2O$ 0.05%. The possible biosynthetic intermediates of PRM T1 including PRM T2 at a final concentration of 0.5~0.8 μM were separately fed to the growing culture and incubation was continued for additional 30~72 hours. Since strain B-54 did not produce any detectable amount of pradimicins apparently due to the block in the early part of the biosynthetic pathway, the fate of these intermediates was readily monitored by HPLC.

Table 4. Bioconversion of possible biosynthetic intermediates of PRM T1 by growing cultures of blocked mutant B-54.

Substrate added	Bioconversion efficiencies (mol. %)		Recovery of substrate (mol. %)
	PRM T2	PRM T1	
4	48.3	51.8	0
5	37.7	62.3	0
6	39.1	60.7	0
1	48.4	51.6	0
7	35.6	64.7	0
3	91.6	8.5	0
2	5.3	94.7	0
PRM T2	79.9	20.1	79.9
PRM T1	5.0	95.5	95.5

Method: Strain B-54 was preincubated at 28°C for 48 hours in 4 ml of medium composed of glucose 3%, Bact soytone 1.5% and $CaCl_2 \cdot 2H_2O$ 0.05%, and each substrate at a final concentration of 0.5~0.8 μM was added. Incubation was continued for additional 72 hours and the product was analyzed by HPLC on YMC-ODS A 301-5 (4.6 i.d. × 100 mm) using acetonitrile - 0.1 M phosphate buffer (17~25 : 83~75, pH 3.5) as eluent at a flow rate of 1 ml/minute with detection at 460 nm.

Table 3 summarizes the result of the bioconversion experiment with **4** showing the incorporation of **4** into PRM T2 and PRM T1 and the possible intermediates **5**, **1**, **3**, and **2**. Table 4 summarizes the results of similar bioconversion experiments with 8 potential intermediates of PRM T1. The efficient conversion of these intermediates allowed us to propose a plausible biosynthetic pathway of PRM T1 and PRM T2 (Fig. 2).

The bioconversion experiments presented here provided evidence that both PRM T1 and PRM T2 have 5*S*,6*S* configuration since the stereochemistry of **5** has been defined previously[6]. When the

biosynthetic pathway of PRM T1 and PRM T2 is compared with that of PRM A, the major difference is the step introducing a substituent at C-11 after incorporation of the amino acid side chain. It is also important to note that the L-xylosyl moiety is introduced into both **2** and **3**. This implies the possibility of microbial modification of pradimicin analogs and derivatives having a phenolic hydroxyl group at C-11 using the mutant B-54.

Experimental

General Procedures

The melting points are uncorrected. Spectral data were recorded on the following instruments: ^{1}H and ^{13}C NMR, JEOL JMN-GX 400; IR, JASCO IR-810 spectrometer; UV-vis, JASCO UVIDEC-610 spectrometer; low and high resolution FAB-MS, JEOL JMS-AX 505H spectrometer; optical rotation, JASCO DIP-140 digital polarimeter. For TLC analysis, E. Merck precoated Kieselgel $60F_{254}$ plates (0.25 mm) were used.

Methanolysis of PRM T1

A solution of PRM T1 (300 mg) in 50 ml of 10% HCl - MeOH was refluxed for 3 hours. The reaction mixture was cooled, filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in 1 ml of acetic anhydride and 2 ml of pyridine, and stirred at 25°C for 15 hours. The solvent was removed *in vacuo* and the residue chromatographed on a column of silica gel (Kieselgel 60, 230 ~ 400 mesh) using 1 : 7 acetone - hexane as eluent to give 12 mg of methyl α-D-fucopyranoside-2,3,4-triacetate: $[\alpha]_D^{27} = +145°$ (*c* 2.42, $CHCl_3$); FAB-MS *m/z* 305 $(M+H)^+$, and 24 mg of racemic methyl α-xylopyranoside-2,3,4-triacetate: FAB-MS *m/z* 291 $(M+H)^+$. The ^{1}H NMR spectrum in $CDCl_3$ showed signals at δ 2.02 (6H, s, 2 × OAc), 2.07 (3H, s, OAc), 3.39 (3H, s, OCH_3), 3.58 (1H, t, J=10.7 Hz, H_{ax} at C-5), 3.79 (1H, dd, J=10.7, 6.0 Hz, H_{eq} at C-5), 4.83 (1H, dd, J=9.8, 3.4 Hz, 2-H), 4.88 (1H, d, J=3.4 Hz, 1-H), 4.96 (1H, ddd, J=10.7, 9.8, 6.0 Hz, 4-H) and 5.47 (1H, t, J=9.8 Hz, 3-H). Addition of 5 equiv of (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol resolved the peaks at δ 3.58, 3.79 and 3.39.

The aglycone solidified in the methanolysis product was collected by filtration, washed with MeOH and redissolved in 10 ml of 1 N NaOH. The solution was stirred at 25°C for 15 hours and acidified with 1 N HCl. The solid formed was collected by filtration and dried to give 100 mg of **1**: MP 200 ~ 210°C; FAB-MS *m/z* 522 $(M+H)^+$, IR 1730 and 1620 cm^{-1}; UV λ_{max} (0.02 N NaOH - MeOH) 247, 308 and 504 nm; ^{1}H NMR δ ($DMSO-d_6$ - D_2O) 2.36 (3H, s), 3.91 (2H, s), 4.22 (1H, d), 4.28 (1H, d), 6.65 (1H, d), 7.08 (1H, s), 7.22 (1H, d) and 8.09 (1H, s).

Alkaline Hydrolysis of PRM T1

A solution of PRM T1 (500 mg) in 80 ml of 1 N NaOH was heated at 65°C for 7 hours. The reaction mixture was cooled and adjusted to pH 3.0 with 6 N HCl. The solid formed was collected by centrifugation (3,000 rpm) and purified by chromatography on a column of YMC GEL ODS-A60 (1.35 liters) using acetonitrile - 0.01 M phosphate buffer (7 : 93, pH 3.5) as eluent to afford **2** (294 mg, 68% yield): MP 200 ~ 210°C (dec); FAB-MS *m/z* 800 $(M+H)^+$; UV (0.02 N NaOH - MeOH, 1 : 1) λ_{max} nm (ε) 246 (32,500), 308 (21,300), 505 (15,300); IR (KBr) 1730 and 1620 cm^{-1}; ^{1}H NMR δ ($DMSO-d_6$) 1.14 (3H, d), 2.37 (3H, s), 3.09 (1H, dd), 3.14 (1H, dd), 3.18 (1H, dd), 3.33 (1H, ddd), 3.56 (1H, dd), 3.61 (1H, m), 3.63 (1H, m), 3.73 (2H, m), 3.94 (2H, d), 4.44 (1H, d), 4.52 (1H, d), 4.61 (1H, d), 4.63 (1H, d), 6.65 (1H, d), 7.21 (1H, s), 7.24 (1H, d), 8.07 (1H, s), 8.36 (1H, t, exchangeable with D_2O).

Methanolysis of 2

A solution of **2** (250 mg) in 50 ml of 10% HCl - MeOH was refluxed for 3 hours. The reaction mixture was cooled, filtered and the filtrate was concentrated *in vacuo*. The residue was acetylated in 1 ml of acetic anhydride and 2 ml of pyridine at 25°C for 15 hours. The solvent was removed *in vacuo* and the residue chromatographed on silica gel using 1 : 7 acetone - hexane to give 15.6 mg of methyl α-D-xylopyranoside-2,3,4-triacetate: $[\alpha]_D^{27} = +116°$; FAB-MS *m/z* 291 $(M+H)^+$, and 29.1 mg of methyl α-D-fucopyranoside-

2,3,4-triacetate.

Alkaline Hydrolysis of PRM T2

A solution of PRM T2 (300 mg) in 50 ml of 1 N NaOH was heated at 65°C for hours. The reaction mixture was cooled and adjusted to pH 3.0 with 6 N HCl. The solid formed was collected by centrifugation (3,000 rpm) and purified by chromatography on a column of YMC GEL ODS-A60 (1.1 liters) using acetonitrile - 0.01 M phosphate buffer (7:93, pH 3.5) as eluent to afford **3** (180 mg, 72% yield): MP 225~235°C (dec); FAB-MS *m/z* 668 $(M+H)^+$; HRFAB-MS *m/z* 668.1643 (Calcd for $C_{32}H_{29}NO_{15}+H$: 668.1615); UV (0.02 N NaOH - MeOH, 1:1) λ_{max} nm (ε) 246 (32,300), 308 (22,400) and 504 (16,800); IR (KBr) 1730 and 1610 cm^{-1}; ^1H NMR δ (DMSO-d_6) 1.14 (3H, d), 2.35 (3H, s), 4.45 (1H, d), 4.54 (1H, d), 4.56 (1H, d), 6.65 (1H, d), 7.17 (1H, s), 7.22 (1H, d), 8.04 (1H, s), 8.57 (1H, t, exchangeable with D_2O).

Methanolysis of PRM T2

A solution of PRM T2 (300 mg) in 60 ml of 10% HCl - MeOH was refluxed for 1.5 hours. The reaction mixture was cooled, filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in 1 ml of acetic anhydride and 2 ml of pyridine, and stirred at 25°C for 15 hours. The solvent was removed *in vacuo* and the residue chromatographed on a column of silica gel (Kieselgel 60) using 1:7 acetone - hexane as eluent to give 23.3 mg of methyl α-D-fucopyranoside-2,3,4-triacetate: $[\alpha]_D^{28} = +119°$ (*c* 2.10, $CHCl_3$); FAB-MS *m/z* 305 $(M+H)^+$, and 20.9 mg of methyl α-L-xylopyranoside-2,3,4-triacetate: $[\alpha]_D^{28} = -108°$ (*c* 2.05, $CHCl_3$); FAB-MS *m/z* 291 $(M+H)^+$.

5-*O*-Defucosylpradimicin T2 (7)

5-*O*-Defucosylpradimicin T2 (**7**) was produced by a blocked mutant B-10 derived from *Actinomadura* sp. AB1236[8]. Strain B-10 was preincubated at 28°C for 3 days in ten 500-ml Erlenmeyer flasks containing 100 ml of medium composed of glucose 2%, Pharmamedia (Traders Protein) 1% and KH_2PO_4 0.1% on a rotary shaker (200 rpm). PRM T2 at a final concentration of 100 μg/ml was added to the flasks and incubation was continued for additional 11 days. The fermentation broth was centrifuged at 3,000 rpm for 20 minutes and the supernatant (850 ml) mixed with 500 ml of Diaion HP-20. The resin was washed with 2 liters of water, and the product was eluted with 1.5 liters of 60% aq acetone. The eluate was concentrated *in vacuo* and the aq residue was chromatographed on a column of YMC GEL ODS-A60 (1.5 liters, YMC Co., Ltd.) using acetonitrile - 0.01 M phosphate buffer (12:88, pH 5.1) as eluent. The fractions containing the major product were combined and concentrated *in vacuo*, and the aq residue was mixed with 30 ml of Diaion HP-20. The resin was washed with 300 ml of water and the product eluted with 100 ml of 60% aq acetone. The acetone was removed *in vacuo* and the aq residue lyophilized to give 65.9 mg of **7**: FAB-MS *m/z* 653 $(M+H)^+$. The ^1H NMR spectrum in DMSO-d_6 - D_2O showed one-proton doublet at δ 5.18 (J = 7.27 Hz) assignable to the anomeric proton of the xylose moiety at C-11.

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References

1) FURUMAI, T.; T. HASEGAWA, M. KAKUSHIMA, K. SUZUKI, H. YAMAMOTO, S. YAMAMOTO, M. HIRANO & T. OKI: Pradimicins T1 and T2, new antifungal antibiotics produced by an actinomycete. I. Taxonomy, production, isolation, physico-chemical and biological properties. J. Antibiotics 46: 589~597, 1993
2) 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
3) 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
4) 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

5) FURUMAI, T.; S. KAKINUMA, H. YAMAMOTO, N. KOMIYAMA, K. SUZUKI, K. SAITOH & T. OKI: Biosynthesis of the pradimicin family of antibiotics. I. Generation and selection of pradimicin-nonproducing mutants. J. Antibiotics 46: 412~419, 1993
6) TSUNO, T.; H. YAMAMOTO, Y. NARITA, K. SUZUKI, T. HASEGAWA, S. KAKINUMA, K. SAITOH, T. FURUMAI & T. OKI: Biosynthesis of the pradimicin family of antibiotics. II. Fermentation, isolation and structure determination of metabolites associated with the pradimicins biosynthesis. J. Antibiotics 46: 420~429, 1993
7) KAKINUMA, S.; K. SUZUKI, M. HATORI, K. SAITOH, T. HASEGAWA, T. FURUMAI & T. OKI: Biosynthesis of the pradimicin family of antibiotics. III. Biosynthetic pathway of both pradimicins and benanomicins. J. Antibiotics 46: 430~440, 1993
8) FURUMAI, T.; K. SAITOH, M. KAKUSHIMA, S. YAMAMOTO, K. SUZUKI, C. IKEDA, S. KOBARU, M. HATORI & T. OKI: BMS-181184, a new pradimicin derivative. Screening, taxonomy, directed biosynthesis, isolation and characterization. J. Antibiotics 46: 265~274, 1993