Notes

PRADIMICIN Q, A NEW PRADIMICIN AGLYCONE, WITH α-GLUCOSIDASE INHIBITORY ACTIVITY

YOSUKE SAWADA, TAKASHI TSUNO, TOMOKAZU UEKI, HARUAKI YAMAMOTO, YASUO FUKAGAWA and TOSHIKAZU OKI

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

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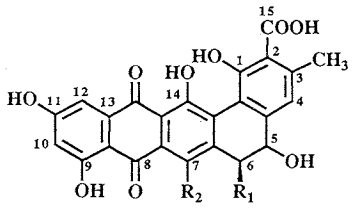
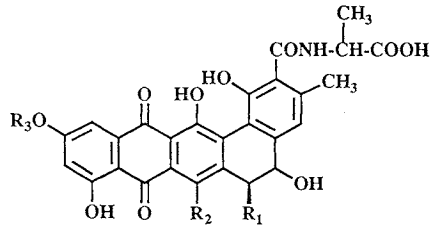
Several pradimicin-family antibiotics have recently been found to possess potent antifungal activity, while their aglycones are inactive against fungi. In the course of the production improvement study of pradimicin L (Fig. 1) by *Actinomadura verrucosospora* subsp. *neohibisca* R103-3[1], a strain numbered A10102 was found to produce a new pradimicin aglycone designated pradimicin Q which exhibited a potent α-glucosidase inhibitory activity *in vitro*. In this paper, we wish to describe the fermentation, isolation, physico-chemical properties and structure of pradimicin Q. Comparative α-glucosidase inhibitory activities of pradimicin Q and other pradimicin aglycones are also reported.

Strain A10102 was isolated from MNNG-treated *A. verrucosospora* subsp. *neohibisca* R103-3 spores according to a procedure as previously described[2] and was fermented at 28°C for 14 days on a rotary shaker operating at 200 rpm in a 500-ml Erlenmeyer flask containing 100 ml of production medium consisting of soluble starch (Nichiden Kagaku) 1%, glucose 1%, L-glutamic acid 0.1%, L-methionine 0.05%, L-arginine 0.05%, $(NH_4)_2SO_4$ 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.05%, $CaCO_3$ 0.3%, K_2HPO_4 0.6% and salts solution 1% (v/v) ($FeSO_4 \cdot H_2O$ 0.1 g, $ZnSO_4 \cdot 7H_2O$ 0.1 g and $MnCl_2 \cdot 4H_2O$ 0.1 g in 1 liter of water), pH 7.0 before autoclaving. The fermentation broth (4.8 liters) was

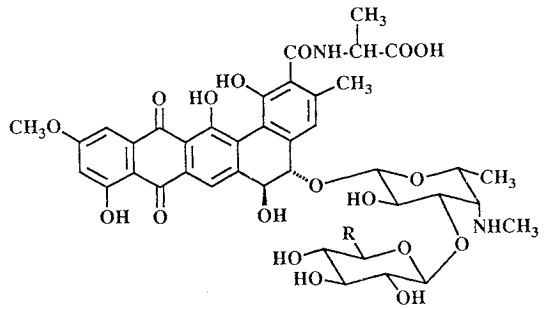
Fig. 1. Structures of pradimicins.



Q: R_1=H R_2=OH
M: R_1=OH R_2=H



N (=benanomicin C): R_1=OH R_2=OCH_3 R_3=CH_3
O: R_1=H R_2=OCH_3 R_3=CH_3
P: R_1=OH R_2=OH R_3=H



Pradimicin A: R=H
Pradimicin L: R=CH_2OH

Correspondence should be addressed to JUN OKUMURA, Bristol-Myers Squibb Research Institute, 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

centrifuged at 5,000 rpm for 10 minutes at room temperature. The supernatant (4.5 liters) was adjusted to pH 2.0 with 6 N HCl and mixed with 2 liters of ethyl acetate. The ethyl acetate layer was separated, rinsed twice with water (200 ml each), and concentrated to give a crude solid (453 mg). The crude solid was then dissolved in CH_3CN-0.15% KH_2PO_4 (pH 3.5) (1:1), and applied onto a column of ODS-A60 (200 ml, YMC Co., Ltd.). Elution was carried out with the same solvent system. Each fraction was monitored by HPLC (Waters M600, column: Cosmosil C_{18}-AR, 4.6×150 mm; elution: CH_3CN-0.15% KH_2PO_4 (pH 3.5) (3:7); flow rate: 0.7 ml/minute; detection: at 254 nm), and pradimicin Q-containing fractions (Rt: 17.1 minutes for pradimicin Q) were pooled and concentrated to give a purple-red solid (190 mg). The solid (50 mg) was dissolved in 2 ml of MeOH-H_2O (3:2) and subjected to Sephadex LH-20 column chromatography using the same solvent system. Fractions containing pradimicin Q were pooled and concentrated *in vacuo* to give needles in free form (35 mg). The purity of pradimicin Q was determined both by HPLC under the above-described conditions and by silica gel TLC (Kieselgel $60F_{254}$, Merck Co.) with MeOAc-*n*-PrOH-28% NH_4OH (45:105:60, v/v) (Rf: 0.23).

Physico-chemical properties of pradimicin Q are summarized in Table 1. The UV and visible spectra of pradimicin Q closely resembled those of

Table 1. Physico-chemical properties of pradimicin Q.

Nature	Purple-red powder
MP (dec.)	>200°C
HRFAB-MS $(M+H)^+$, *m/z*	Found 465.0811 (Calcd: 465.0800)
Molecular formula	$C_{24}H_{16}O_{10}$
UV λ_{max} nm (ε)	
in MeOH	229 (25,600), 288 (19,500), 514 (13,200)
in 0.01 N HCl-MeOH (1:1)	232 (25,800), 289 (20,600), 512 (14,000)
in 0.01 N NaOH-MeOH (1:1)	242 (20,000), 305 (19,600), 549 (15,900)
IR (KBr) cm^{-1}	3197, 1712, 1600, 1488, 1399, 1245, 1187
CD λ nm ($\Delta\varepsilon$/mol·cm) in MeOH	212 (+11.4), 225 (−4.4), 259 (−23.2), 286 (+6.6)

Table 2. ^1H NMR data of pradimicins Q and P (400 MHz, in DMSO-d_6).

Proton	Pradimicin Q	Pradimicin P
3-CH_3	2.504 (s)	2.34 (s)
4-H	6.97 (s)	6.84 (s)
5-H	4.55 (dd, J=4.3, 9.8)	4.53 (d, J=3.4)
6-H_{ax}	2.66 (dd, J=9.8, 15.8)	—
6-H_{eq}	3.10 (dd, J=4.3, 15.8)	5.10 (d, J=3.4)
10-H	6.63 (d, J=2.4)	6.67 (d, J=2.4)
12-H	7.24 (d, J=2.4)	7.29 (d, J=2.4)
16-NH	—	8.37 (d, J=7.0)
17-H	—	4.46 (dq, J=7.0, 7.3)
17-CH_3	—	1.36 (d, J=7.3)

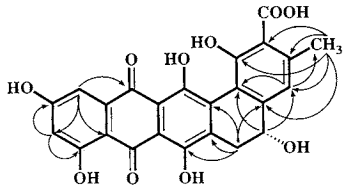
Chemical shifts are given relative to solvent as internal reference at 2.50 ppm. Multiplicity and coupling constants (Hz) are in parentheses.

Table 3. ^{13}C NMR data of pradimicins Q and P.

Position	^{13}C shift		^1H shift	Position	^{13}C shift		^1H shift
	Pradimicin Q	Pradimicin P	Pradimicin Q		Pradimicin Q	Pradimicin P	Pradimicin Q
C-1	156.0 s	152.0 s		C-9	165.5 s	165.4 s	
C-2	118.4 s	127.7 s		C-10	108.2 d	108.4 d	6.63 d (J=2.4)
C-3	140.2 s	137.5 s		C-11	164.5 s	164.4 s	
3-CH_3	21.6 q	18.6 q	2.504 s	C-12	108.5 d	108.6 d	7.24 d (J=2.4)
C-4	118.3 d	123.7 d	6.97 s	C-12a	134.9 s	134.7 s	
C-4a	137.0 s	139.7 s		C-13	185.7 s	186.4 s	
C-5	66.2 d	71.0 d	4.55 dd (J=4.3, 9.8)	C-13a	110.6 s	110.8 s	
C-6	30.7 t	62.8 d	2.66 dd (J=9.8, 15.8), 3.10 dd (J=4.3, 15.8)	C-14	155.0 s	154.0 s	
				C-14a	131.6 s	132.4 s	
C-6a	146.1 s	137.8 s		C-14b	115.3 s	115.2 s	
C-7	153.9 s	155.3 s		C-15	171.5 s	167.0 s	
C-7a	111.2 s	112.4 s		C-17	—	47.6 d	
C-8	187.5 s	187.6 s		17-CH_3	—	16.8 q	
C-8a	109.1 s	109.2 s		C-18	—	173.7 s	

Chemical shifts are given relative to DMSO-d_6 as internal reference for both ^{13}C (39.5 ppm) and ^1H (2.50 ppm). Coupling constants (Hz) are given in parentheses.

Fig. 2. Long range ^{13}C-^{1}H correlations of pradimicin Q.



pradimicin aglycones such as pradimicin P[3]. The FAB-MS spectrum of pradimicin Q revealed a $(M+1)^+$ ion at m/z 465 and a $(M)^-$ ion at m/z 464. The molecular formula of pradimicin Q was determined to be $C_{24}H_{16}O_{10}$ by HRFAB-MS, which is the same as that of pradimicin M[3].

As shown in Table 2, the ^{1}H NMR spectrum of pradimicin Q is similar to that of pradimicin P except for the two upfield protons at C-6 and the lack of protons of the alanine moiety. The methine signals at δ 4.55 and the methylene signals at δ 2.66 and δ 3.10 that are assignable to C-5 (δ_C 66.2) and C-6 (δ 30.7), respectively, from ^{13}C-^{1}H COSY spectral data are all split into a doublet of doublets each, that is the AMX spin system. In the NOESY spectra, NOE's of the methine proton (δ 4.55) to 4-H (δ 6.97) and of 4-H to 3-CH_3 protons were observed. The ^{13}C NMR spectrum of pradimicin Q is similar to that of pradimicin P except for the A ring carbon signals and the upfield shifts (δ 30.7) at C-6 (Table 3). These assignments were confirmed by the correlation spectroscopy *via* long range coupling (COLOC) experiments (Fig. 2). From these spectral data, the structure of pradimicin Q was deduced to be dealanyl 6-dehydroxypradimicin P.

The coupling constant values, 9.8 and 4.3 Hz at C-5 (Table 2), however, indicate vicinal couplings of 5-H_{ax}-6-H_{ax} and 5-H_{ax}-6-H_{eq}, respectively, compared with 5-H_{eq}-6-H_{eq} in pradimicin P. Therefore, the hydroxy group at C-5 of pradimicin Q has the equatorial orientation. The CD data of pradimicin Q (Table 1) shows the same signs as those of pradimicins A and O, suggesting that pradimicin Q has the 5*S* configuration. Thus, the structure of pradimicin Q is deduced to be dealanyl-7,11-didemethyl-(5*S*)-pradimicin O (Fig. 1).

As 5,6-dihydrobenzo[*a*]naphthacenequinone compounds have a common and intense UV absorption maximum around 500 nm (Table 1) which overlaps with that of *p*-nitrophenol released from substrate *p*-nitrophenyl α-D-glucopyranoside, the amount of *p*-nitrophenol was measured after extraction with BuOH at the alkaline pH. Assay conditions are briefly described as follows: 40 μl of α-glucosidase (Sigma G-5003, Type I BAKERS's yeast) (0.1 mg/ml in 100 mM phosphate buffer, pH 6.8 = 0.44 U/assay) for tests or the buffer for the control, 950 μl of 0.7 mM *p*-nitrophenyl α-D-glucopyranoside (Sigma N1377), and 10 μl of a varied concentration of an assay compound in DMSO were mixed and incubated at 37°C for 15 minutes. To the reaction mixture, 1.0 ml of 0.2 N NaOH and 1.0 ml of BuOH were added and mixed vigorously. Absorbance of the BuOH layer at 405 nm was measured spectrophotometrically. α-Glucosidase inhibitory activity was expressed in IC_{50} (μg/ml) at which the test compound inhibited 50% of the enzyme activity. IC_{50} was determined from a standard curve of *p*-nitrophenol which was prepared from the assay without test compound (0% inhibition) and the assay without enzyme (100% inhibition). Pradimicin Q shows the most potent α-glucosidase inhibitory activity among the tested analogs, with IC_{50} of 3 μg/ml (pradimicin N 62 μg/ml), while pradimicins A, L and P are inactive at 100 μg/ml (Table 4). Pradimicins M, N, O, P and Q were also subjected to inhibitory activity assays using β-glucosidase (Sigma G4511), α- and β-mannosidases (Seikagaku Kogyo 100962 and 100963, respectively) and α-(Seikagaku Kogyo 100560) and β-galactosidases (Sigma G1875). None of the compounds show any inhibitory activity on any enzyme at 100 μg/ml except for β-galactosidase. The β-galactosidase activity was inhibited by pradimicin M (39.9%), N (32.4%), O (55.4%), P (36.0%) and Q (44.8%), each at 100 μg/ml.

Table 4. Comparative α-glucosidase inhibitory activities of pradimicin Q and related compounds.

Compound	IC_{50} (μg/ml)
Pradimicin A	>100
L	>100
M	69
N[a]	62
O	17
P	>100
Q	3
Pradimicinone I (AG-2)[4]	>100
Pradinone I (Dealanyl pradimicinone I)[4]	96

Data are shown in the mean of duplicate determinations.

[a] Pradimicin N is identical with benanomicin C based on its physico-chemical and spectral data. IC_{50} of benanomicin C was reported to be 60 μg/ml[5].

Pradimicin Q exhibited neither *in vitro* antifungal nor antibacterial activities at 100 μg/ml. The cytotoxicities (IC_{50}) against human colon HCT-116 cells and mouse melanoma B16-F10 cells were

determined to be 75 and 100 μg/ml, respectively.

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