Communications to the Editor

PRADIMICIN†, A NOVEL CLASS OF POTENT ANTIFUNGAL ANTIBIOTICS

Sir:

In the course of screening for new antibiotics active against fungi, an actinomycete strain No. P157-2 that had been isolated from a soil sample collected in Fiji Island was found to produce novel antibiotics, pradimicins A and B[1,2]. Pradimicin A, the major component showed moderate *in vitro* activity against a wide variety of fungi and yeasts including clinically important pathogens. More interestingly, it exhibited marked *in vivo* therapeutic activity against systemic fungal infections caused by *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans* strains in mice. Degradation studies revealed that pradimicin A has a unique structure containing the following moieties: D-Alanine, D-xylose, 4,6-dideoxy-4-methylamino-D-galactose and a substituted 5,6-dihydrobenzo[a]naphthacenequinone. This communication presents production, isolation, chemical and biological properties and structures of pradimicins A and B. Based on the taxonomic studies performed, strain P157-2 is classified as a heretofore undescribed species of the genus *Actinomadura* and named *Actinomadura hibisca* sp. nov. (ATCC 53557), and the details will be reported in a separate paper.

Pradimicin was produced in a 200-liter tank fermentor using a medium consisting of glucose 3%, soybean meal 3%, Pharmamedia 0.5%, yeast extract 0.1% and $CaCO_3$ 0.3%. The tank was operated at 28°C with agitation of 250 rpm and aeration of 120 liters/minute. Antibiotic production was monitored by bioassay using *C. albicans* A9540 as a test organism and visible absorption at 500 nm in 0.01 N NaOH - MeOH (1:1). After 5~6 days of fermentation, antibiotic potency reached the maximum of 500~700 μg/ml.

The harvested broth (108 liters) was centrifuged and the supernate was acidified to pH 2.0. After the biologically inactive precipitate was removed, the filtrate was adjusted to pH 5.0 which resulted in the formation of dark red precipitate of crude pradimicin. This solid was stirred in a mixture of BuOH - MeOH - 1% NaCl (3:1:4, pH 2.0). The organic layer was separated and extracted with alkaline water (pH 8.0). The aqueous layer was adjusted to pH 2.0 and subjected to column chromatography on Diaion HP-20 eluted with 60% aq Me_2CO (pH 3.0). The red, active eluate was concentrated *in vacuo* to semi-pure solid which was purified by reversed phase silica gel chromatography, developed with CH_3CN - 0.15% KH_2PO_4 (22:78, pH 3.5). The effluent containing the main component, based on bioassay and TLC (silica gel, MeOAc - *n*-propanol - 28% NH_4OH, 45:105:60), was concentrated *in vacuo* to an aqueous solution which was passed through a Diaion HP-20 column for desalting. Concentration of the active eluate yielded a red homogeneous sample of pradimicin A·HCl (30.6 g). A second active eluate was worked up in a similar manner to afford a minor congener pradimicin B·HCl (1.96 g). Pradimicin A·HCl was crystallized from MeOAc - *n*-propanol - 0.1 N NaOH mixture to give fine needles of the monosodium salt.

Pradimicin A (**1**) is red, amphoteric crystals: MP 193~195°C; $[\alpha]_D^{26}$ +685° (*c* 0.1, 0.1 N HCl); molecular formula $C_{40}H_{44}N_2O_{18}$ based on the elemental analysis (calcd for $C_{40}H_{44}N_2O_{18}\cdot 4H_2O$: C 52.63, H 5.74, N 3.07; found: C 52.99, H 5.18, N 3.11) and high-resolution fast atom bombardment (HRFAB)-MS, (VG 70SE), calcd for $C_{40}H_{45}N_2O_{18}$ *m/z* 841.26674 (M+H), found *m/z* 841.26510. The UV and visible spectrum of **1** in 50% MeOH showed absorption maxima at 231 (ε 28,300), 284 (22,700) and 482 nm (9,600) which shifted to 234 (ε 31,100), 299 (26,600) and 459 nm (11,100) in 0.01 N HCl - 50% MeOH and to 240 (ε 33,300), 318 (14,700) and 500 nm (15,100) in 0.01 N NaOH - 50% MeOH. The presence of a carboxylic acid was indicated based on the IR spectrum which exhibited a carbonyl band at 1720 cm^{-1} in its HCl salt and at 1610 cm^{-1} in its sodium salt or zwitter ionic form. The properties of pradimicin B (**2**) is similar to those of **1**. MP 195~198°C; $[\alpha]_D^{26}$ +440° (*c* 0.1, 0.1 N

† Originally called BU-3608 or BMY-28567 (pradimicin A) and BMY-28634 (pradimicin B).

Table 1. ^{13}C NMR spectra of pradimicin A (**1**), AG-11 and AG-2 (100 MHz in DMSO-d_6).

Carbon No.	m	Chemical shift (ppm)		
		1	AG-11	AG-2
C-1	q	16.4	16.3	
C-2	q	17.6	17.3	17.4
C-3	q	20.0	20.0	19.8
C-4	q	36.6	36.8	
C-5	d	48.2	47.7	47.9
C-6	q	56.2	55.9	55.7
C-7	d	63.4	64.0	
C-8	t	66.0		
C-9	d	67.9	67.9	
C-10	d	69.6	71.0	
C-11	d	70.2	71.2	71.6
C-12	d	71.9	71.8	72.4
C-13	d	73.8		
C-14	d	76.1		
C-15	d	80.4		
C-16	d	82.7	82.1	
C-17	d	104.4	104.0	103.9
C-18	d	104.5	104.8	
C-19	d	105.3		
C-20	d	106.3	105.9	105.7
C-21	s	110.5	110.2	110.2
C-22	d	111.6	111.0	110.7
C-23	d	116.9	116.7	114.9
C-24	s	119.0	118.8	118.5
C-25	s	119.3	119.0	118.6
C-26	s	126.9	126.6	125.9
C-27	s	132.2	131.9	131.8
C-28	s	133.1	133.2	133.3
C-29	s	136.5	136.3	136.2
C-30	s	137.7	137.6	137.9
C-31	s	138.0	137.9	140.5
C-32	s	143.7	143.6	145.3
C-33	s	157.6	157.3	157.1
C-34	s	164.1	163.9	163.8
C-35	s	166.0	165.7	165.5
C-36	s	166.4	166.7	166.8
C-37	s	168.9	168.2	168.1
C-38	s	174.6	174.3	174.2
C-39	s	180.5	180.2	180.2
C-40	s	187.5	187.3	187.2

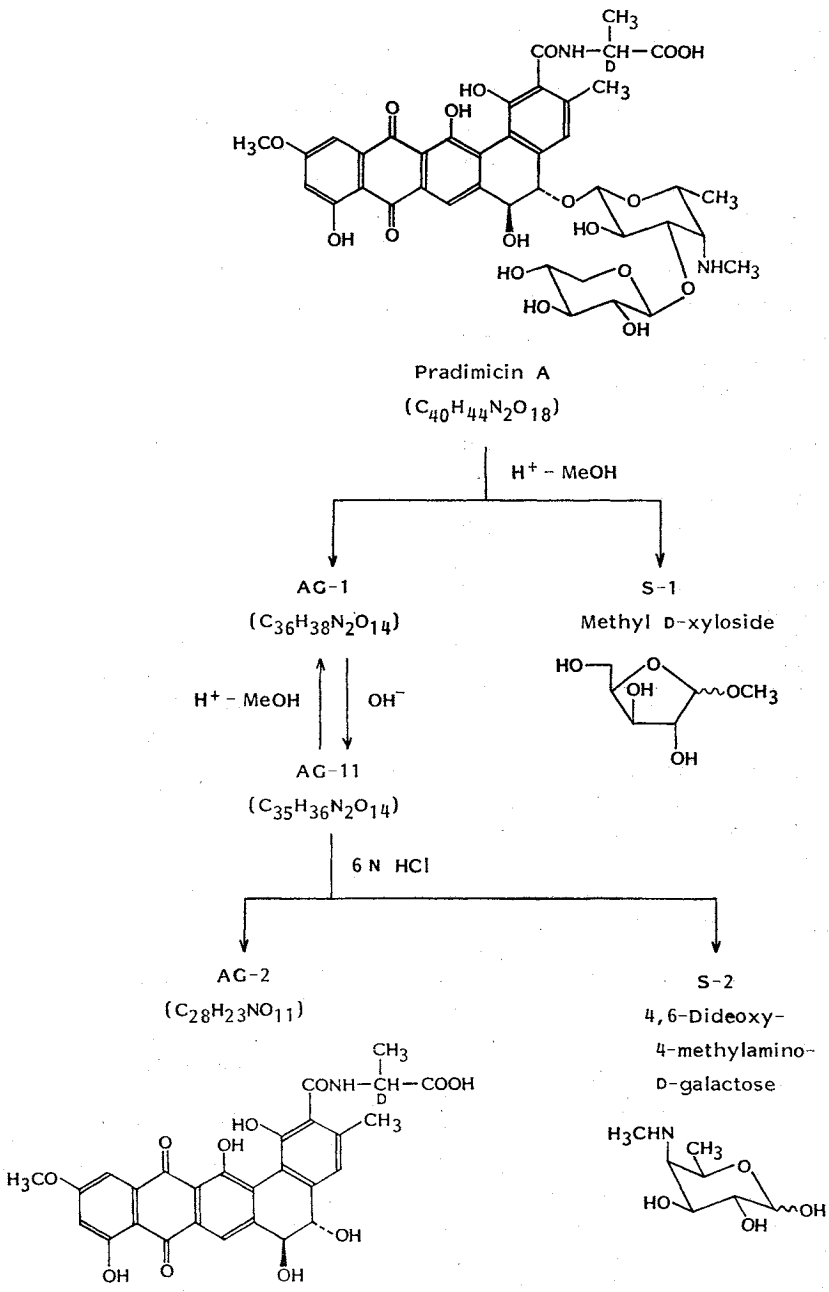
m: Multiplicity, s, singlet; d, doublet; t, triplet; q, quartet.

HCl). Both **1** and **2** are soluble in DMF, DMSO and acidic and alkaline water, slightly soluble in water, MeOH, EtOH and BuOH but insoluble in other organic solvents. The ^{13}C NMR spectra of **1** (Table 1) revealed the presence of two anomeric carbons (δ 104.5 and 105.3) and two quinone carbonyls (δ 180.5 and 187.5) together with three *C*-methyl, one *N*-methyl and one *O*-methyl groups. These physico-chemical and spectral data suggested that **1** consisted of a chromophore aglycone and two sugars.

1 was hydrolyzed with 1.5 N methanolic hydrogen chloride under reflux to a chromophore fragment (AG-1, $C_{36}H_{38}N_2O_{14}$) and methyl D-xyloside (S-1). Upon treatment with 0.1 N NaOH, AG-1 afforded a bio-active demethyl compound (AG-11, $C_{35}H_{36}N_2O_{14}$) in a quantitative yield. Conversely, AG-11 was changed to AG-1 by treatment with acidic MeOH. Thus, comparison of molecular formulae and spectral characteristics indicated that AG-1 was a methyl ester of the aglycone AG-11 formed during methanolysis. Examination of their physico-chemical data revealed that AG-11 was identical with pradimicin B (**2**). When subjected to more severe acid degradation (6 N HCl, 110°C, 18 hours), AG-11 released a smaller aglycone (AG-2) by splitting off an amino sugar (S-2). A small amount of D-alanine was isolated from the hydrolysates. Though S-2 was not obtained in the hydrolysis presumably due to its instability in acid, it was isolated as the methyl glycoside ($C_8H_{17}NO_4$) upon acid methanolysis of *N*-acetylpradimicin A and identified as 4,6-dideoxy-4-methylamino-D-galactose[3]. The aglycone AG-2 was isolated as red powder: MP 221~223°C, $[\alpha]_D^{26}$ −140° (*c* 0.1, MeOH); $C_{28}H_{23}NO_{11}$; FAB-MS *m/z* 550 $(M+H)^+$.

AG-1, AG-11 and AG-2 showed almost the same UV and visible spectra as those of **1** at various pH's, indicating that they retained the chromophore of the antibiotic. The carbon skeleton of the chromophore was elucidated by zinc dust distillation carried out for AG-2. The hydrocarbon obtained showed a UV and visible spectrum (maxima at 220, 252, 258, 292, 302, 316, 374, 398, 422 and 449 nm) and the molecular ion at *m/z* 292, indicating that it is a methylbenzo[a]naphthacene. The ^{13}C NMR spectrum of AG-2 (Table 1) exhibited 4 carbonyls (δ 168.1, 174.2, 180.2, 187.2) and 18 aromatic carbons together with two *C*-methyl, one *O*-methyl and three methine carbons. Further NMR studies using homo- and heteronuclear two-dimensional spectrometry allowed to assign the structure of AG-2 as shown in Scheme 1. The sites and manner of the two sugar linkages were elucidated by extensive ^1H NMR analysis on **1** and AG-11 and the complete structure of pradimicin A was

Scheme 1. Degradation of pradimicin A.



established (Scheme 1). The detailed structure studies will be reported in a separate paper.

The MICs of **1** against various fungi and yeasts were determined by serial agar dilution method. As shown in Table 2, **1** showed antifungal activity against various fungi and yeasts examined.

The *in vivo* effectiveness of **1** was assessed in experimental infections of mice produced by inoculation of *C. albicans*, *A. fumigatus*, or *C. neoformans*. Test organisms were cultured for 18 hours at 28°C in YGP medium (yeast extract, glucose, peptone, K_2HPO_4, $MgSO_4$) and then suspended in saline. Male ICR mice weighing 20 to 24 g were infected intravenously with

Table 2. *In vitro* antifungal activity of pradimicin A (**1**).

Test organisms	MIC (μg/ml)
Aspergillus flavus FA 21436	6.3
A. fumigatus IAM 2530	3.1
A. nidulans CS-17	12.5
Candida albicans A9540	6.3
C. albicans YA25578	6.3
Cladosporium trichoides CS-38	3.1
Cryptococcus neoformans IAM 4514	0.8
Epidermophyton floccosum CS-16	6.3
Fusarium moniliforme A2284	6.3
Mucor spinosus IFO 5317	>100
Penicillium chrysogenum MBS6746	6.3
P. citrinum CS-23	50
P. expansum CS-22	1.6
Piricularia oryzae D91	6.3
Blastomyces dermatitidis D40	6.3
Sporothrix schenckii IFO 8158	1.6
Torulopsis glabrata CS-103	3.1
Trichophyton mentagrophytes D155	6.3
T. mentagrophytes No. 4329	12.5
T. rubrum A22789	3.1
Trichosporon cutaneum CS-4	1.6

Medium: Sabouraud dextrose agar (pH 7.0).

about 20 times the median lethal inoculum size of the test fungi. The antibiotic at various dose levels was administered to groups of 5 mice each either intravenously or intramuscularly and the dose that protects 50% of the animals from infection (PD_{50}, mg/kg) was calculated from survival rates on the 20th day after the fungal challenge. All control animals died within 7 to 15 days after infection. **1** exhibited pronounced therapeutic efficacy curing mice from these fungal infections; PD_{50}; 7.2 mg/kg by a single iv and 11 mg/kg by twice daily for 2 days im for *C. albicans* A9540, 5.7 mg/kg by twice daily for 2 days im for *A. fumigatus* IAM 2034, and 3.5 mg/kg by twice daily for 2 days im for *C. neoformans* IAM 4514. The LD_{50} of **1** was 140 mg/kg following iv administration to male ICR-SP mice.

As described above, pradimicin A is a new antifungal antibiotic with pronounced *in vivo* activity against various fungal infections in mice. From the analyses of ^{1}H and ^{13}C NMR spectra and degradation studies, a novel, unique structure has been assigned to the antibiotic.

Addendum in Proof

After we reported pradimicin A (BMY-28567) in the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy, Oct. 4~7, 1978 in New York, and the First International Conference on Drug Research in Immunologic and Infectious Diseases. Antifungal Drugs: Oct. 8~10, 1987 in Garden City, closely related antibiotic benanomicins A and B have been published in this journal, Vol. 41 No. 6 pp. 807~811, 1988.

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(Received May 12, 1988)

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