and *Claviceps* spp., respectively. In addition, we have found that field-produced sclerotia formed by inoculation of corn ears in field test plots contain quantities of nominine similar to those found in laboratory-produced sclerotia. Taken together, these results suggest a possible ecological role for sclerotial metabolites and provide further evidence that fungal sclerotia are a unique and promising source of new bioactive natural products.

Experimental Section

General Procedures. Sclerotia from a strain of *A. nomius* (NRRL 13137) were obtained from the USDA Northern Regional Research Center in Peoria, IL. The sclerotia were prepared by solid substrate fermentation on autoclaved corn kernels using general procedures which have been previously described[3] and were stored at 4 °C until extraction. Proton and carbon NMR data were obtained in $CDCl_3$ on a Bruker WM-360 spectrometer, and chemical shifts were recorded using the signal for the residual protiated solvent (7.24 ppm) as a reference. Carbon multiplicities were established by a DEPT experiment. One-bond C–H correlations were obtained using an XHCORR pulse sequence optimized for 135 Hz. Proton signals studied with the selective INEPT technique were individually subjected to three separate experiments, optimizing for 7, 10, or 13 Hz. HREIMS data were obtained on a VG ZAB-HF instrument. Details of other experimental procedures and insect bioassays have been described elsewhere.[13,14]

Isolation and Properties of Nominine (2). Sclerotia of *A. nomius* (500–750 μm diameter, 53 g) were ground with a mortar and pestle and triturated repeatedly with hexane (5 × 200 mL). The combined hexane extracts were filtered and evaporated to afford 117 mg of a light yellow oil. This residue was subjected to reversed-phase semipreparative HPLC (5μ C_{18} column; 250 × 10 mm; 90:10 MeOH–H_2O at 2.0 mL/min) to afford 23.7 mg of nominine (**2**) as an off-white powder. The retention time for **2** under these conditions was 22.2 min. Compound 1: mp 54–55 °C; $[\alpha]_D$ +23.6° (*c* 0.85, MeOH); ^1H NMR and ^{13}C NMR ($CDCl_3$), Table I; EIMS (70 eV) 405 (M^+; rel intensity 57), 387 (100), 318 (15), 304 (52), 302 (70), 288 (17), 248 (15), 232 (15), 196 (42), 180 (40), 168 (28), 156 (16); HREIMS obsd 405.3035, calcd for $C_{28}H_{39}NO$ 405.3031.

Liquid Culture of *A. nomius*. A sterilized medium suitable for production of sclerotia on agar in petri dishes (1.5% glucose and 0.5% yeast extract; 50 mL) was inoculated with *A. nomius* and aerated by agitation on an orbital shaker at 200 rpm for 28 days. Although the fungus produced substantial mycelial growth under these conditions, sclerotia were not formed, and no trace of nominine was detected in organic extracts of the mycelium or the culture filtrate by analytical HPLC.

Detection of Nominine in Sclerotia from Field-Inoculated Corn. Several silking corn ears on growing corn plants at the USDA Northern Regional Research Center field plot were toothpick-wound inoculated with a conidial suspension of *A. nomius*. When the ears reached full maturity, small quantities of sclerotia were harvested from the ears and manually separated from all other fungal and plant material. Extraction of these sclerotia with $CHCl_3$ and analysis of the extract by analytical HPLC under the conditions above indicated the presence of nominine at a level similar to that found in laboratory-produced sclerotia.

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The Structures of Pradimicins A, B, and C: A Novel Family of Antifungal Antibiotics

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The structures of the novel antifungal antibiotics pradimicins A, B, and C, elaborated by a new strain of *Actinomadura hibisca*, have been determined on the basis of chemical degradations and spectral analysis. Acid hydrolysis cleaved pradimicin A to yield D-xylose, 4,6-dideoxy-4-(methylamino)-D-galactose, an aromatic chromophore fragment, and D-alanine. Extensive homo- and heteronuclear 2D NMR experiments assisted by the degradation results allowed us to assign *N*-[[(5*S*,6*S*)-5-*O*-[4,6-dideoxy-4-(methylamino)-3-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl]-5,6,8,13-tetrahydro-1,6,9,14-tetrahydroxy-11-methoxy-3-methyl-8,13-dioxobenzo[*a*]-naphthacen-2-yl]carbonyl]-D-alanine for the structure of pradimicin A. Pradimicins B and C are desxylosyl and des-*N*-methyl analogues of pradimicin A, respectively.

Introduction

Although enormous screening efforts have been made in the past 30 years, there are relatively few antifungal antibiotics with clinical efficacy, particularly against systemic fungal infections. In our efforts to discover microbial metabolites active against fungal infections, we have found that cultured broth of *Actinomadura hibisca* No. P157-2 (ATCC 53557), isolated from a soil sample from Fiji Island, contained red pigments that strongly protected mice from lethal infections caused by *Candida*, *Aspergillus*, and *Cryptococcus* strains.[1] The active principals were precipitated from the broth filtrate at pH 5.0 and purified by column chromatography to yield three components, pradimicins A (**1a**), B (**1b**), and C (**1c**).[2] In the in vitro assay,

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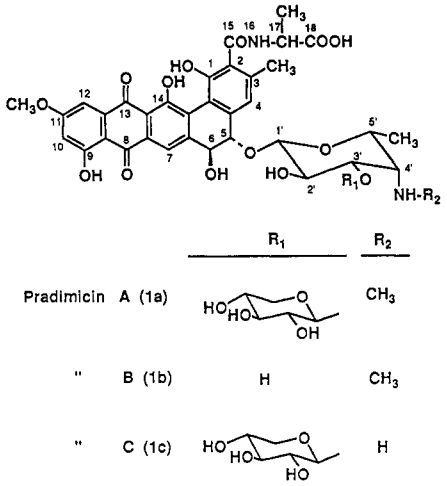

Figure 1. Structures of pradimicins A, B, and C.

these antibiotic components inhibited growth of a variety of fungi and yeasts with the minimum inhibitory concentration (MIC) ranging from 0.8 to 6.3 mcg/mL, while Gram-positive and Gram-negative bacteria were not sensitive. The three antibiotics also showed strong protective effects in mice experimentally infected with *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* strains. We report herein the structure elucidation of pradimicins A (**1a**), B (**1b**), and C (**1c**), which have a novel 5,6-dihydrobenzo[*a*]naphthacene chromophore substituted with D-alanine and sugars as shown in Figure 1.

Results and Discussion

The major component of the complex was **1a**, and it was isolated as an orange red amorphous powder of zwitterionic nature: mp 193–195 °C dec; $[\alpha]^{26}_D$ +685° (*c* 0.1, 0.1 N HCl); IR (KBr) 3400, 1605, and 1520 cm^{-1}; UV λ_{max} (50% MeOH) 231 nm (ϵ 28 300), 284 (22 700), and 482 (9600). Sodium salt of **1a** was obtained as dark red needles from a mixture of methyl acetate and 1-propanol. The molecular formula of $C_{40}H_{44}N_2O_{18}$ was established for **1a** based on the combustion analysis and high-resolution fast atom bombardment mass spectrometry (HR-FAB-MS, MH^+: obsd *m/z* 841.265 10, calcd 841.266 74).[3] **1b**: mp 195–198 °C dec; $[\alpha]^{26}_D$ +440° (*c* 0.1, 0.1 N HCl); $C_{35}H_{36}N_2O_{14}$; *m/z* 709 (MH^+). **1c**: mp 220–225 °C dec; $[\alpha]^{26}_D$ +619° (*c* 0.1, 0.1 N HCl); $C_{39}H_{42}N_2O_{18}$; *m/z* 827 (MH^+). The UV and IR spectra of **1b** and **1c** are very similar to those of **1a**, demonstrating that they share a common chromophore. The ^{13}C NMR spectrum of **1a** displayed 40 signals composed of CH_3-C × 3, CH_3-N × 1, CH_3-O × 1, CH_2-O × 1, CH-N(or O) × 12, CH= × 4, >C= × 14, amide or carboxylic acid × 2 (δ 168.9 and 174.6) and quinone carbonyl × 2 (δ 180.5 and 187.5). Acid methanolysis (1.5 N HCl–MeOH, reflux, 4 h) cleaved **1a** to a chromophore fragment (**2**), mp 245–248 °C dec, $C_{36}H_{38}N_2O_{14}$, *m/z* 723 (MH^+), and

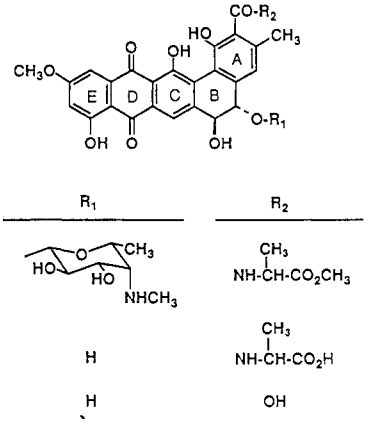

Figure 2. Structures of degradation products **2**, **3**, and **5**.

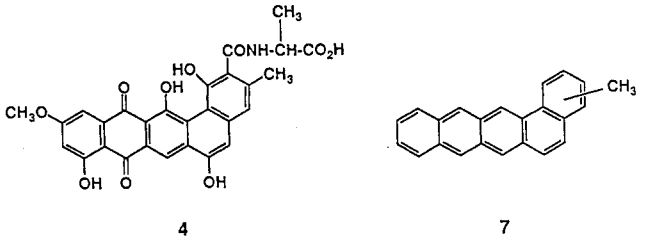

Figure 3. Structures of degradation products **4** and **7**.

methyl D-xyloside. The NMR spectrum of **2** contained an OCH_3 group (δ_H 3.68, s, and δ_c 51.4) which had not been observed in **1a**. Upon heating with 0.1 N NaOH for 1 h, **2** split off the OCH_3 group yielding **1b**, the bioactive minor component of pradimicin, which conversely converted to **2** by acid methanolysis. Vigorous acid hydrolysis of **1a** or **2** (6 N HCl, 115 °C, 14 h) afforded a major aglycon **3** (mp 221–223 °C dec; $[\alpha]^{26}_D$ −140° (*c* 0.1, MeOH), $C_{28}H_{23}NO_{11}$; *m/z* 550 (MH^+)), two minor aglycones (**4** and **5**) ($C_{28}H_{21}NO_{10}$, *m/z* 532 (MH^+), mp >250 °C, $C_{25}H_{18}O_{10}$, *m/z* 479 (MH^+), mp 207–210 °C dec), and D-alanine.[4,5] The presence of an amino sugar in **1b** had been postulated by the calculated molecular formula difference between **1b** and **3**. Although it could not be isolated from the above hydrolyzate possibly due to decomposition during hydrolysis, it was obtained as a mixture of α and β anomers (78:22, **6a** and **6b**) by acid methanolysis of *N*-acetylpradimicin A. The ^1H–^1H 2D NMR experiments and optical rotational value allowed us to assign the methyl 4,6-dideoxy-4-(methylamino)-D-galactopyranoside structure to the sugar. Compound **3** retained the UV spectrum and, in the ^{13}C NMR, all 22 sp^2 carbons (18 aromatic and 4 carbonyl carbons), two C-CH_3, one O-CH_3, and three methines observed for the parent antibiotic. Upon distillation with zinc dust, **3** afforded a methylbenzo[*a*]naphthacene (**7**), $C_{23}H_{16}$, *m/z* 292 (M^+), UV λ_{max} 220, 252, 258, 292, 302, 316, 355, 374, 398, 422, and 449 nm, demonstrating a benzo[*a*]naphthacene nucleus to the antibiotic. The ^{13}C signals of **3** (also of **1a**) closely resembled some of those of 10-dihydrosteffimycin A[6] (rings D and E in

(2) While this publication was being written, the production and structure of new antifungal antibiotic benanomicins were published. Although stereochemistry of C5 and C6 of benanomicins has not been reported, one of the components, benanomicin B, was identified with pradimicin C by a direct comparison. Takeuchi, T.; Hara, T.; Naganawa, H.; Okada, M.; Hamada, M.; Umezawa, H.; Gomi, S.; Sezaki, M.; Kondo, S. *J. Antibiot.* **1988**, *41*(6), 807. Gomi, S.; Sezaki, M.; Kondo, S.; Hara, T.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1988**, *41*(8), 1019.

(3) We observed abundant $(M + 3H)^+$ ions of pramidicins A, B, and C and their hydroysis products ions in FAB-MS and secondary-ion MS. This was due to reduction of the quinone of these compounds to the hydroquinone in the mass spectrometer which adsorbed one proton. Misra, R.; Pandy, R. C.; Silverton, J. V. *J. Am. Chem. Soc.* **1982**, *104*, 4478. Cooper, R.; Unger, S. *J. Antibiot.* **1985**, *38*(1), 24.

(4) The D configuration was assigned based on the HPLC behavior on a chiral HPLC column (MIC Gel ODS-1HU, Mitsubishi Kasei). Elution: 2 mM *N,N*-dipropyl-L-alanine and 1 mM copper acetate solution, pH 5.7.

(5) In the acid hydrolysis, major aglycon **3** precipitated rapidly (85% yield), and it was resistant to further hydrolysis. HPLC analysis of the hydrolyzate indicated the presence of aglycone **5** and D-alanine in a comparable ratio (ca. 5% yield). The peptide bond was found to be unusually stable to acid and alkaline hydrolysis (6 N HCl and 6 N NaOH, 110 °C). The ^1H and ^{13}C NMR data evidenced the presence of 1 M D-alanine in this molecule.

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Table I. ^{13}C NMR Data of 1a, 1b, 1c, 2, and 3 (100 MHz, DMSO-d_6)

carbon	1a	1b	1c	2	3
1	157.6	157.3	157.5	157.9	157.1
2	126.9	126.6	126.9	125.7	125.9
3	136.5	136.3	136.3	136.8	136.2
4	116.9	116.7	116.8	116.6	114.9
4a	137.7	137.6	137.5	137.5	140.5
5	82.7	82.1	82.5	82.0	71.6
6	71.9	71.8	71.9	71.6	72.4
6a	143.7	143.6	143.6	143.6	145.3
7	111.6	111.0	111.2	111.2	110.7
7a	132.2	131.9	132.0	131.7	131.8
8	187.5	187.3	187.2	187.1	187.2
8a	110.5	110.2	110.3	110.2	110.2
9	164.1	163.9	163.9	163.8	163.8
10	104.4	104.0	104.3	104.1	103.9
11	166.0	165.7	165.8	165.6	165.5
12	106.3	105.9	106.0	105.7	105.7
12a	138.0	137.9	137.8	137.6	137.9
13	180.5	180.2	180.3	180.2	180.2
13a	119.3	119.0	119.0	118.7	118.6
14	166.4	166.7	166.5	165.4	166.8
14a	133.1	133.2	133.0	132.4	133.3
14b	119.0	118.8	118.8	118.5	118.5
15	168.9	168.2	168.2	168.1	168.1
17	48.2	47.7	48.0	47.5	47.8
18	174.6	174.3	174.5	173.0	174.2
3-CH_3	20.0	20.0	19.9	20.0	19.8
11-OCH_3	56.2	55.9	56.0	55.8	55.7
17-CH_3	17.6	17.3	17.5	17.0	17.4
18-$COOCH_3$				51.4	
1′	104.5	104.8	104.4	104.6	
2′	70.2	71.2	69.8	71.0	
3′	80.4	71.0	79.8	70.6	
4′	63.4	64.0	54.3	63.9	
5′	67.9	67.9	67.6	67.5	
5′-CH_3	16.4	16.3	16.4	16.0	
4′-NCH_3	36.6	36.8		36.5	
1″	105.3		105.1		
2″	73.8		73.5		
3″	76.1		76.0		
4″	69.6		69.4		
5″	66.0		65.8		

Figure 2, C8–C13 in Table I) and those of cosmocarcin A[7] (rings C and D, C6a–C8a and C12a–C14a). The ^1H NMR data also supported the similarity to those antibiotics indicating that **3** possesses a 2-methoxy-4,9-dihydroanthraquinone partial structure. The extensive homo- and heteronuclear two-dimensional NMR studies including long-range ^1H–^{13}C COSY and NOESY revealed the structure of the remaining part of the core moiety. The experiments were conducted with **2**, which was the most soluble of the pradimicin analogues in the NMR solvent (DMSO-d_6). Analysis of the data allowed the unambiguous assignment of all the carbon (Table I) and proton signals of **2**. In the long-range ^1H–^{13}C COSY, C3-methyl protons displayed cross peaks with C2, C3, and C4; H4 with C2, C14b, and the methyl carbon attached to C3; H5 with C4a, C6, C6a, C14b, and C1′ (anomeric carbon of **6**); and H6 with C4a, C6a, and C7, establishing the substitution pattern of rings A, B, and C. (Figure 4). The spectrum also substantiated the assigned structure of rings C, D, and E and the D-alanine bonded to C2-carboxylic acid. The NOESY spectrum of **2** solidly supported this structure (Figure 4). In the ^1H NMR spectrum of **1a** and **2**, H5 coupled to H6 with J value of ca. 10.0 Hz, indicating that they were in a trans-diaxial orientation. A negative first ($\Delta\epsilon$ = −30.6 at 225.4 nm) and positive second Cotton effect ($\Delta\epsilon$ = +16.14 at 205.8 nm) observed for **3** indicated a

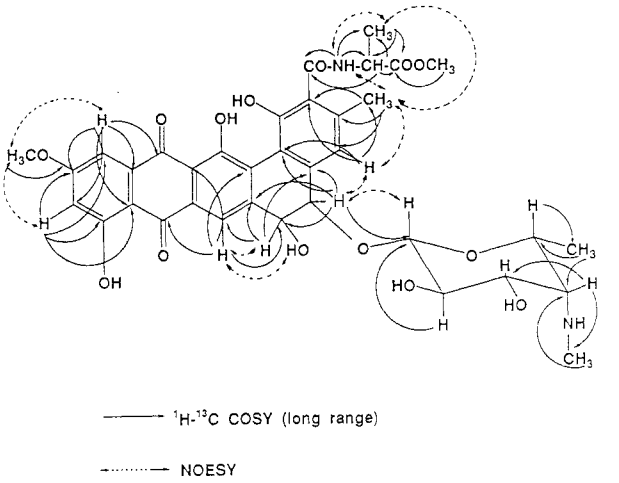

Figure 4. Long-range ^1H–^{13}C COSY and NOESY spectra of **2**.

negative helicity of the axis between the plane of ring A and rings C, D, and E.[8,9] These results proved a 5*S*,6*S* absolute configuration of ring B of the antibiotic. The β-pyranoside linkage of the sugar to C5-OH was assigned by the magnitude of the coupling of the anomeric proton (H1′, δ 4.63, d, J = 8.1 Hz). The molecular formula of **4**, $C_{28}H_{21}NO_{10}$, indicated that **4** was a dehydration product of **3**. A pronounced bathochromic shift in its UV spectrum suggested that the dehydration extended the conjugation of the chromophore. The ^1H NMR spectrum of **4** showed an aromatic proton (δ 7.20) in addition to the four aromatic protons observed for **3**. Instead, the two low-field methines assigned to H5 and H6 in **3** were missing in the spectrum of **4**, verifying that the dehydration took place at C5-OH and C6-H. The UV absorption of **5** appeared at slightly longer wavelengths (λ_{max} 224, 274, 320, and 509 nm in alkaline solution) than those of **1a**, **2**, and **3**. The NMR demonstrated that **5** possessed an identical aglycon structure with **3** but lacked the signals assignable to D-alanine of **3**.

The ^1H and ^{13}C NMR of **1a** assisted by two-dimensional NMR experiments evidenced a β-pyranoside configuration of the D-xylose (H1″, δ 4.44, d, J = 7.3 Hz). Previous mild hydrolysis experiment has identified **1b** as desxylosyl-**1a**. In comparative ^{13}C NMR analysis, C3′ of **1a** appeared 8.9 ppm lower field than that of **1b** showing that the xylose was linked to C3′-OH in **1a**.

The ^1H and ^{13}C NMR spectra of **1c** correspond well with those of **1a**, differing only in that the N-CH_3 (δ_H 2.61 and δ_C 36.6) in **1a** was missing and the C4′ signal shifted upfield by 9.1 ppm in **1c**. *N*-Desmethyl structure for **1c** was confirmed by the isolation of methyl 4,6-dideoxy-4-amino-D-galactoside[10] in acid methanolysis of *N*-acetyl **1c**. It was concluded from the results of the above investigation that pradimicins A (**1a**), B (**1b**), and C (**1c**) have the structures as illustrated in Figure 1, the degradation products **2**, **3** and **5** as in Figure 2, and **4** and **7** as in Figure 3.

Experimental Section

Thin-layer chromatography (TLC) was performed on precoated silica gel plates (Kieselgel 60F_{254}, Merck, 0.25 mm thick). The IR spectra were determined on a JASCO IR-810 spectrometer

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and the UV spectra on a JASCO UVIDEC-610C spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a JEOL JMN-GX 400 spectrometer operated in the Fourier transform mode using tetramethylsilane and/or dioxane as the internal standard. Electron-impact and secondary-ion mass spectra (EI-MS and SI-MS) were obtained with a Hitachi M80B mass spectrometer modified with an im-beam and SI-MS inlet. Fast atom bombardment mass spectra (FAB-MS) were run with a JMS-DX 303 HF spectrometer (JEOL) and high-resolution fast atom bombardment mass spectra (HR-FAB-MS) on a VG70SE spectrometer. Optical rotation were determined with a JASCO Model DIP-140.

Isolation of Pradimicins A (1a), B (1b), and C (1c). The fermentation broth (108 L) was centrifuged, and the supernatant was adjusted to pH 2.0 by 6 N HCl. After the brown precipitate was removed by filtration, the filtrate was neutralized to deposit a dark red crude solid of pradimicin. This solid was thoroughly stirred with a mixture of *n*-BuOH–MeOH–1% NaCl, pH 2.0 (3:1:4, 73 L). The organic layer was separated and extracted with aqueous NaOH (pH 8.0, 30 L). The aqueous layer was adjusted to pH 2.0 by 1 N HCl and subjected to column chromatography on Diaion HP-20 eluted with 60% aqueous acetone (pH 3.0). The red, active eluate was concentrated in vacuo to afford a semipure solid of the antibiotic (62 g). Three grams of the solid was subjected to reversed-phase silica gel chromatography (ODS-60, Yamamura Chemical Lab., 8 × 100 cm) developed with CH_3CN–0.15% KH_2PO_4, pH 3.5 (22:78, v/v). The major red eluate containing **1a** was concentrated in vacuo to an aqueous solution, which was desalted by Diaion HP-20 chromatography with 60% acetone (pH 3.0) elution, yielding a red homogeneous solid of **1a**·HCl (1.52 g). In this reversed-phase chromatography, the eluates before and after **1a** were pooled and further purified by reversed-phase silica gel and Diaion HP-20 chromatography to afford pure **1b**·HCl (140 mg) and **1c**·HCl (98 mg), respectively. Crystallization of **1a**·HCl from MeOAc–*n*-PrOH–0.1 N NaOH gave fine needles of the monosodium salt. The salt dissolved in water and adjusted to pH 5.0 by 0.1 N HCl, to yield a precipitate of the zwitterionic form of **1a**. Aqueous solutions of **1b**·HCl and **1c**·HCl were adjusted to pH 5.0 by 0.1 N NaOH to precipitate the zwitterionic form of **1b** and **1c**, respectively.

1a: mp 193–195 °C dec; $[\alpha]^{26}_D$ +685° (*c* 0.1, 0.1 N HCl); UV (50% MeOH) λ_{max} 231 nm (ϵ 28300), 284 (22700), 482 (9600); (0.01 N HCl–50% MeOH) λ_{max} 234 (31100), 299 (26600), 459 (11100); (0.01 N NaOH–50% MeOH) λ_{max} 240 (33300), 318 (14700), 500 (15100); IR (KBr) 3400, 1605, 1450, 1390, 1295, 1260, 1160, 1050 cm^{-1}; ^{13}C NMR (Table I); HR-FAB-MS (glycerol) *m/z* 841.2651 (MH^+); SI-MS (glycerol) *m/z* 843 $(M + 3H)^+$, (mNBA) *m/z* 841 (MH^+). Anal. Calcd for $C_{40}H_{44}N_2O_{18}·H_2O$: C, 55.94; H, 5.40; N, 3.26. Found: C, 55.99; H, 5.59; N, 3.24.

1b: mp 195–198 °C dec; $[\alpha]^{26}_D$ +440° (*c* 0.1, 0.1 N HCl); UV (MeOH) λ_{max} 234 nm (ϵ 30100), 286 (24100), 473 (10100); (0.01 N HCl–MeOH) λ_{max} 234 (31100), 296 (27500), 460 (11100); (0.01 N NaOH–MeOH) λ_{max} 241 (33300), 316 (14200), 504 (15000); IR (KBr) 3380, 1610, 1450, 1390, 1295, 1260, 1165, 1130, 1070 cm^{-1}; ^{13}C NMR (Table I); SI-MS (glycerol) *m/z* 711 $(M + 3H)^+$; (mNBA) *m/z*, 709 (MH^+). Anal. Calcd for $C_{35}H_{36}N_2O_{14}·H_2O$: C, 57.85; H, 5.27; N, 3.85. Found: C, 57.79; H, 5.31; N, 3.85.

1c: mp 220–225 °C; $[\alpha]^{26}_D$ +619° (*c* 0.1, 0.1 N HCl); UV (50% MeOH) λ_{max} 230 nm (ϵ 31400), 285 (23400), 481 (9900); (0.01 N HCl–50% MeOH) λ_{max} 234 (32700), 298 (27800), 459 (11500); (0.01 N NaOH–50% MeOH) λ_{max} 240 (32200), 320 (14900), 499 (15400); IR (KBr) 3370, 1605, 1445, 1390, 1295, 1260, 1160, 1040 cm^{-1}; ^{13}C-NMR (Table I); SI-MS (mNBA) *m/z* 827 (MH^+).

Acid Methanolysis of 1a. A solution of **1a** (944 mg) in 1.5 N methanolic hydrogen chloride (60 mL) was refluxed for 4 h at 80 °C. The reaction mixture was diluted with water (200 mL) and extracted with *n*-butanol three times (100 mL each). The extract was concentrated in vacuo to give a crude solid of **2** (864 mg). A part of the solid (430 mg) was chromatographed on a column of reversed-phase silica gel (Lichroprep RP-18, 40-63 μm, Merck, 4 × 45 cm) developed with CH_3CN–0.15% KH_2PO_4 pH 3.5 (40:60, v/v). The eluate was collected in fractions which were examined by HPLC. (column: Microsorb Short One C18, 4.6 × 100 mm, 3 μm. Rainin Instrument Co. Elution: CH_3CN–0.15 KH_2PO_4, pH 3.5 (52:48). Flow rate: 1.2 mL/min. Detection: UV 254 nm. Retention time: **1a**, 2.03 min; **2**, 4.45 min.) The appropriate fractions were concentrated to an aqueous solution, which was charged on a column of Diaion HP-20 (200 mL) for desalting. The column was washed with water (500 mL) and eluted with 80% acetone (pH 3.0). Evaporation of the red effluent yielded pure **2** hydrochloride (144 mg). This salt was dissolved in water (15 mL) and adjusted to pH 5.0 with 0.1 N NaOH. The resultant precipitate was collected by filtration, washed with acetone, and dried in vacuo to give an amphoteric red powder of **2** (127 mg): mp 245–248 °C dec; $[\alpha]^{26}_D$ –340° (*c* 0.1, 0.1 N HCl); FAB-MS (glycerol) *m/z* 723 (MH^+); SI-MS (glycerol) *m/z* 725 $(M + 3H)^+$, (mNBA) *m/z* 723 (MH^+); UV (MeOH) λ_{max} 234 nm (ϵ 28700), 283 (23600), 476 (10000); IR (KBr) 3400, 1735, 1625, 1440, 1385, 1255, 1160, 1070 cm^{-1}; ^{13}C NMR (Table I). Anal. Calcd for $C_{36}N_{38}N_2O_{14}·2H_2O$: C, 56.99; H, 5.58; N, 3.69. Found: C, 56.68; H, 5.05; N, 3.67.

The aqueous layer of the hydrolyzate was neutralized by Amberlite IR-45 resin (OH^-) and concentrated in vacuo to a small volume, which was loaded on a column of Diaion HP-20, which was developed with water. Evaporation of the anthrone-positive eluate yielded a pale-yellow solid (144 mg). This solid was chromatographed on a column of Sephadex G-10 eluted with H_2O, yielding methyl D-xyloside (131 mg): $[\alpha]^{26}_D$ +60° (*c* 2.0, H_2O); EI-MS *m/z* 164 (M^+). It was identical with an authentic sample in TLC and ^1H NMR spectrum.

Alkaline Hydrolysis of 2. A solution of **2** (230 mg) in 0.1 N NaOH (30 mL) was refluxed for 1 h. The reaction solution was acidified to pH 3.0 and applied to a column of Diaion HP-20 (250 mL). After washing with water, the column was eluted with 80% acetone (pH 3.0) to give pure **1b** hydrochloride (220 mg). This HCl salt was dissolved in water and adjusted to pH 5.0, yielding an amphoteric amorphous powder of **1b** (127 mg).

Acid Hydrolysis of 1a. A solution of **1a** (6.3 g) in 6 N HCl (275 mL) was heated at 115 °C for 14 h in a sealed container. After cooling the precipitate was collected by filtration. A part of the filtrate (1.2 mL) was passed through a column of Diaion HP-20 (20 mL). The ninhydrin-positive effluent was concentrated in vacuo to afford a white solid containing alanine (2.2 mg). The stereochemistry of the alanine was determined to be "D" by chiral HPLC. (Column: MCI Gel ODS 1HU, 4.6 × 150 mm, 5 μm, Mitsubishi Kasei. Elution: 2 mM *N,N*-dipropyl-L-alanine, 1 mM copper acetate, pH 5.7. Flow rate: 0.8 mL/min. Detection: UV 254 nm, t_R: D-Ala 4.8 min, L-Ala 6.6 min.) The precipitate obtained above was dissolved in aqueous NaOH (pH 11). The solution was adjusted to pH 6.0 by 1 N HCl, and the resultant precipitate was filtered off. The filtrate was loaded on a column of Diaion HP-20 (2.6 L). The column was washed with water and eluted with 60% acetone. Evaporation of the acetone eluate afforded 3.7 g of a red powder containing **3**, **4**, and **5**. This solid was purified by Sephadex LH-20 chromatography (3.6 L) with 50% MeOH as eluant. The first red eluate containing homogeneous **3** was concentrated in vacuo. **3** (3.2 g): mp 221–223 °C dec; $[\alpha]^{26}_D$ –140° (*c* 0.1, MeOH); FAB-MS (glycerol) *m/z* 550 (MH^+); SI-MS (glycerol) *m/z* 552 $(M + 3H)^+$; IR (KBr) 3370, 1600, 1445, 1390, 1295, 1255, 1160, 1130 cm^{-1}; UV (MeOH) λ_{max} 234 nm (ϵ 34500), 287 (29000), 478 (11500); (0.01 N HCl–MeOH) λ_{max} 233 nm (35300), 296 (29900), 458 (12600); (0.01 N NaOH–MeOH) λ_{max} 240 nm (37400), 314 (14800), 504 (14600); ^{13}C NMR (Table I). Anal. Calcd for $C_{28}H_{23}NO_{11}·^1/_2H_2O$: C, 60.22; H, 4.33; N, 2.51. Found: C, 60.28; H, 4.40; N, 2.73.

Concentration of the second red eluate afforded **4** (20 mg): UV (50% MeOH) λ_{max} 237 nm (ϵ 22000), 278 (24400), 366 (11500), 516 (9800); (0.01 N HCl–50% MeOH) λ_{max} 278 nm (14800), 342 (7100), 477 (5000); (0.01 N NaOH–50% MeOH) λ_{max} 243 nm (22600), 288 (29400), 376 (10500), 538 (9700); IR (KBr) 3400, 1615, 1480, 1440, 1365, 1290, 1240 cm^{-1}; FAB-MS (glycerol) *m/z* 532 (MH^+); molecular formula $C_{28}H_{21}NO_{10}$. The third red effluent from the Sephadex column was evaporated to dryness (87 mg), which was charged on a column of reversed-phase silica gel (ODS-60, Yamamura Chem. Lab. 22 × 450 mm). The column was developed with CH_3CN-0.03M phosphate buffer pH 7.0 (20:80), and the eluate was examined by HPLC. (Column: YMC A-301-3, Yamamura Chem. Lab. Elution: CH_3CN–0.03 M phosphate buffer pH 7.0 (25:75). Flow rate: 1.0 mL/min. Detection: UV 254 nm. t_R: 5.40 min.) The appropriate fractions were concentrated in vacuo and then desalted on a column of Diaion HP-20 to yield a homogeneous red powder of **5** (40 mg):

mp 207-210 °C dec; FAB-MS (glycerol) m/z 479 (MH^+); $C_{25}H_{18}O_{10}$; UV (50% MeOH) λ_{max} 237 nm (ϵ 29600), 283 (20100), 471 (9600); (0.01 N HCl-50% MeOH) precipitation; (0.01 N NaOH-50% MeOH) λ_{max} 224 (26900), 274 (23000), 320 (10400), 509 (11600); IR (KBr) 3400, 1600, 1440, 1380, 1290, 1255, 1185, 1160, 1120 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.58 (3 H, s), 3.89 (3 H, s), 4.17 (1 H, dd, J = 3.9 and 11.1), 4.26 (1 H, dd, J = 3.4 and 11.1), 5.69 (1 H, br s), 5.91 (1 H, br s), 6.67 (1 H, br d, J = 2.1), 6.86 (1 H, s), 7.05 (1 H, br d, J = 2.1), 8.02 (1 H, s), 13.05 (1 H, br s), 14.01 (1 H, s); ^{13}C NMR (DMSO-d_6) δ 186.3, 181.0, 170.1, 165.7, 165.7 164.5, 160.0, 145.9, 142.5, 137.5, 137.5, 132.1, 130.7, 125.9, 119.5, 117.8, 115.9, 113.2, 110.5, 105.9, 105.0, 72.2, 71.7, 55.9, 22.9.

Zinc Dust Distillation of 3. A mixture of **3** (50 mg) and zinc dust (500 mg) was placed in the bulb of a long glass tube (7 × 500 mm), which was then sealed. The bulb was heated over a burner to a red glow and kept for 30 s. After cooling, the tube was broken above the bulb and the upper piece containing the distillate was rinsed with diethyl ether. The ether extract was evaporated to dryness, which was developed on a preparative TLC plate (SiO_2, hexane-benzene, 9:1). The yellow band (R_f 0.41) was cut off and eluted from the silica gel with CH_2Cl_2. Evaporation of the solvent yielded a yellow liquid of **7**: UV λ_{max} (n-heptane) nm 220, 252, 258, 292, 302, 316, 355, 374, 398, 422, 449; EI-MS m/z 292 (M^+). These data were consistent with a methyl benzo[a]naphthacene.

Isolation of Amino Sugar 6. Pradimicin A (600 mg) was treated with Ac_2O (6 mL) in MeOH (130 mL) at room temperature overnight. Concentration of the mixture in vacuo afforded a red solid of N-acetyl **1a** (558 mg). This solid (407 mg), without further purification, was hydrolyzed with 5.2 N HCl-MeOH (90 mL) under reflux temperature for 2.5 h. The reaction mixture was neutralized with 6 N NaOH and concentrated to an aqueous solution, which was loaded on a column of Diaion HP-20 (100 mL). The column was eluted with water, and the ninhydrin-positive eluate was evaporated. The residue was chromatographed on Amberlite CG-50 (H^+, 60 mL) with elution of 0.01 N HCl. The ninhydrin-positive fractions were pooled, concentrated to dryness (21.2 mg), charged on a column of Sephadex LH-20 (80 mL), and developed with 50% MeOH. Evaporation of eluate containing the sugar afforded a pale-yellow solid (**6**α and **6**β, 7.5 mg): $[\alpha]^{26}_D$ +87.5° (c 0.3, H_2O); EI-MS m/z 191 (M^+), 160 ($M - OCH_3$)$^+$.

All protons of **6**α and **6**β was unequivocally assigned by the ^1H-^1H COSY experiment. Thus, **6** was identified as an anomeric mixture of methyl 4,6-dideoxy-4-(methylamino)-D-galactopyranoside. Pradimicin C was hydrolyzed in the same way as **1a** and yielded a pale-yellow solid of methyl 4,6-dideoxy-4-amino-D-galactoside mixture (6.2 mg, $\alpha:\beta$ = 78:22): $[\alpha]^{26}_D$ +89.8° (c 0.29, H_2O); SI-MS (glycerol) m/z 178 (MH^+), 200 ($M + Na$)$^+$.

Identity with methyl 4,6-dideoxy-4-amino-D-galactopyranoside was confirmed by a direct comparison with an authentic synthetic sample.[10]

Supplementary Material Available: ^1H NMR chemical shift data of **1a**, **1b**, **1c**, **2**, **3**, and **4** in dimethyl-d_6 sulfoxide and those of **6**α, **6**β, and methyl 4,6-dideoxy-4-amino-D-galactopyranoside in deuteroxide (3 pages). Ordering information is given on any current masthead page.

Biosynthesis of Pradimicin A

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The biosynthesis of pradimicin A (**1**) has been studied by feeding sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetates and D- and L-[1-^{13}C]alanines to the producing organism *Actinomadura hibisca* sp. P157-2 (ATCC 53557). ^{13}C NMR spectroscopy established that the aglycon moiety of **1** is derived from 1 alanine unit and 12 acetate units, condensed in the "head-to-tail" fashion typical of polyketide biogenesis. Of particular interest is the efficient incorporation of D-alanine into **1**, suggesting that D-alanine might act as the direct precursor for the D-alanine side chain of **1**.

Introduction

Pradimicin A (**1**), a new antibiotic, has been found in the culture filtrate of *Actinomadura hibisca* sp. P157-2 (ATCC 53557).[1-3] The antibiotic is active in vitro against a wide variety of fungi and yeasts, some Gram-positive bacteria, and viruses. More interestingly, **1** demonstrates in vivo therapeutic activity against systemic fungal infections caused by *Candida albicans* A9540, *Aspergillus fumigatus* IAM2530, and *Cryptococcus neoformans* IAM4514 in mice. The closely related antibiotics benanomicins A (**2**) and B (**3**) have been reported to be produced by an actinomycete, MH193-16F4.[4] Structurally, all of these compounds contain a glycosylated benzo[a]naphthacenequinone that has a D-alanine side chain. As part of our microbial modification program, we initiated a biosynthetic study of pradimicin A by *A. hibisca* sp. P157-2. This paper presents the spectroscopic analysis of ^{13}C-labeled samples of **1**, which established the biosynthesis of the aglycon of **1**.

Results

[1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetates and D- and L-[1-^{13}C]alanines were fed to cultures of *A. hibisca* sp. P157-2 to establish the biosynthetic origin of the aglycon moiety of **1**. The ^{13}C-enriched samples of **1** thus formed were isolated and the positions of the ^{13}C-enriched carbon atoms determined by ^{13}C NMR spectroscopy.

Acetate Connectivity in 1. Accurate chemical shift assignment of each carbon of **1** was essential in determining which pairs of carbons originate from the same molecule of acetate. In the initial ^{13}C NMR experiments chemical shifts of some of the carbons in **1** crossed over or coalesced at certain pH's, which seemed to occur due to the zwitterionic nature of **1**. However, when **1** was isolated as a water-insoluble solid by adjusting an aqueous solution of

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