Pergamon

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Pramanicin, a Novel Antimicrobial Agent from a Fungal Fermentation

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Abstract: The antimicrobial agent pramanicin (**1**), and a related fatty acid (**6**), were isolated from a corn-based solid or a lactose-containing liquid fermentation of a sterile fungus found growing in grass. The structures of these compounds were determined by a variety of spectral means including UV, IR, and NMR spectroscopy, as well as mass spectrometry. A number of chemical derivatives are also presented here. Pramanicin represents a new class of antimicrobial agents containing a highly functionalized head group and functionalized fatty side chain.

The search for novel antifungal agents has led to the discovery of a wide variety of compounds which can be described in a general fashion as having a polar, highly functionalized head group and simple fatty side chains. Included in this group are restricticin[1a], pneumocandins[1b], sphingofungins[1c], papulacandins[1d], L-657,398[1e], and lipoxymycin[1f]. In spite of their general structural similarity, the target of these antifungal agents is highly variable and includes the sterol biosynthesis pathway, sphingolipid biosynthesis pathway and cell wall biosynthesis. In this paper the isolation, structure determination and chemical derivatization of **1**, an antimicrobial agent which contains both a highly functionalized head group and functionalized side chain, as well as the naturally-occuring side chain itself (**6**), will be described.

RESULTS AND DISCUSSION

Isolation and Structure Determination of 1

The initial isolation of pramanicin (**1**) was fairly straightforward, consisting of extraction, partition, and silica gel and preparative HPLC chromatography. In subsequent isolations it was demonstrated that Sephadex LH-20 (CH_2Cl_2/hexane/MeOH 10:10:2) chromatography could be substituted for the preparative HPLC step, resulting in a better chromatographic yield and no loss of purity.

A listing of the ^{13}C and ^{1}H NMR data derived from the ^{13}C NMR, ^{1}H NMR, DEPT[2], and HMQC[3] experiments in CD_3OD is shown in Table 1. Assignments are indicated on the structure in Figure 1. The ^{1}H NMR data in DMSO-d_6 is also shown in Table 1.

Table 1. ^{13}C and ^{1}H NMR Assignments for **1**.

Atom	^{13}C NMR (CD_3OD)	^{1}H NMR (CD_3OD) 400 MHz	^{1}H NMR (DMSO-d_6) 500 MHz
7	197.88 C		
2	174.94 C		
9	145.13 CH	6.64 dd (15.6, 7.0)	6.36 dd (15.7, 7.5)
8	127.84 CH	7.05 dd (15.6, 0.7)	6.97 bd (15.7,)
3	88.09 C		6.3 bs (OH)
4	78.90 CH	4.15 d (7.2)	3.94 dd (7.2, 5.3) 5.71 bd (5.3) (OH)
11	62.88 CH	2.93 ddd (6.2, 4.9, 1.9)	2.96 ddd (6.0, 5.2, 1.9)
6	61.99 CH_2	3.79 dd (11.7, 2.8) 3.55 dd (11.7, 5.4)	3.57 1H m 3.31 1H m 4.77 1H t (5.3) (OH)
5	60.26 CH	3.47 ddd (7.2, 5.4, 2.8)	3.18 ddd (7.2, 5.4, 2.9)
10	57.79 CH	3.31 ddd (~7 obs, 1.9, 0.7)	3.38 dd (7.5, 1.9)
12	33.05 CH_2	1.60 m	1.55 m
14-18	33.05 CH_2	1.29 bs	1.27 bs
14-18	30.66 CH_2	1.29 bs	1.27 bs
14-18	30.63 CH_2	1.29 bs	1.27 bs
14-18	30.51 CH_2	1.29 bs	1.27 bs
14-18	30.44 CH_2	1.29 bs	1.27 bs
13	26.97 CH_2	1.45 m	1.48 m
19	23.73 CH_2	1.29 bs	1.27 bs
20	14.43 CH_3	0.89 t (6.8)	0.84 t (7.0)
1			7.99 s (NH)

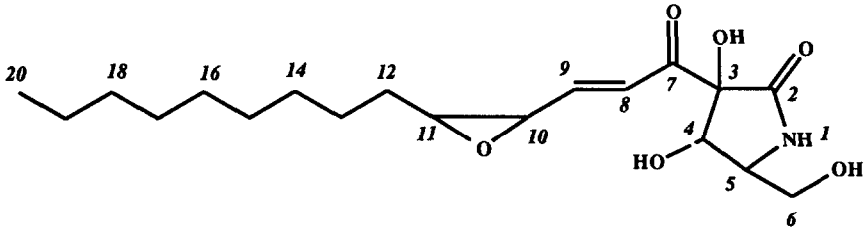

Figure 1. Structure and numbering system for **1**.

Structurally significant fragment ions obtained from **1** and its trimethylsilyl (TMSi) derivative are shown in Figure 2. The methane CI spectrum of the neat compound was of better quality than the corresponding EI spectrum but both contained essentially the same structural information. HR-MS values obtained on the TMSi derivative are listed in Table 2. The number of TMSi groups present in each fragment were determined by comparison of H_9-TMSi and D_9-TMSi spectra.

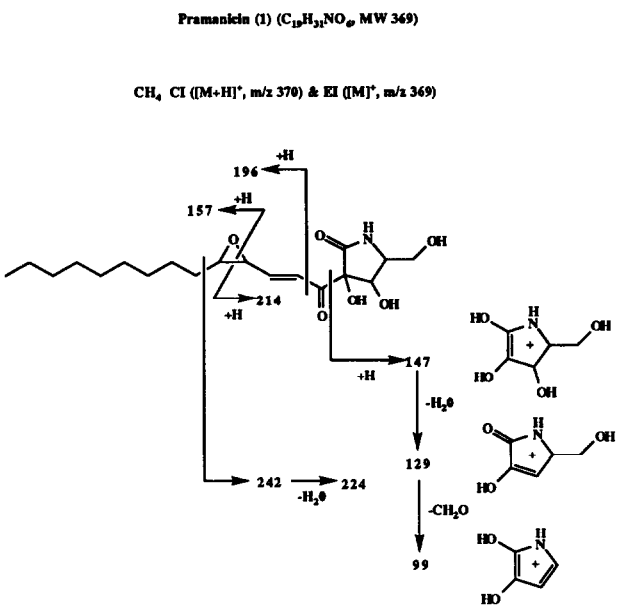

Figure 2. MS fragmentation summary for pramanicin (**1**).

Table 2. HR-MS on H-TMSi Derivative of **1**.

Found	Calculated	Formula
657.3754	657.3733	$C_{19}H_{31}NO_6$+$(SiC_3H_8)_4$
554.3133	554.3153	$C_{18}H_{28}NO_5$+$(SiC_3H_8)_3$
530.2264	530.2246	$C_{10}H_{12}NO_6$+$(SiC_3H_8)_4$
435.2147	435.2113	$C_5H_9NO_4$+$(SiC_3H_8)_4$
398.1630	398.1639	$C_8H_8NO_4$+$(SiC_3H_8)_3$
345.1637	345.1612	$C_5H_7NO_3$+$(SiC_3H_8)_3$
243.1089	243.1111	$C_4H_5NO_2$+$(SiC_3H_8)_2$

The proposed structure was derived from the spectral data based on the following arguments. Two partial structures, shown in Figure 3, were indicated by a COSY[4] experiment. Mass spectral fragmentation suggested that the *m/e* 242 ($C_{10}H_{12}NO_6$) fragment was made up of the polar head group *m/e* 147 ($C_5H_9NO_4$) and a $C_5H_3O_2$ piece which contained a carbonyl, a double bond and an epoxide, since there were three unsaturations and no functionality that would react with TMSi. Carbon-hydrogen coupling constants, derived from an HMBC[5] experiment, of 172 (C-11) and 180 (C-10) Hz confirmed the presence of the epoxide in partial structure A.

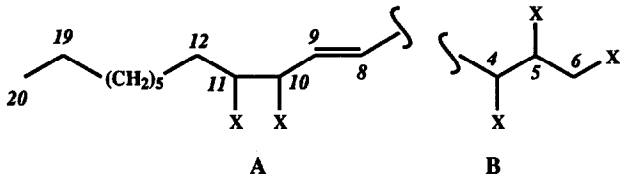

Figure 3. Partial structures of **1** derived from a COSY experiment.

Placement of the carbonyl adjacent to C-8 was suggested by MS, UV (λ_{max} 245 nm, ε 14,428) and IR (1708 cm^{-1}) spectroscopy, and confirmed by the HMBC experiment. The HMBC experiment also indicated that the quaternary carbon (C-3) at 88.09 ppm could be placed between partial structures A and B as indicated in Figure 4.

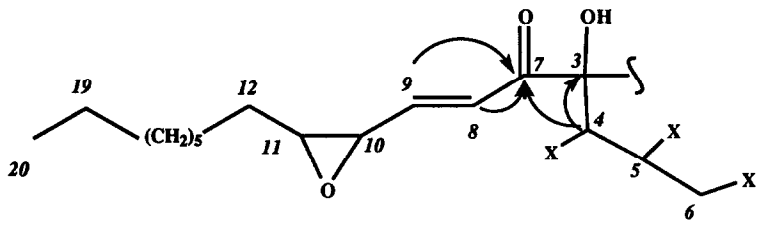

Figure 4. Partial structures of **1** derived from an HMBC experiment.

The only two functionalities left to be placed were a carbonyl and an NH. Based on the IR absorption (1688 cm^{-1}) and the ^{13}C NMR chemical shift (174.94 ppm) of the carbonyl, the two functionalities were thought to constitute an amide. The molecular formula, $C_{19}H_{31}NO_6$, indicated the presence of five unsaturations. The unsaturation for the amide carbonyl plus the three unsaturations shown in Figure 4 indicated that there was one additional unsaturation, suggesting a five-membered ring lactam. The chemical shift (88.09 ppm) of the C-3 carbon suggested that it may be between two carbonyls (see the predicted ^{13}C spectrum derived from molecular modeling[6a,b] in Figure 5), and the chemical shift of the proton on C-5 (3.47 ppm) suggested that it is adjacent to an NH rather than a carbonyl. The carbonyl and NH were, therefore, placed between C-3 and C-5 as shown in the structure in Figure 5. The presence of the amide was confirmed by a ^{1}H-^{15}N HMQC experiment in which the proton at 7.99 ppm correlated with a nitrogen at 126.2 ppm. A value of the one bond ^{1}H-^{15}N coupling constant, $^{1}J_{HN}$ = 92 Hz, was obtained via a coupled 1D HMQC experiment.

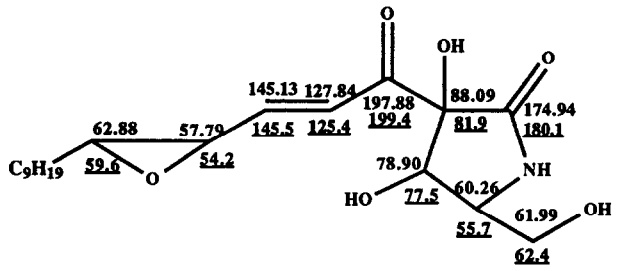

Figure 5. Observed ^{13}C NMR chemical shifts are shown along with predicted chemical shifts (underlined) based on molecular modeling[6a,b] of **1**.

The important correlations to confirm the proposed five-membered lactam were not seen in the HMBC experiment in CD_3OD. However, in DMSO-d_6 the hydroxyl and NH protons were not exchanged and a number of key correlations that support the proposed structure were observed in an HMBC experiment as shown in Figure 6.

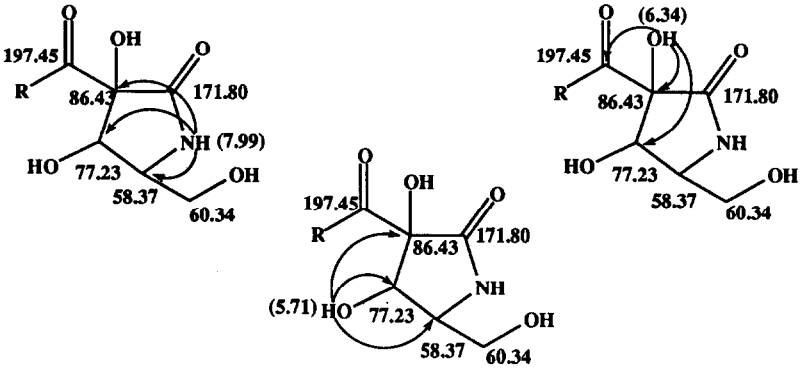

Figure 6. Key HMBC correlations for **1** in DMSO-d_6. proton chemical shifts are in parentheses.

In addition, two key SIMBA[7] (selective inverse multiple bond analysis) correlations, illustrated in Figure 7, confirm the proposed structure.

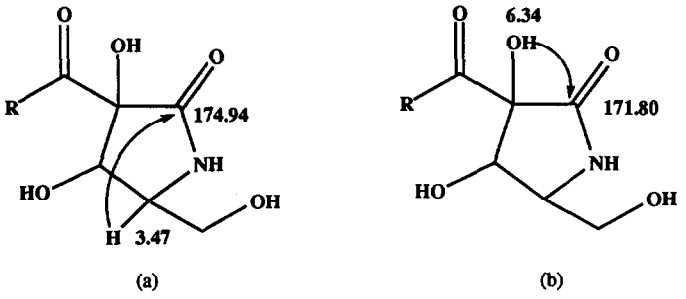

Figure 7. SIMBA experiments on **1** in CD_3OD (a) and DMSO-d_6 (b).

The small coupling constant between the epoxide protons on carbons 10 and 11 (~2 Hz) suggested that the epoxide had a *trans* orientation. A ROESY[8] experiment supported the *trans* stereochemistry with correlations between H-9 and H-11, and H-10 with the H-12 and H-13, protons, but no correlation between H-10 and H-11 (Figure 8). Reaction of **1** with KSeCN[9a,b] yielded the dienone **2** (Figure 9), a method expected to conserve the stereochemistry in the resulting double bond, resulted in a *trans* double bond (J=15.1 Hz), in support of the proposed *trans* orientation of the epoxide.

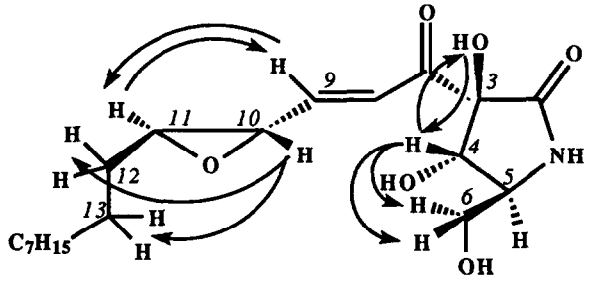

Figure 8. Key ROESY correlations for **1** in DMSO-d_6.

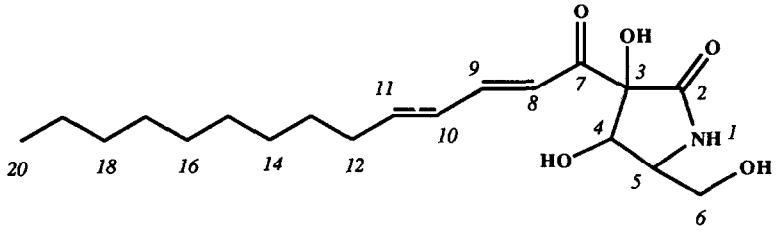

Figure 9. Structure of the dienone **2**.

The relative stereochemistry of the lactam ring was also determined via interpretation of ROESY correlations as indicated in Figure 8. The strong correlations between the hydroxyl proton on C-3, the C-4

proton, and the C-6 protons placed all these groups on the same side of the molecule, while the lack of significant interaction between H-4 and H-5 indicate that H-5 is on the opposite side of the molecule. In one conformer with this relative stereochemistry, the dihedral angle between H-5 and the N-H is ~90°, which is consistent with the lack of coupling between these two protons.

Acid Instability of 1

It was noted that in a mixture of MeOH and dilute aqueous HCl (pH 2) **1** slowly yielded a mixture of three compounds but favoring production of **3** and **4**. On the other hand, in a mixture of MeOH and strongly acidic water (1 N HCl), **1** was rapidly converted to **5** and small quantities of **3** and **4**. The structures of **3**, **4**, and **5** are shown in Figure 10.

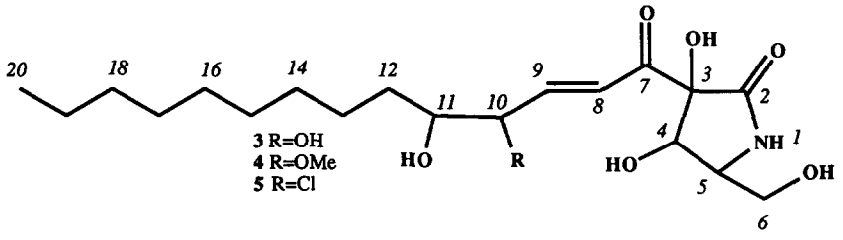

Figure 10. The structures of the acid reaction products of **1**.

The Structure of Component 6

A second natural product (**6**) was isolated from a liquid fermentation of this fungus, and shown to have the structure indicated in Figure 11. The protons on carbons 2 to 5 were apparent, although the signal for the proton on C-2 was extremely broad. Connectivity for this section was established via single-frequency decoupling. Neither C-1 nor C-2 was visible in the 300 MHz ^{13}C NMR, however, H-2 at 6.24 ppm correlated with C-2 at 126.3 ppm in the HMQC experiment, while C-1 at 169.5 ppm was apparent from the correlation with H-3 in the HMBC experiment.

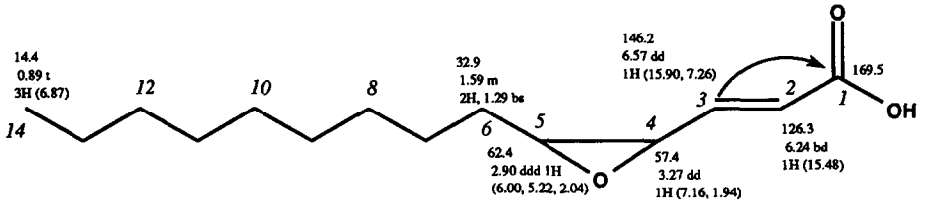

Figure 11. The structure of component **6**.

Antimicrobial Activity

Pramanicin (**1**) showed antifungal MIC's in the range 4 to >100 μM against *Candida* sp., 100 μM against *Aspergillus fumigatus*, and 20 μM against *Cryptococcus neoformans*. The acapsular form of *Cryptococcus neoformans* (MY2062) was, however, much less resistant to pramanicin. Antibacterial activity was also observed against *Bacillus subtilis* with an MIC of 4 μM.

Table 3. Antimicrobial Activity of **1**.

Organism	Merck Culture Number	MIC Pramanicin μM
Candida albicans	MY 1055	20.0
Candida parapsilosis	MY 1010	4.0
Candida tropicalis	MY 2167	20.0
Candida krusei	MY 2168	> 100.0
Cryptococcus neoformans	MY 2061	20.0
Cryptococcus neoformans	MY 2062	0.062
Aspergillus fumigatus	MF 5668	100.0
Bacillus subtilis	MB 964	4.0

EXPERIMENTAL SECTION

IR absorption spectra were obtained with a Perkin-Elmer model 1750 Infrared Fourier Transform Spectrophotometer using a multiple internal reflectance cell (MIR, ZnSe) on neat 10-20 μg samples. UV absorption spectra were measured with a Beckman DU-70 Spectrophotometer.

Nominal mass resolution mass spectral data was obtained on a Finnigan-MAT TSQ700 instrument. HR-MS measurements were performed on a JEOL SX102A instrument using perfluorokerosene as internal standard. Trimethylsilyl (TMSi) derivatives were prepared by reaction with a 1:1 mixture of bistrimethylsilyltrifluoroacetamide (BSTFA) and pyridine at 50°C for 30 min.

NMR spectra were recorded at 300 MHz on a Varian XL-300 spectrometer, 400 MHz on a Varian Unity 400 spectrometer or at 500 MHz on a Varian Unity 500 spectrometer. ^{1}H NMR spectra were recorded in CD_3OD or DMSO-d_6 at 25°C using the solvent peaks at δ3.30 or δ2.49, respectively, as internal reference downfield of tetramethylsilane (TMS) at zero ppm. ^{13}C NMR spectra were recorded at 100 MHz in CD_3OD at 25°C where chemical shifts are given in ppm downfield of TMS using the solvent peak at 49.0 ppm as internal reference. Proton-proton chemical shift correlation spectra (COSY) were recorded in CD_3OD using the standard pulse sequence[4]. Proton-carbon chemical shifts correlations were obtained using inverse detection and HMQC[3], HMBC[5], and SIMBA[7] pulse sequences. The HMBC spectra (CD_3OD, 400 MHz; DMSO-d_6, 500 MHz) were optimized for a long range $^nJ_{CH}$ of 7 Hz while the SIMBA spectra (DMSO-d_6, 500 MHz) were optimized for a long range $^nJ_{CH}$ of 4 Hz and utilized either a 6.2 or 12.4 mS gaussian pulse for the selective 90 degree ^{13}C pulse. ROESY[8] spectra were obtained using the pulse sequence described by Kessler *et al.*[13] with a mixing time of 200 mS. ^{1}H-^{15}N HMQC spectra (500 MHz) were obtained in approximately 15 minutes on a 7 mg sample of pramanicin dissolved in 220 μL of DMSO-d_6 at 25°C utilizing a 3 mm micro inverse probe (Nalorac Cryogenics Corp). ^{15}N chemical shifts were referenced to external 2.9 M $^{15}NH_4Cl$ in 1M HCl which has been assigned a chemical shift of 24.9 ppm with respect to liquid NH_3 (0 ppm).[14]

Description of the Producing Organism

The producing organism (MF5868=BW283, Merck Microbial Resources Culture Collection) was isolated from internal tissues of surface-sterilized culms of an unidentified grass collected in Sussex Co., New

Jersey. The fungus was grown on a variety of mycological media, under different light regimes, and on cellulosic materials such as sterilized leaves and filter paper but, in all cases, failed to sporulate and thus could not be identified.

In agar culture, the fungus exhibits the following morphology: Colonies on oatmeal agar (Difco) at 25°C, 12 hr photoperiod attaining 35 mm in 7 days, slighty raised, with advancing zone appressed, even, with lanose to floocose, dull, obscurely zonate, at first white but soon pale gray, Pale Gull Gray (capitalized color names from Ridgway[10]), Light Gull Gray, Light Olive-Gray, Olive-Gray, to gray bluish gray, Gull Gray, Storm Gray to dull olivaceous black, Dusky Green-Gray in age developing small hemispherical or pulvinate mycelial masses that resemble protoperithecia or conidiomata, mycelial masses forming droplets of a clear exudate, with surfaces becoming lacunose in age due to collapse or evaporation of exudate droplets with reverse dull gray.

Colonies on Emerson's Yp Ss agar (Difco) at 25°C, 12 hr photoperiod attaining 44-48 mm in 7 days, similar in appearance to colonies on oatmeal agar except margin is more appressed, surface is more zonate, with less development of sterile mycelial masses, reverse dull gray to olivaceous black, zonate.

Colonies on Blakeslee's malt agar at 25°C, 12 hr photoperiod attaining 60-61 mm in 7 days, similar in appearance above, but less zonate. No growth at 37°C.

The vegetative hyphae were dematiaceous, branched, simple-septate hyphae, 1.5-4 μm in diameter. Hyphal cells are multinucleate when viewed by fluorescent staining with 4',6'-diamidino-2-phenyindole[11]. These vegetative characteristics suggest that the fungus belongs to the Ascomycotina.

Fermentation

A vegetative culture of MF5868 was prepared by inoculating a 54-ml portion of seed medium[12] in a 250-ml unbaffled, cotton-plugged Erlenmeyer flask with 2-ml of mycelia in 10% glycerol that had been stored at -80°C. This seed culture was incubated for 3 days at 25°C with 50% relative humidity on a rotary shaker at 220 rpm with a 5-cm throw under constant fluorescent light. Pelleted growth was blended for 5-10 seconds in a Waring blender, and 2-ml portions were then used to inoculate second stage seed cultures that were incubated for 2 days as stated above. Each portion of production medium was then inoculated with a 2-ml portion of the second stage culture. Production cultures, either with a solid cracked corn-based medium (component/ 250-ml flask: cracked corn, 10.0 g; Ardamine PH, 2.0 mg; KH_2PO_4, 1.0 mg; $MgSO_4 \cdot 7H_2O$, 1.0 mg; Na tartrate, 1.0 mg; $FeSO_4 \cdot 7H_2O$, 0.1 mg; $ZnSO_4 \cdot 7H_20$, 0.1 mg; distilled water, 10.0 ml. pH was not adjusted prior to autoclaving for 20 min. Immediately before use, the medium was moistened with 15 ml of distilled water and autoclaved again for 20 min.) or with 50 ml of a liquid medium (lactose, 75 g/l; yellow corn meal, 20 g/l; Ardamine PH, 5 g/l; L-leucine, 3.5 g/l; 2-(N-morpholino)-ethanesulfonic acid, 16.2 g/l. pH of medium was adjusted to 6.0 with NaOH prior to autoclaving) in 250-ml unbaffled Erlenmeyer flasks were incubated at 25°C with 50% relative humidity for up to 21 days. The flasks containing the solid cracked corn-based medium were grown under static conditions while the liquid fermentation flasks were shaken. All other incubation parameters remained the same as above. The medium components used in this study were obtained from the following sources: cracked corn (Bay-mor), Ardamine PH (Champlain Industries Inc.), yellow corn meal (Quaker Oats Co.). Other components were standard reagent-grade compounds and salts. All media were steam sterilized at 121°C at 15 psi for 20 minutes.

Isolation of Pramanicin (1)

After 21 days of growth in solid cracked corn-based medium, as described above, the contents of 18 flasks were mixed with 50-ml portions of methyl ethyl ketone (MEK) and shaken at 220 rpm for 1 hour at 25°C. The titer of (**1**) via HPLC was 200 μg/ml. The mixture was filtered and the resulting 700 ml of filtrate was diluted to 900 ml with additional MEK. Six hundred and fifty ml of hexane was added to 650 ml of this MEK extract, which resulted in a 65 ml lower, aqueous-rich layer and an 1120 ml MEK/hexane upper layer, which contained **1**. The upper layer was concentrated to dryness *in vacuo* and partitioned between 65 ml MeOH and 65 ml hexane. This yielded an upper hexane layer of 24 ml and a lower MeOH/hexane layer of 118 ml, which contained the compound of interest.

The lower layer from the second partition was concentrated to dryness, reconstituted in 10 ml EtOAc, and chromatographed on a 460 ml silica gel 60 (EM Science, 230-400 mesh) column using a step gradient (EtOAc, EtOAc/MeOH 98:2, EtOAc/MeOH 90:10, EtOAc/MeOH 50:50). The compound of interest was located in composite rich cut eluting with EtOAc/MeOH 90:10.

The silica gel rich cut was concentrated to dryness, reconstituted in 2 ml of MeOH/H_2O (95:5), centrifuged, and a 0.5 ml portion chromatographed on a 2.12 X 25 cm Zorbax RX C-8 column using MeOH/H_2O 70:30. A 12.6 mg sample (λ_{max} MeOH 245, $E^{\%}$ 391) of **1** was obtained upon lyophilization of the rich cut from preparative HPLC.

Pramanicin (1)

Pramanicin formed a crystalline, white solid (m. p. 110-113°C) from MeOH/H_2O. UV λ_{max} 245 nm (ε 14,428). IR 3000-3600, 1708, 1688 cm^{-1}. $[\alpha]^{25}_D$ =-35° (*c* 0.21 in MeOH).

Conversion of 1 to Dienone 2

A solution of **1** (5.3 mg, 14.4 μmol) in 300 μL of solvent (MeOH/H_2O, 10:1) was placed in a 1 mL reaction vial equipped with magnetic stirring bar. A solution of KSeCN (12.15 mg, 84.3 μmol) in 200 μL of solvent (MeOH/H_2O, 10:1) was added in one portion to the reaction vial with stirring. The reaction mixture was stirred at room temperature for 12 h, while being monitored by HPLC. After 12 h the reaction mixture was diluted with 2.0 ml of CH_2Cl_2 and the organic layer separated and washed with water (2 X 2.0 mL) to remove the inorganic salts. The CH_2Cl_2 layer was concentrated to dryness and purified by HPLC using MeOH/H_2O (70:30) as eluant on a Zorbax RX C-8 column. The retention time of **2** was 10.65 mins (1.6 mg, 31%).

HREIMS $C_{19}H_{31}NO_5$ (calc. *m/z* 353.2202, found 353.2266). IR 3345, 1700 cm^{-1}. UV λ_{max} MeOH 289 nm (ε 18,519). ^1H NMR (CD_3OD, 400 MHz) δ 0.89 (t, J=7 Hz, 20-H_3), 1.23-1.36 (16 H, bm), 2.06 (q, J=7 Hz, 12-H_2), 3.59 (dd, J=11.5, 5.5 Hz, 6b-H), 3.66 (ddd, J= 11.5, 5.5, 2.5 Hz, 5-H), 3.85 (dd, J=11.5, 2.5 Hz, 6a-H), 4.27 (d, J=7.0 Hz, 4-H), 6.13 dt (J=15.1, 6.7 Hz, 11-H), 6.19 dd (J=15.2, 10.6 Hz, 10-H), 6.81 (d, J=15.25 Hz, 8-H), 7.32 (dd, J=15.25, 10.37, Hz, 9-H).

Reactions of 1 with Methanolic Acid

Fifteen mg of **1**, in 1.5 ml MeOH/aqueous HCl (pH 2.0) 2:1, was stirred for 120 hrs at room temperature, the pH adjusted to 6.4, and lyophilized. This dried preparation was reconstituted in 0.5 ml MeOH/H_2O (70:30) and chromatographed on a 0.94 X 25 cm Zorbax RX C8 column at 2 ml/min using

MeOH/H_2O (70:30) as the mobile phase and UV detection at 245 nm. A 3.0 mg sample of **3**, a 4.3 mg sample of **4**, and a 1.8 mg sample of **5** were recovered after concentration of the appropriate fractions from the preparative HPLC.

Component **3**, MW 387 (369 + 18) via FABMS and EIMS of the penta- and hexasilyl derivatives. ^1H NMR (CD_3OD, 300 MHz) δ 0.89 t (J=6.8 Hz, 20-H_3), 1.29 bs, 3.50 m (5-H), 3.57 d (J=5.4 Hz, 6-H), 3.80 m (6-H), 4.12 ddd (J=6.0, 4.5, 1.5 Hz, 10-H), 4.17 d (J=7.2 Hz, 4-H), 6.97 dd (J=15.6, 1.5 Hz, 8-H), 7.08 dd (J=15.6, 4.5 Hz, 9-H).

Component **4**, HRFABMS $C_{20}H_{35}NO_7Li$ (calc. *m/z* 408.2573, found 408.2553). via FABMS and EIMS of the pentasilyl derivative. ^1H NMR (CD_3OD, 300 MHz) δ 0.89 t (J=6.8 Hz, 20-H_3), 1.29 bs, 3.34 s (10-OMe), 3.49 m (5-H), 3.55 dd (J=11.6, 5.6 Hz, 6-H), 3.72 ddd (J=6.0, 4.8, 0.6 Hz, 10-H), 3.80 dd (J=11.4, 2.4 Hz, 6-H), 4.17 d (J=7.2 Hz, 4-H), 6.84 dd (J=15.9, 6.2 Hz, 9-H), 6.96 dd (J=15.9, 0.8 Hz, 8-H).

Component **5**, HREIMS $C_{19}H_{32}NO_6Cl$ (calc. *m/z* 405.1918, found 405.1843). ^1H NMR (CD_3OD, 300 MHz) δ 0.89 t (J=6.8 Hz, 20-H_3), 1.29 bs, 4.16 d (J=7.2 Hz, 4-H), 4.52 dd (J=4.8, 7.7 Hz), 6.89 dd (J=15.5, 7.7 Hz, 9-H) 6.99 d (J=15.5 Hz, 8-H).

Component 6

After 8 days of growth, 1.5 liters of culture in liquid medium 2 were mixed with 1.4 volumes of MEK and shaken at 220 rpm for 1 hr at 25°C. A 1930 ml portion of hexane was added to 1930 ml of the MEK extract from a liquid fermentation of this fungi. The lower aqueous layer was discarded and the upper layer was concentrated and partitioned into two layers by the addition of 350 ml hexane and 150 ml MeOH. The lower layer was concentrated to dryness and chromatographed on silica gel using a step gradient of EtOAc/MeOH. The first 90:10 EtOAc/MeOH eluate was concentrated *in vacuo* and chromatographed on a 2.12 X 25 cm Zorbax RX C-8 column using MeOH/H_2O 70:30, followed by a 90:10 MeOH/H_2O wash. A fraction from the 90:10 MeOH/H_2O wash contained 2.9 mg of **6**.

HREIMS $C_{14}H_{24}O_3$ (found 240.1746, calc. 240.1725). UV λ_{max} MeOH 208 nm (ε 12,960). IR 1722. ^1H NMR (CD_3OD, 400 MHz) δ 0.89 t (J=6.87 Hz, 14-H_3), 1.29 bs, 1.46 m 2H (7-H_2), 1.59 m 2H (6-Ha), 2.90 ddd (J=6.00, 5.22, 2.04 Hz, 5-H), 3.27 dd (J=7.16, 1.94 Hz, 4-H), 6.24 bd (J=15.48 Hz, 2-H), 6.57 dd (J=15.90, 7.26 Hz, 3-H). ^{13}C NMR (CD_3OD, 300 MHz), 146.23 (3-C), 62.49 (5-C), 57.45 (4-C), 33.07 (6-C), 33.00, 30.66, 30.66, 30.52, 30.44 (8-C, 9-C, 10-C, 11-C, 12-C), 26.95 (7-C), 23.75, (13-C), 14.44 (14-C). HMQC (CD_3OD, 400 MHz) 146.2 (3-C), 6.57 dd (3-H); 126.3 (2-C), 6.24 bd (2-H); 62.4 (5-C), 2.90 ddd (5-H); 57.4 (4-C), 3.27 dd (4-H); 32.9 (6-C), 1.59 m (6a-H), 1.29 bs (6b-H); 30.5 (8-C, 9-C, 10-C, 11-C, 12-C), 1.29 bs (8-H, 9-H, 10-H, 11-H, 12-H); 26.9 (7-C), 1.46 m (7a,b-H); 23.6 (13-C), 1.29 bs 13a,b-H); 14.4 (14-C), 0.89 t (14-H). HMBC (CD_3OD, 400 MHz) 6.57 dd (3-H), 62.4 (5-C), 57.4 (4-C), 169.5 (1-C).

MIC Determinations

Antifungal activity was determined using microtiter dish broth dilution assays in which the organisms were added to CM media (1% peptone, 0.5% yeast extract, 0.05% dextrose) at 1 X 10^3 colony forming units per ml. The dishes were incubated at 30° C for 18 hour and read for visible growth. The lowest concentration to prevent visible growth was defined as the MIC (minimum inhibitory concentration).

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