Vertilecanins: New Phenopicolinic Acid Analogues from *Verticillium lecanii*

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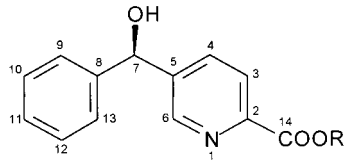
Five new phenopicolinic acid analogues (**1**−**5**) have been isolated from solid-substrate fermentation cultures of *Verticillium lecanii*. The most abundant component (vertilecanin A; **1**) displays antiinsectan activity against *Helicoverpa zea*. These compounds were obtained by chromatographic fractionation of the EtOAc culture extract and identified by analysis of NMR and MS data. The known fungal metabolites 2-decenedioic acid and 10-hydroxy-8-decenoic acid were also isolated from these cultures.

Mycoparasitism within fungal communities has frequently been observed and reported.[1,2] Our initial studies of fungi that colonize *Aspergillus flavus* sclerotia have led to the isolation of a number of antifungal agents, including some with activity against the host species.[3−5] During our ongoing chemical studies of mycoparasitic and fungicolous fungi, a strain of *Verticillium lecanii* (Zimm) Viegas (deuteromycetes; NRRL 26576) originally isolated from a mushroom (*Amanita bisporigera*) was grown in solid-substrate fermentation on rice. *V. lecanii* is known both as a mycoparasite and as a pathogen of insects, and the EtOAc extract of these fermentations exhibited antiinsectan activity against *Helicoverpa zea* (corn earworm). Fractionation of the extract by Sephadex LH-20 column chromatography, followed by semipreparative reversed-phase HPLC, afforded five new phenopicolinic acid derivatives. The structures of these metabolites, which we named vertilecanin A (**1**), vertilecanin A methyl ester (**2**), vertilecanin B (**3**), vertilecanin B methyl ester (**4**), and vertilecanin C (**5**), were determined by analysis of NMR and MS data. The known compounds 2-decenedioic acid and 10-hydroxy-8-decenoic acid were also isolated.



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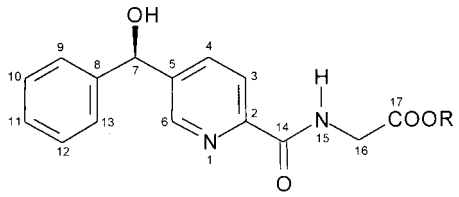
2 R = CH_3



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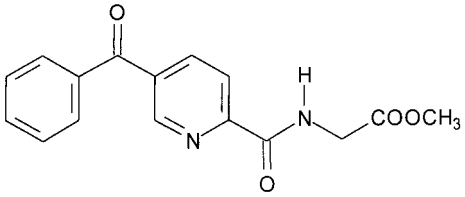

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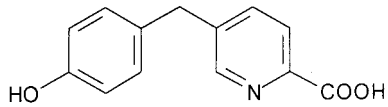

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Results and Discussion

The HREIMS data for vertilecanin A (**1**) showed a molecular ion at *m*/*z* 229.0745, consistent with the molecular formula $C_{13}H_{11}O_3N$ (nine unsaturations). The ^1H and ^{13}C NMR data for **1** (Table 1) contained signals for a phenyl group, a 2,5-disubstituted pyridine ring, and an isolated sp^3 oxymethine unit. DEPT results indicated the presence of two exchangeable protons, one of which was assigned to a carboxylic acid group (^{13}C NMR signal at δ 165.5; IR band at 1718 cm^{-1}). The remaining exchangeable proton must be attributed to a hydroxy group.

An HMBC experiment recorded in CD_3OD allowed straightforward assembly of the structure of vertilecanin A. HMBC correlations (Table 1) of the isolated oxygenated methine proton H-7 to C-9/13 of the phenyl ring and to C-4 and C-6 of the pyridine ring linked carbons C-5 and C-8 to C-7. The signal for H-3 of the pyridine unit correlated with carboxylic acid carbon C-14, placing this group at C-2 of the pyridine ring. On the basis of these data, the structure of vertilecanin A was assigned as **1**.

To determine the absolute configuration at C-7, the carboxylic acid functionality was first converted to the corresponding methyl ester by treatment with trimethylsilyldiazomethane in CH_3OH to give **2**. This product was then converted to *R*/*S*-phenylbutyrate ester derivatives by treatment with a sample of *R*-phenylbutyryl chloride that contained a small quantity of the *S*-isomer. Formation of the desired phenylbutyrate ester products was confirmed by downfield shifts of H-7 (e.g., from δ 5.90 to δ 6.97 in the *R*-isomer) and by the appearance of additional proton signals for the new acyl group. The ^1H NMR data for the derivatives were compared, and downfield shifts of the H-3

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Table 1. NMR Data for Vertilecanin A (**1**) and Vertilecanin B (**3**)

position	**1** δ_H (mult., *J* in Hz)[a]	**1** δ_C[b]	**1** HMBC correlations[b] (C#)	**3** δ_H (mult., *J* in Hz)[a]	**3** δ_C[a]	**3** HMBC correlations[a] (C#)
2		148.2			149.5	
3	8.10 (d, 8.1)	125.6	2, 5, 14	8.06 (d, 8.1)	122.4	5
4	8.04 (dd; 8.1, 2.1)	137.1	2, 7	7.97 (dd; 8.1, 2.0)	136.1	2, 6
5		145.5			144.7	
6	8.74 (br s)	147.9	2, 4, 5	8.67 (d, 2.0)	147.8	2, 4, 5
7	6.04 (s)	74.0	4, 5, 6, 8, 9/13	6.00 (s)	73.7	4, 6, 8, 9/13
8		144.4			145.1	
9/13	7.45 (br d, 7.2)[c]	127.4	7, 11, 13/9	7.46 (d, 7.6)[c]	127.3	7, 11, 13/9
10/12	7.33 (t, 7.2)[c]	129.4	8, 12/10	7.33 (t, 7.6)[c]	129.3	8, 12/10
11	7.25 (m)[c]	128.6	9/13	7.24 (t, 7.6)[c]	128.2	9/13
14		167.4			164.8	
15−NH				8.61 (br m)		
16				4.21 (br s)	41.2	14, 17
17				-	171.3[d]	

[a] Recorded in acetone-d_6 solution. [b] Recorded in CD_3OD solution. [c] Signals appear as distorted multiplets due to magnetic inequivalence. [d] Chemical shift measured from HMBC data.

(δ_{S-R} +0.08) and H-4 (δ_{S-R} +0.09) signals for the *S*-phenylbutyrate ester relative to those of the *R*-derivative were observed. Thus, by application of Helmchen's rules,[6] the configuration at C-7 was determined to be *R*. This new compound bears close resemblance to phenopicolinic acid (**6**), a dopamine β-hydroxylase inhibitor originally isolated from cultures of a *Paecilomyces* sp.[7]

HRESIMS analysis of a second related metabolite (**2**) indicated the molecular formula $C_{14}H_{13}O_3N$ (nine unsaturations). The ^1H NMR spectrum was nearly identical to that of **1** except for the presence of an additional methoxy resonance (δ 3.88) showing a long-range HMBC correlation to the carboxy carbon at δ 166.1 (see Experimental Section). The data for this compound also matched those obtained for the methyl ester prepared from **1** (see above), supporting the assignment of **2** as the methyl ester of vertilecanin A.

A molecular formula of $C_{15}H_{14}O_4N_2$ (10 unsaturations) was consistent with the HRESIMS data for another related metabolite, vertilecanin B (**3**). The ^1H and ^{13}C NMR data for **3** (Table 1) were very similar to those of vertilecanin A (**1**), but included new signals corresponding to a methylene group and an amide functionality. The downfield-shifted methylene proton doublet was coupled to the secondary amide proton signal at δ 8.60. HMBC correlations (Table 1) of the methylene signal to the amide (δ 164.8) and carboxy (δ 171.3) carbons suggested the presence of an acylated glycine subunit. Although no correlations were observed between H-3 and C-14 or between NH-15 and C-2, the acylated glycine subunit was attached to the pyridine ring at C-2 on the basis of NMR similarities and structural analogy to compounds **1** and **2**. These observations led to the assignment of structure **3** for vertilecanin B.

A molecular formula of $C_{16}H_{16}O_4N_2$ (10 unsaturations) was deduced from the HRESI mass spectrum of compound **4**. The ^1H NMR data for **4** were nearly identical to those of **3**, except for the presence of an additional methoxy resonance at δ 3.68. Irradiation of the methoxy proton signal in a selective INEPT experiment resulted in polarization transfer to the carboxy carbonyl signal (δ 170.9), indicating the presence of a methyl ester group. This compound was therefore identified as vertilecanin B methyl ester (**4**).

The ^{13}C NMR spectrum of a fifth related metabolite resembled that of compound **4**, except that the C-7 oxygenated methine carbon signal in **4** was replaced by a ketone carbonyl signal, suggesting that **5** differs from **4** by replacement of the secondary alcohol unit with a ketone group. These data, together with HRESIMS and HMBC and 1D NMR data otherwise paralleling those for **4**, supported the assignment of structure **5** for vertilecanin C.

The absolute stereochemistry at C-7 for compounds **2**−**4** was presumed to be *R* in each case by analogy to vertilecanin A (**1**). However, since the optical rotations of vertilecanins A and B (and the corresponding methyl esters) were of opposite sign, this stereochemical assignment was verified by conversion of vertilecanin A (**1**) to vertilecanin B methyl ester (**4**). Treatment of vertilecanin A with glycine methyl ester in the presence of DCC afforded a sample of **4**. After HPLC purification, this semisynthetic product gave an optical rotation that matched the rotation observed for the sample of **4** isolated from the *V. lecanii* extract.

The known compound 2-decenedioic acid was also isolated as a major constituent of the EtOAc extract. 2-Decenedioic acid has been previously isolated from *Penicillium notatum*,[8] as well as nonfungal sources. Interestingly, it has also been reported as a nematicidal product from the nematode-trapping fungus *Pleurotus ostreatus*.[9] The related metabolite 10-hydroxy-8-decenoic acid was obtained as a minor component. This metabolite has been reported as a constituent of injured fruit bodies of several mushrooms, including *Lepista nebularis* and *Cantharellus tubaeformis*.[10,11] Both compounds were identified through analysis of their NMR and mass spectral data and by comparison with literature values.

Although it cannot be ruled out that some portion of the isolated quantities of vertilecanin A and B methyl esters (**2** and **4**) could be artifacts, the evidence suggests that these compounds were not formed during the isolation process. The EtOAc extract was dissolved in MeOH for partitioning at an early stage. However, exposure of **1** and **3** to methanol for lengthy periods did not produce any trace of **2** or **4** by ^1H NMR analysis. In addition, ^1H NMR signals apparently corresponding to methoxy groups were detectable in the crude EtOAc extract prior to any exposure to MeOH. Finally, samples of both **2** and **4** were isolated in a scale-up procedure that avoided the use of solvent mixtures containing methanol for chromatography.

None of the isolated compounds showed significant activity in assays against *H. zea* at the 100 ppm dietary level. However, vertilecanin A (**1**) is a major component of the crude extract, and when tested at a level commensurate with its abundance in the extract, significant activity was observed (49% reduction in weight gain relative to controls when tested at 1500 ppm). Thus, vertilecanin A appears to account for most, if not all, of the antiinsectan activity

of the original extract. Vertilecanin A also shows some modest antibacterial activity, producing an 8 mm zone of inhibition in a standard disk assay against *Bacillus subtilis* (ATCC 6051) at 100 μg/disk. Vertilecanin B (**3**) and 10-hydroxy-8-decenedioic acid were inactive at this concentration.

Experimental Section

General Experimental Procedures. Reversed-phase HPLC employed a Rainin Microsorb C_{18} column (1 × 25 cm, 5 μm particles). ^{1}H and ^{13}C NMR data were obtained using $CDCl_3$, CD_3COCD_3, or CD_3OD solutions with a Bruker AC-300 spectrometer operating at 300 or 75.5 MHz or a Bruker WM-360 operating at 360 or 90 MHz. HMQC and HMBC spectra were recorded on a Bruker AMX-600 spectrometer. Residual protonated solvent signals were used as internal references. FAB mass spectra were obtained using a VG ZAB-HF double-focusing mass spectrometer. Details of other general experimental procedures have been described elsewhere.[12]

Fungal Material. A mature basidiocarp of *Amanita bisporigera* (No. 55255) was collected at the Sand Ridge State Park, Forest City, Tazwell Co., IL, by Dr. Harry D. Thiers on August 8, 1995. This collection of *A. bisporigera* was returned to the laboratory in a plastic bag, placed in the freezer (−7 °C), and freeze-dried (Virtis, Inc.) within 7 days after the date of collection. To isolate microfungal colonists, the dried basidiocarp was reduced to a powder by mixing at "low" speed in a sterilized food homogenizer for 2 min. The powdered fungal tissue was transferred to a plastic bag and returned to the freezer. Direct plating of stromatal filings was accomplished by sprinkling a small portion (100−200 mg) of this powder over the surface of each of two plates of dextrose peptone yeast extract agar (DPYA) containing streptomycin (25 mg/L) and tetracycline (1.25 mg/L). Plates were incubated in the dark at 25 °C for 5 days, and representative cultures were isolated from each colony type showing a distinctive morphology on DPYA. After 7−12 days incubation, the tube cultures isolated from the powdered basidiocarp of *A. bisporigera* was segregated into groups of presumptive species and maintained for identification and rice fermentation (25 °C). One of these cultures was identified as *Verticillium lecanii* (NRRL 26576).

V. lecanii was grown on several slants of potato dextrose agar (PDA) for 14 days (25 °C). A hyphal fragment-spore suspension (propagule density, 10^3/mL of sterile distilled water) prepared from potato dextrose agar slants served as the inoculum. Fermentations of *V. lecanii* were carried out in eight 500 mL Erlenmeyer flasks, each containing 50 g of rice (Botan Brand; J.F.C. International). Distilled water (50 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. After the flasks had been autoclaved and cooled to room temperature, they were inoculated with 1.0 mL of the hyphal fragment-spore suspension and incubated for 40 days at 25 °C.

Extraction and Isolation. Following incubation, the fermented rice substrate in each flask was first fragmented with a spatula and then extracted repeatedly with EtOAc (3 × 50 mL). The EtOAc extracts were combined, filtered, and evaporated to give a yellow oil (3.3 g). This extract was partitioned between hexane and 10% H_2O in MeOH, and the antifungal activity was concentrated in the H_2O−MeOH fraction (609 mg). This fraction was then subjected to Sephadex LH-20 column chromatography, eluting sequentially with 250 mL each of CH_2Cl_2−hexane (1:1), CH_2Cl_2−hexane (4:1), and CH_2Cl_2−acetone (3:2, then 1:1, then 1:4). Fractions were pooled on the basis of TLC analysis to afford five fractions. Fraction 3 (175 mg; eluted with 3:2 CH_2Cl_2−acetone) was rechromatographed on a silica gel column (2 × 30 cm) with a stepwise gradient eluting from 0% to 40% (v/v) (2% increments) MeOH in $CHCl_3$. Subfractions 2 (21 mg) and 4 (37 mg), eluting with 2% and 20% CH_3OH−$CHCl_3$, respectively, were further purified by semipreparative reversed-phase HPLC. HPLC separation of subfraction 2 (Beckman Ultrasphere C_{18} column, 1 × 25 cm, 5 μm particles, 2.0 mL/min, 20−85% CH_3CN in 0.1% HCOOH/H_2O for 30 min) afforded 10-hydroxy-8-decenoic acid (2.2 mg, t_R 19.6 min). Subfraction 4 was chromatographed using 10−40% CH_3CN in 0.1% HCOOH−H_2O for 30 min (monitored by UV absorption at 215 nm, flow rate 2.5 mL/min) to afford vertilecanin A (**1**; 7.8 mg, t_R 22.7 min). Fraction 6 from the Sephadex column (35 mg; eluted with 1:4 CH_2Cl_2−acetone) was subjected to reversed-phase HPLC using the same column and a gradient of 10−40% CH_3CN in 0.1% HCOOH−H_2O for 40 min, to yield vertilecanin B (**3**, 2.7 mg, t_R 32.5 min). Sephadex column fraction 5 (45.1 mg; eluted with 1:1 CH_2Cl_2−acetone) was separated further by reversed-phase HPLC (20−80% CH_3CN-0.1% HCOOH−H_2O) to yield 2-decenedioic acid (16.1 mg, t_R 27.6 min).

A second, larger fermentation afforded 2 g of EtOAc extract, which was partitioned as described above. The antiinsectan activity was again concentrated in the H_2O−CH_3OH fraction (1.3 g). This material was then subjected to Sephadex LH-20 column chromatography eluting with 250 mL each of 4:1 hexane−CH_2Cl_2, followed by 1:1 hexane−CH_2Cl_2, CH_2Cl_2, CH_2Cl_2−Me_2CO (2:3, then 3:2, then 1:4), and finally with 100% MeOH. The antiinsectan fraction 3 (42 mg, eluting with 100% CH_2Cl_2) was further purified by semipreparative reversed-phase HPLC (2.5 mL/min, 10−50% CH_3CN in 0.1% HCOOH−H_2O for 40 min, 50−100% over 20 min). This step afforded vertilecanin A methyl ester (**2**, 2.1 mg, t_R 31.9 min), vertilecanin B methyl ester (**4**, 18.1 mg, t_R 34.2 min), and vertilecanin C (**5**, 3.4 mg, t_R 42.7 min). A 48 mg portion of Sephadex column fraction 5 (1.1 g; eluted with 3:2 CH_2Cl_2−Me_2CO) was subjected to reversed-phase HPLC using a gradient of 0−45% CH_3CN in 0.1% HCOOH−H_2O for 45 min, to yield vertilecanin A (**1**, 36.7 mg, t_R 25.4 min) and vertilecanin B (**3**, 2.7 mg, t_R 37.5 min).

Vertilecanin A (1): white solid; mp 155−157 °C; $[\alpha]_D$ −11° (*c* 0.04 g/100 mL, CH_3OH, 23 °C); UV λ_{max} (CH_3OH) 208 (ϵ 5800), 228 (1900), 270 (1100) nm; IR ν_{max} (CaF_2) 3345, 1718, 1350 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; EIMS *m/z* 229 (M^+, 81), 185 (30), 167 (8), 150 (13), 124 (100), 105 (74), 79 (75); HREIMS, obsd M^+ at *m/z* 229.0745, calcd for $C_{13}H_{11}NO_3$, 229.0739.

Vertilecanin A methyl ester (2): white solid; $[\alpha]_D$ −7° (*c* 0.02 g/100 mL, CH_3OH, 24 °C); UV λ_{max} (CH_3OH) 210 (ϵ 2400), 228 (ϵ 1700), 270 (ϵ 1000) nm; IR ν_{max} (CaF_2) 3345, 1718, 1595, 1260 cm^{-1}; ^{1}H NMR (600 MHz, CD_3COCD_3) δ (mult, *J* in Hz, H#) 8.02 (br d, 8.1, H-3), 7.96 (dd; 8.1, 1.9, H-4), 8.75 (d, 1.9, H-6), 6.00 (d, 3.9, H-7), 7.45 (br d,* 7.5, H-9/H-13), 7.33 (distorted t, 7.5, H-10/H-12), 7.25 (distorted t, 7.5, H-11), 3.88 (s, OMe), 5.29 (d, 3.9, OH); ^{13}C NMR (90.5 MHz, CD_3COCD_3) δ 147.7 (C-2), 125.3 (C-3), 135.4 (C-4), 145.2 (C-5), 149.0 (C-6), 73.7 (C-7), 145.0 (C-8), 127.3 (C-9/13), 129.3 (C-10/12), 128.3 (C-11), 166.1 (C-14), 52.5 (OMe, C-15); HMBC correlations (CD_3COCD_3) H-3 ⇒ C-5; H-4 ⇒ C-2; H-6 ⇒ C-2, 4, 5; H-7 ⇒ C-4, 5, 6, 9/13; H-9/13 ⇒ C-7, 11, 13/9; H-10/12 ⇒ C-8, 12/10; H-11 ⇒ C-9/13; H_3-15 ⇒ C-14; OH ⇒ C-7, 8; HRFABMS (thioglycerol + PEG + TFA), obsd $(M+H)^+$ at *m/z* 244.0986, calcd for $C_{14}H_{14}O_3N$+H, 244.0974.

Conversion of Vertilecanin A (1) to Vertilecanin A Methyl Ester (2). To a solution of 11.2 mg of **1** in 1 mL of CH_3OH was added a 2 M solution of $TMSCHN_2$ in hexane until the solution remained yellow. After stirring for 24 h, the solution was concentrated under N_2 flow to give the methyl ester **2** (11.2 mg, 93% yield). The ^{1}H NMR data for this material matched those for the natural product.

Formation of the *R*- and *S*-2-Phenylbutyryl Esters of Vertilecanin A Methyl Ester (2). (*R*)-2-Phenylbutyryl chloride was prepared according to a literature procedure.[13] (The resulting material contained a small amount of the *S*-isomer.) To a solution of 1,3-dicyclohexylcarbodiimide (16.5 mg) in THF (5 mL), (*R*)-2-phenylbutyryl chloride (14 μL), compound **2** (5.5 mg), and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine (1.0 mg) were added. After 48 h, TLC analysis confirmed the disappearance of the starting material. The solvent was then evaporated under N_2 flow. The residue was dissolved in 3.0 mL of Et_2O, and the resulting solution was extracted sequentially with 2% CH_3COOH (4 mL), 3% $NaHCO_3$ (4 mL), and H_2O (2 × 2 mL). The organic phase was dried ($MgSO_4$) and

evaporated to give the crude reaction product, which was subjected to semipreparative reversed-phase HPLC using a gradient from 40% to 100% CH_3CN in H_2O over 30 min to afford 6.1 mg of the acylated product.

(*R*)-2-Phenylbutyryl ester of 2: ^{1}H NMR (300 MHz, CD_3COCD_3) δ (mult, *J* in Hz, H#) 7.34 (ov. m, ϕ), 7.29 (ov. m, ϕ), 7.26 (ov. m, ϕ'), 8.03 (dd; 7.8, 0.6, H-3), 7.90 (br dd; 7.8, 1.8, H-4), 8.74 (d, 1.8, H-6), 6.97 (s, H-7), 3.74 (t, 7.8, H-2′), 2.10 (m, H_a-3′), 1.83 (m, H_b-3′), 0.85 (t, 7.5, H_3-4′), 3.88 (s, 15-OMe).

(*S*)-2-Phenylbutyryl ester of 2: ^{1}H NMR (300 MHz, CD_3COCD_3) δ (mult, *J* in Hz, H#) 7.34 (ov. m, ϕ), 7.29 (ov. m, ϕ), 7.26 (ov. m, ϕ'), 8.11 (dd; 7.8, 0.6, H-3), 7.99 (dd; 7.8, 1.8, H-4), 8.58 (d, 1.8, H-6), 6.99 (s, H-7), 3.74 (t, 7.8 Hz, H-2′), 2.10 (m, H_a-3′), 1.83 (m, H_b-3′), 0.85 (t, 7.5 Hz, H_3-4′), 3.86 (s, 15-OMe).

Vertilecanin B (3): colorless oil; $[\alpha]_D$ +13° (*c* 0.03 g/100 mL, 24 °C, CH_3OH); UV λ_{max} (CH_3OH) 210 (ϵ 3300), 228 (ϵ 2500), 270 (ϵ 1500) nm; IR ν_{max} (CaF_2) 3352, 2927, 1663, 1599, 1489, 1457 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; EIMS *m*/*z* 286 (M^+, 2), 242 (51), 212 (18), 185 (100), 128 (53), 77 (26); positive ion HRESIMS, obsd $(M + H)^+$ at *m*/*z* 287.1048, calcd for $C_{15}H_{14}N_2O_4$+H, 287.1055.

Vertilecanin B methyl ester (4): white solid; mp 119−121 °C; $[\alpha]_D$ +9.7° (*c* 0.035 g/100 mL, 25 °C, CH_3OH); UV λ_{max} (CH_3OH) 210 (ϵ 3600), 232 (ϵ 2900), 270 (ϵ 1600) nm; IR ν_{max} (CaF_2) 3624, 3539, 3064, 2948, 1753, 1680, 1633, 1525, 1474, 1378, 1227, 1089 cm^{-1}; ^{1}H NMR (300 MHz, CD_3COCD_3) δ (mult, *J* in Hz, H#) 8.05 (br d, 7.9, H-3), 7.96 (dd; 7.9, 2.0, H-4), 8.66 (d, 2.0, H-6), 5.99 (s, H-7), 7.44 (m, H-9/13), 7.32 (m, H-10/12), 7.23 (m, H-11), 8.71 (br m, 15 N−H); 4.19 (d, 6.0, H-16), 3.68 (s, 18-OMe); ^{13}C NMR (75 MHz; CD_3COCD_3) δ 149.2 (C-2), 122.4 (C-3), 136.1 (C-4), 144.9 (C-5), 147.8 (C-6), 73.7 (C-7), 145.1 (C-8), 127.3 (C-9/13), 129.2 (C-10/12), 128.2 (C-11), 165.1 (C-14), 41.6 (C-16), 170.9 (C-17), 52.2 (C-18); selective INEPT correlations (CD_3COCD_3) H-3 ⇒ C-2, 5, 6,* 14; H-4 ⇒ C-2, 5, 6, 7; H-6 ⇒ C-2, 3,* 4, 5, 7, 14*; H-7 ⇒ C-4, 5, 6, 8, 9/13; H-9/13 ⇒ C-7, 13/9; H-10/12 ⇒ C-8, 9/13, 11, 12/10; H-11 ⇒ C-8, 9/13, 10/12; H_2-16 ⇒ C-14, 17; H_3-18 ⇒ C-17. *Denotes a four-bond correlation. EIMS *m*/*z* 300 (M^+, 10), 268 (21), 241 (100), 212 (23), 184 (51), 166 (7), 107 (29), 77 (55); HREIMS, obsd M^+ at *m*/*z* 300.1116, calcd for $C_{16}H_{16}N_2O_4$, 300.1100.

Vertilecanin C (5): colorless oil; UV λ_{max} (CH_3OH) 210 (ϵ 5800), 254 (ϵ 4800), 272 (ϵ 4400) nm; IR ν_{max} (CaF_2) 3416, 3384, 3049, 2936, 1752, 1684, 1667, 1522, 1283 cm^{-1}; ^{1}H NMR (600 MHz, CD_3COCD_3) δ (mult, *J* in Hz, H#) 8.27 (dd; 7.8, 0.8, H-3), 8.33 (dd; 7.8, 2.0, H-4), 8.94 (dd, 2.0, 0.8, H-6), 7.87 (m, H-9/13), 7.60 (m, H-10/12), 7.73 (m, H-11), 8.83 (br m, 15 N−H); 4.25 (d, 6.0, H-16), 3.72 (s, 18-OMe); ^{13}C NMR (90.5 MHz, CD_3COCD_3) δ 152.7 (C-2), 122.5 (C-3), 139.5 (C-4), 136.4 (C-5), 150.0 (C-6), 194.7 (C-7), 137.5 (C-8), 130.8 (C-9/13), 129.6 (C-10/12), 134.2 (C-11), 164.3 (C-14), 41.7 (C-16), 170.8 (C-17), 52.3 (C-18); HMBC correlations (CD_3COCD_3) H-3 ⇒ C-4, 5; H-4 ⇒ C-2, 6, 7; H-6 ⇒ C-2, 4, 5, 7; H-9/13 ⇒ C-7, 11, 13/9; H-10/12 ⇒ C-8, 11, 12/10; H-11 ⇒ C-9/13; H_2-16 ⇒ C-14, 17; H_3-18 ⇒ C-17; EIMS *m*/*z* 298 (M^+, 8), 266 (26), 239 (100), 210 (32), 182 (49), 105 (76), 77 (74); HREIMS, obsd M^+ at *m*/*z* 298.0978, calcd for $C_{16}H_{14}N_2O_4$, 298.0954.

Conversion of Vertilecanin A (1) to Vertilecanin B Methyl Ester (4). A solution of NaOMe was prepared by addition of NaOMe (5.2 mg, 0.096 mmol, 2.2 equiv) to MeOH (5 mL) at 0 °C. Glycine methyl ester hydrochloride (16.6 mg, 0.132 mmol, 3 equiv) was added in one portion, and the resulting mixture was allowed to cool at 0 °C. After stirring for 15 min, a sample of vertilecanin A (**1**; 20 mg, 0.88 mmol, 2 equiv) was added, followed by addition of *N*,*N*′-dicyclohexylcarbodiimide (DCC; 27.2 mg, 0.132 mmol, 3 equiv), and the reaction mixture was allowed to stir for 24 h at 0 °C under an N_2 atmosphere. The resulting mixture was filtered and rinsed with MeOH, and the filtrate was concentrated under N_2. The oily residue (8 mg of 50 mg total) was subjected to RP HPLC (Alltech HS Hyperprep 100 BDS C_{18}; 10 × 250 mm; flow rate, 2 mL/min; 20−100% CH_3CN in H_2O over 40 min) to provide vertilecanin B methyl ester (**4**; 3.2 mg; t_R 22.4 min), which was identical (NMR, HPLC t_R, $[\alpha]_D$) with the sample of **4** isolated from the *V. lecanii* extract.

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