Ophiocerins A−D and Ophioceric Acid: Tetrahydropyran Derivatives and an Africane Sesquiterpenoid from the Freshwater Aquatic Fungus *Ophioceras venezuelense*

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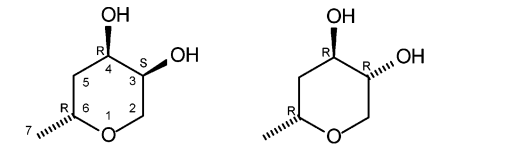
Four new tetrahydropyran derivatives called ophiocerins A−D (**1**−**4**) and a new africane sesquiterpenoid (ophioceric acid; **5**) have been isolated from cultures of the aquatic fungus *Ophioceras venezuelense*, together with the known compound regiolone. The structures and relative stereochemistry of these compounds were determined by analysis of 1D and 2D NMR data, while absolute stereochemical assignments for **1**−**4** were proposed by application of the exciton chirality CD method.

During our ongoing studies of freshwater fungi as sources of new bioactive metabolites,[1−4] chemical investigations of the aquatic fungus *Ophioceras venezuelense*[5] (Magnaporthaceae) have produced three diasteromeric tetrahydropyran derivatives named ophiocerins A−C (**1**−**3**), an isocrotonyl derivative of a fourth tetrahydropyran (ophiocerin D; **4**), a new africane sesquiterpenoid (ophioceric acid; **5**), and the known compound regiolone.[6] Details of the isolation and structure determination of these compounds are presented here.



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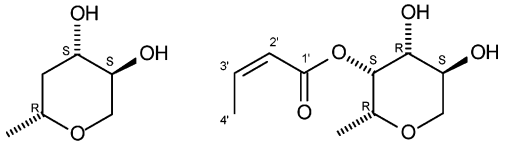

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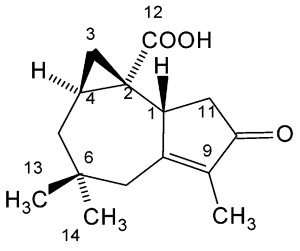

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Results and Discussion

Solid-substrate fermentation cultures of *O. venezuelense*[5] grown on rice were extracted with ethyl acetate. The resulting extract showed modest activity against *Candida albicans* and was partitioned with acetonitrile and hexane to yield an acetonitrile-soluble mixture that was fractionated over Sephadex LH-20. The activity was lost during this process. However, spectral analysis of the resulting fractions revealed the presence of several major components that could not be matched to known metabolites, despite the fact that three of them appeared to have molecular masses below 150 Da. Further purification of these fractions by reversed-phase HPLC and silica gel flash chromatography yielded compounds **1**−**5**, as well as regiolone.[6]

Ophiocerin A (**1**) crystallized from either acetonitrile or a mixture of hexane and acetone. Compound **1** was assigned the molecular formula $C_6H_{12}O_3$ (one unsaturation) on the basis of HREIMS, ^{1}H NMR (Table 1), and ^{13}C NMR (Table 2) data. The ^{13}C NMR and DEPT spectra revealed the presence of one methyl group, two aliphatic methylene units (one oxygenated), and three oxygenated sp^3 methine carbons. These groups accounted for all but two hydrogens and the unsaturation required by the molecular formula. Therefore, **1** must contain two OH groups and one ring. Chemical shift considerations, homonuclear decoupling experiments, and HMQC data led to straightforward identification of a single spin-system in the molecule. At this stage, two possible structures could be envisioned. To distinguish between the 3,4-dihydroxy-7-methyltetrahydropyran structure **1** and a similar tetrahydrofuran structure, the diacetate of **1** was prepared. The ^{1}H NMR spectrum of the diacetate contained two new acetate methyl signals (δ_H 2.10 and 1.99) and two oxygenated methine signals that were downfield-shifted relative to their positions in the spectrum of **1** (to 4.89 and 5.37 from δ_H 3.69 and δ_H 4.04, respectively). This required the oxygenated methine units in ophiocerin A to bear free OH groups and indicated that it must possess the tetrahydropyran structure shown in **1**. The ^{1}H NMR data for **1** and its diacetate were consistent with adoption of a chair conformation for the tetrahydropyran ring. The 11 Hz coupling constant between H-2ax and H-3 observed in the NMR spectrum of the diacetate implied that H-3 is axial, while the relatively small *J*-values observed between H-4 and its neighbors indicated that H-4 is equatorial. The 11 Hz coupling constant between H-5ax and H-6 in **1** (Table 1) supported an equatorial orientation for the methyl group. Thus, the relative stereochemistry was assigned as shown in **1**.

Ophiocerins B (**2**) and C (**3**) were assigned the same molecular formula as **1** ($C_6H_{12}O_3$; one unsaturation) on the basis of EIMS and NMR data. The ^{1}H NMR (Table 1), ^{13}C

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Table 1. ^{1}H NMR Spectral Data for Ophiocerins A−D (**1**−**4**) in $CDCl_3$[a]

position	**1** δ (mult; J_{H-H})	**2** δ (mult; J_{H-H})	**3** δ (mult; J_{H-H})	**4** δ (mult; J_{H-H})
2ax	3.52 (m)	3.68 (dd; 2.2, 12)	3.10 (dd; 10, 11)	3.18 (dd; 10, 11)
2eq	3.67 (m)	3.92 (dd; 1.8, 12)	3.91 (dd; 5.1, 11)	3.99 (dd; 5.4, 11)
3	3.69 (m)	3.43 (m)	3.44 (ddd; 5.1, 8.8, 10)	3.83 (ddd; 5.4, 9.6, 10)
4	4.04 (m)	3.92 (m)	3.49 (m)	3.67 (dd; 3.5, 9.6)
5eq	1.85 (ddd; 2.2, 3.7, 14)	1.57 (ddd; 2.7, 3.5, 14)	1.94 (ddd; 1.7, 4.7, 13)	5.18 (dd; 3.5, 1.1)
5ax	1.49 (ddd; 2.5, 11, 14)	1.77 (ddd; 3.2, 11, 14)	1.31 (ddd; 11, 11, 13)	
6	3.78 (ddq; 2.2, 6.3, 11)	3.83 (ddq; 2.7, 6.3, 11)	3.49 (m)	3.64 (dq; 1.1, 6.4)
7	1.12 (d; 6.3)	1.15 (d; 6.3)	1.18 (d; 6.2)	1.14 (d; 6.4)
2'				5.89 (dq; 1.8, 11)
3'				6.41 (dq; 7.3, 11)
4'				2.14 (dd; 1.8, 7.3)

[a] Chemical shifts were found to be somewhat concentration-dependent. Spectra were recorded at 400 MHz using ~30 mg/mL (**4**) or ~50 mg/mL (**1**, **2**, **3**) solutions. OH signals were observed in some instances, typically as broad signals, but varied in appearance, and are not listed here.

Table 2. ^{13}C NMR Spectral Data for Ophiocerins A−D (**1**−**4**) in $CDCl_3$[a]

position	**1** δ	**2** δ	**3** δ	**4** δ
2	65.8	67.2	69.7	69.7
3	67.3	68.4	72.1	67.7
4	67.0	67.4	72.7[b]	74.7
5	39.0	36.0	40.6	72.9
6	67.2	68.3	73.2[b]	73.8
7	20.8	21.1	21.2	16.7
1′				167.0
2′				119.9
3′				147.0
4′				15.7

[a] Spectra were recorded at 100 MHz using the same concentrations as in ^{1}H NMR. [b] These assignments are interchangeable; all other ^{13}C NMR assignments were verified by HMQC experiments.

NMR (Table 2), and DEPT data for both **2** and **3** were similar to those of **1**, suggesting that the three compounds are diastereomers of each other, and this was confirmed by decoupling experiments. The relative stereochemistry of **2** and **3** was again proposed on the basis of ^{1}H NMR *J*-values. The vicinal coupling constants for both C-2 protons in **2** were small, indicating that H-3 must be equatorial. The *J*-values for H_2-5, H-6, and H_3-7 in **2** (Table 1) were nearly identical to those in **1**, suggesting the same relative stereochemistry at positions C-4 and C-6. In **3**, vicinal *J*-values for H_2-2 suggested an axial orientation for H-3, as in **1**. In this case, however, an axial orientation for H-4 was proposed on the basis of the relatively large vicinal couplings with H-3 and H-5ax. The *J*-values for H_2-5 and H-6 were consistent with an axial orientation for H-6 as in **1** and **2**.

The molecular formula of ophiocerin D (**4**) was assigned as $C_{10}H_{16}O_5$ on the basis of HRESIMS (*m/z* 239.0908 $[M + Na]^+$), ^{1}H NMR (Table 1), and ^{13}C NMR (Table 2) data. ^{1}H NMR decoupling, ^{13}C NMR, DEPT, and HMQC data revealed the presence of a structure similar to those of compounds **1**−**3**, but in this instance, the C-5 aliphatic CH_2 was replaced by a third oxygenated methine unit, and olefinic and carbonyl signals consistent with an isocrotonyl group were also observed. The *cis*-configuration of the isocrotonyl group was assigned on the basis of the 11 Hz *J*-value between H-2′ and H-3′. The downfield chemical shift of the new oxygenated methine proton (δ_H 5.18) required location of the isocrotonyl group at C-5 to form structure **4**. The relative stereochemistry of **4** was again assigned by analysis of its ^{1}H NMR coupling constants (Table 1). The structures and relative configurations assigned for **1**−**4** were also fully supported by analysis of the spectral data obtained for the corresponding dibenzoates, which were formed by treatment of samples of each compound with benzoyl chloride in pyridine.

The absolute stereochemistry of **1**−**4** was assigned by CD spectroscopy using the exciton chirality method.[7,8] The CD spectra of *O*-dibenzoyl derivatives of **1**−**4** were recorded and evaluated for the signs of their bisignate Cotton effects. These were correlated with the chirality at C-3 and C-4, using the signs of the Cotton effects as indicators according to published protocols.[7] The dibenzoate derivative of **1** gave a negative bisignate Cotton effect, while those of **3** and **4** gave positive values. As expected, bisignate effects were observed for these three compounds, and the higher wavelength CD Cotton effects were clearly observable at the usual wavelength of approximately 230 nm; however, the peaks and troughs were not of equivalent intensity. This phenomenon is known to occur with other dibenzoates and may be due to a strong background ellipticity arising from the extremely strong benzoate absorption at ca.195 nm, or may suggest that other factors are influencing the CD curve at lover wavelengths[8] (particularly in the case of **4**, which has a pre-existing α,β-unsaturated ester chromophore). A bisignate curve was not obtained for **2**, presumably because of the *trans*-diaxial relative orientation of the two benzoate units. The J_{H3-H4} value of 2.7 Hz measured for the dibenzoate derivative of **2** was consistent with a *trans*-diaxial relationship for the dibenzoate units expected from the relative stereochemistry of the starting material, as well as from the results of molecular modeling calculations (Spartan '02. Wavefunction, Inc.). The intensity of coupling between two interacting chromophores is angle-dependent.[9] In this context, there is purported to be no exciton coupling when the projection angles of interacting moments are 0° or 180°, but the intensity is maximal when the angle of interaction moments is approximately 70°.[9] This would be consistent with observation of a bisignate Cotton effect for **1**, **3**, and **4** and the absence of such an effect in the spectrum of **2**, although it is unclear how the absence of coupling between two chromophores might influence the CD spectrum. Thus, the absolute stereochemistry of **2** was proposed by analogy with **1** and **3**, rather than on the basis of its CD curve, and the absolute configurations of ophiocerins A−D were assigned as shown in **1**−**4**.

Ophioceric acid (**5**) was isolated as a white powder and was assigned the molecular formula $C_{15}H_{20}O_3$ (six unsaturations) by analysis of EIMS, ^{1}H NMR, and ^{13}C NMR data (Table 3). The ^{13}C NMR and DEPT spectra revealed the presence of three methyl groups, four methylene units, two sp^3 methines, two quaternary carbons, two nonprotonated olefinic carbons, a ketone group, and a carboxylic acid or ester carbonyl. These data also required the presence of one exchangeable proton. Since there are no oxygenated sp^3 carbons in the molecule (and only three oxygen atoms), the carboxy carbonyl must be present as part of an acid

Table 3. ^{1}H NMR, ^{13}C NMR, and HMBC Data for Ophioceric Acid (**5**) in $CDCl_3$

position	δ_H (mult; J in Hz)	δ_C	HMBC (H → C #)
1	2.48 (m)	43.4	1, 8, 9, 10, 12[a]
2		26.0	
3α	0.78 (dd; 6.0, 4.4)	24.0	1, 2, 4, 5, 12
3β	1.75 (dd; 9.2, 4.4)		1, 2, 5, 12
4	1.60 (m)	25.5	12
5α	1.25 (dd; 15, 12)	41.6	3, 4, 6, 13, 14
5β	2.05 (dd; 15, 5.2)		4, 6, 7, 13, 14
6		34.4	
7α	2.32 (d; 15)	42.9	1, 6, 8, 9, 13, 14
7β	2.50 (dd; 15, 13)		1, 5, 6, 8, 9, 13, 14
8		171.6	
9		136.8	
10		208.6	
11α	2.48 (m)[b]	39.1	1, 8, 9, 10, 12[a]
11β	2.68 (dd; 13, 5.2)[b]		1, 2, 8, 10, 12
12		178.6	
13	0.95 (s)	31.3	5, 6, 7, 14
14	1.09 (s)	29.2	5, 6, 7, 13
15	1.70 (s)	8.22	8, 9, 10

[a] Correlations for H-1 and H-11α could not be unambiguously distinguished due to signal overlap. [b] These assignments are interchangeable.

group. These units accounted for three unsaturations, thereby requiring ophioceric acid to be tricyclic.

All carbon atoms were assigned to their respective protons by analysis of HMQC data. COSY correlations revealed the presence of an isolated methylene unit (H_2-7) and two other spin systems in **5** (H-1/H_2-11 and H_2-3/H-4/H_2-5). Both of the latter units were linked to C-2 on the basis of HMBC correlations of H_2-3 to C-1 and C-2 and of H_2-11 to C-2. Additional HMBC correlations from the singlet methyl signals for H_3-13 and H_3-14 to C-5, C-6, and C-7 revealed that both were connected to C-6 and that C-6 was, in turn, connected to C-5 and C-7. HMBC correlations of H_2-11 to C-1, C-8, C-9, and C-10 and of H_3-15 to C-8, C-9, and C-10 enabled assignment of a cyclopentenone unit, accounting for one of the rings. Correlations of H_2-7 to C-8, C-9, and C-1 provided additional evidence for the connectivity between C-8 and C-1. Chemical shifts for C-8, C-9, and C-10 are consistent with the presence of the cyclopentenone moiety.

The further correlations of H_2-3 and H-4 to C-12 require a bond between C-2 and C-12, in addition to the linkage between C-2 and C-1. These data create fused three- and seven-membered rings and, together with the cyclopentenone moiety, complete the proposed gross structure of ophioceric acid (**5**). Chemical shifts and J-values for H_2-3 and H-4 and δ-values for C-2, C-3, and C-4 were consistent with the presence of the cyclopropane ring.[9]

NOESY correlations of one of the protons at δ 2.48 to H-3α and H-5α and of H-5α to H-3α support the assignment of the relative stereochemistry as depicted in **5**. Although the signals for H-1 and H-11α overlap at δ 2.48, the distance from any proton at C-11 to the protons at C-3 and C-5 could not result in the very strong cross-peaks observed to these signals from the proton signal at δ 2.48, making these correlations more likely to arise from H-1 rather than H-11α. Comparison of NMR shifts and J-values with the closest known literature analogue (possessing an angeloyloxy substituent at C-5 and a methyl group in place of the acid moiety),[10] as well as other related compounds,[11−16] provided additional evidence for the proposed relative stereochemistry. The absolute stereochemistry of **5** was not assigned. To our knowledge, there is no report in the literature describing the absolute stereochemistry of any africane sesquiterpenoid.

Ophiocerins A−D (**1**−**4**) represent the major components in the extract, but showed neither antifungal (*Candida albicans*) nor antibacterial (*Staphylococcus aureus*, *Escherichia coli*) activity in standard disk assays at 200 μg/disk. Ophioceric acid (**5**) was also inactive in these assays. Thus, the source of the activity observed in the original extract remains undefined.

Biosynthetically, compounds **1**−**4** could be envisioned to arise from either polyketide or monosaccharide precursors, as they bear resemblance to relatively rare triketide products such as triacetic acid lactone,[17,18] as well as to sugar-derived molecules such as deoxyhexoses,[19] anhydroglucitol,[20] dihydropyrans,[21] or kojic acid.[22,23] The absolute stereochemistry assigned at C-6 for **1**−**4** would be consistent with derivation of all four compounds from the D-series of sugars and is therefore suggestive of a monosaccharide origin. The presence of an isocrotonyl group is also somewhat unusual, although it has been reported as a component of trichothecin and trichothecinols A−C isolated from *Trichothecium roseum*,[24] as well as in a trichothecinol of plant origin,[25] and in isocrotonylpterosin B isolated from the bracken fern *Pteridium aquilinum*.[26] The isocrotonyl moiety could arise from early steps of polyketide or fatty acid biosynthesis.[27]

Despite their relative simplicity, compounds **1**−**4** have not been previously described from a natural source. The only reported precedent for a compound with the same 6-methyltetrahydropyran-3,4-diol gross structure as **1**−**3** was obtained during chemical studies of kojic acid as a product of catalytic hydrogenation and was characterized only by elemental analysis, derivatization, and IR spectroscopy.[28] A few synthetic, protected forms of tetrahydro-6-methylpyran-3,4-diols have also been described.[29,30] The free triol analogue of **4** (1,5-anhydro-D-fucitol) and its enantiomer have been reported as synthetic products[31,32] and as a byproduct of the chemical hydrolysis of suitable glycosides under reducing conditions.[33] Although **4** could be named as a derivative of 1,5-anhydro-D-fucitol, and compounds **1**−**3** could be formally labeled as 1,5-anhydro-4,6-dideoxyhexitols (or as 6-methyltetrahydropyran-3,4-diols), we propose the common names ophiocerins A−D for these metabolites.

Ophioceric acid (**5**) belongs to the africane family of sesquiterpenoids. Such compounds are rare in nature. Prior examples include 8β-angeloyloxysenoxyri-4-en-3-one from the roots of *Senecio oxyriifolius*,[10] africanol,[11] $\Delta^{9(15)}$-africanene,[12,13] 10α-hydroxy-Δ9(15)-africanene,[14] and africananane-9,15-diol,[15] all from soft corals, and the leptographiols, from the ascomycete fungus *Leptographium lundbergii*.[16] To our knowledge, this is only the second report of a sesquiterpenoid of this class from a fungal source. In addition, compounds **1**−**5** appear to be the first natural products described from any member of the genus *Ophioceras*.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Rudolph Research Autopol III automatic polarimeter. UV measurements were performed with a Varian Cary 100 Bio UV−vis spectrophotometer. CD spectra were recorded using a Cary 17 spectrophotometer-CD optical module. IR measurements employed a Perkin-Elmer Spectrum BX FT-IR instrument. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-400 instrument. HMQC spectra were recorded at 600 MHz (^{1}H dimension, Bruker AMX-600). All NMR spectra were referenced to the solvent signals ($CDCl_3$; Aldrich) at δ_H 7.24/δ_C 77.0. Mass spectra were recorded with either an ion trap ThermoFinnigan LCQ (ESIMS) or a Micromass Autospec mass spectrometer (HRESIMS). HPLC was

performed using a semipreparative Alltech C_{18} HS Hyperprep 100 BDS column (250 × 10 mm, 8 μm particles) or a Dynamax 60A C_{18} column (250 × 21.4 mm, 8 μm particles) using a Beckman 110B solvent delivery module and a Beckman 168 diode array detector.

Fungal Material. *Ophioceras venezuelense*[5] is a freshwater pyrenomycete fungus in the family Magnaporthaceae. It has been found in Venezuela and Costa Rica, and it occurs on submerged wood and herbaceous debris in freshwater habitats. Cultures of *O. venezuelense* were obtained from single ascospores collected from a fast-flowing freshwater stream at La Selva Biological Station in Heredia, Costa Rica, in May 2000. Methods employed have been described previously.[34,35] A subculture of the isolate employed in this work is deposited at the Department of Plant Biology, University of Illinois Fungal Collection with the collection number A447-1B.

Fermentation, Extraction, and Isolation. Ascospores were subcultured onto 250 g of rice and incubated at 25 °C under 12 h light/12 h dark conditions. After five weeks, the fermentation mixture was broken up with a spatula and extracted twice with EtOAc (2 × 250 mL). The combined, filtered EtOAc solution was evaporated to afford 915 mg of an extract that was partitioned between CH_3CN and hexane to obtain a CH_3CN-soluble fraction (703 mg). This material was fractionated through a Sephadex LH-20 column (385 × 35 mm) using a step gradient of 185 mL of hexane−CH_2Cl_2 (1:4), 490 mL of CH_2Cl_2−acetone (3:2), 615 mL of CH_2Cl_2−acetone (1:4), and 250 mL of MeOH, collecting a total of 17 fractions. A 29 mg fraction eluted with CH_2Cl_2−acetone (3:2) was processed by reversed-phase HPLC (C_{18}-Alltech HS Hyperprep, 250 × 10 mm, 8 μm) using a CH_3CN−H_2O gradient (20% CH_3CN for 10 min, then 20%−40% CH_3CN over 5 min, 40% CH_3CN for 15 min, 40%−100% CH_3CN over 5 min, and 100% for 8 min) at 2 mL/min with UV detection at 230 nm to afford ophioceric acid (**5**; 2 mg, t_R 37.2 min). A 22 mg fraction eluted with CH_2Cl_2−acetone (3:2) was processed by reversed-phase HPLC using CH_3CN−H_2O gradient elution (10% CH_3CN for 20 min, then 10%−30% CH_3CN for 30 min, 30%−100% CH_3CN for 5 min, and 100% for 20 min) at 2 mL/min and UV detection at 220 nm to afford regiolone (2.3 mg; t_R 52.2 min). Regiolone was identified by comparison of its MS and ^{1}H NMR data with those reported in the literature.[6] The sign of the optical rotation obtained matched that reported for the (−)-isomer, but the minimal magnitude obtained upon repeated measurements relative to reported values suggested that the sample was either scalemic or racemic and contaminated by trace optically active impurities. Reversed-phase HPLC of another fraction (69 mg) that eluted with 3:2 CH_2Cl_2−acetone using a different CH_3CN−H_2O gradient elution (20% CH_3CN over 15 min, 20%−100% CH_3CN over 30 min, and then 100% CH_3CN over 5 min; 2 mL/min; UV detection at 220 nm) yielded **4** (18 mg; t_R 12.4 min). A third fraction (240 mg) from the CH_2Cl_2−acetone (3:2) system yielded **1** (58 mg) and **2** (96 mg) upon flash chromatography on silica gel using $CHCl_3$−CH_3CN (6:4) as the mobile phase. Similarly, flash chromatography (hexane−2-propanol−MeOH, 14:2:1) of a 137 mg fraction that eluted from the original Sephadex column with CH_2Cl_2−acetone (1:4) yielded **2** (8 mg), crude **3** (42 mg), and a mixture of **2** and **3** (62 mg) in a 3:1 ratio estimated by comparison of their respective proton signals in the ^{1}H NMR spectrum.

Compound **3** coeluted with a trace of another substance that absorbed strongly at 254 nm. Therefore, the sample of crude **3** was further purified by preparative reversed-phase HPLC using CH_3CN−H_2O gradient elution (20%−100% CH_3CN over 25 min, and then 100% CH_3CN over 5 min; 10 mL/min; UV detection at 254 nm) to afford pure **3** (40 mg, collected between 3 and 10.8 min).

Ophiocerin A (1): white crystals; mp 63−65 °C; $[\alpha]_D$ −24° (*c* 0.1, CH_2Cl_2); IR (film on NaCl plate) ν_{max} 3391, 1384, 1094, 1054, 1011 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 132 (M^+; rel int 3), 114 (17), 99 (8), 97 (14), 89 (10), 60 (100), 45 (79); HREIMS obsd *m*/z 132.0787 [M^+], calcd for $C_6H_{12}O_3$ 132.0786; obsd *m/z* 114.0677 [M − H_2O]$^+$, calcd for $C_6H_{10}O_2$ 114.0681.

Ophiocerin B (2): pale yellow oil, $[\alpha]_D$ −37° (*c* 0.1, CH_2Cl_2); IR (film on NaCl plate) ν_{max} 3390, 1446, 1384, 1266, 1077, 1005 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 132 (M^+; rel int 2), 117 (7), 99 (13), 89 (45), 71 (38), 60 (100), 45 (88).

Ophiocerin C (3): white crystals; mp 82−83 °C; $[\alpha]_D$ +45° (*c* 0.1, CH_2Cl_2); IR (film on NaCl plate) ν_{max} 3285, 1453, 1379, 1241, 1147, 1091, 1073, 1023 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 117 ([M − CH_3]$^+$; rel int 9), 99 (13), 89 (63), 71 (40), 60 (100), 45 (98).

Ophiocerin D (4): white crystals; mp 96−98 °C; $[\alpha]_D$ +40° (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} 209 (ϵ 7500) nm; IR (film on NaCl plate) ν_{max} 3423, 1720, 1646, 1442, 1181, 1099, 1076 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 173 ([M − C_2H_3O]$^+$; rel int 8), 87 (10), 69 (100), 57 (12), 45 (12); HRESIMS obsd *m/z* 239.0908 [M + Na]$^+$, calcd for $C_{10}H_{16}O_5Na$ 239.0895.

Ophioceric Acid (5): yellow oil; $[\alpha]_D$ +87° (*c* 0.2, CH_3OH); UV (MeOH) λ_{max} 241 (ϵ 6000) nm; IR (film on NaCl plate) ν_{max} 3600−2660 (br), 1694, 1683, 1634, 1193, 1159 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 3; EIMS (70 eV) *m/z* 248 (M^+; rel int 32), 233 (100), 215 (7), 191 (5), 187 (6), 159 (7), 105 (9), 91 (20), 77 (19), 53 (18); HREIMS obsd *m*/z 248.1414 [M^+], calcd for $C_{15}H_{20}O_3$ 248.1412.

Acetylation of 1. A sample of **1** (1 mg) was mixed with 0.5 mL of acetic anhydride and two drops of pyridine. After the mixture was stirred at room temperature for 14 h, 0.5 mL of H_2O was added, and the resulting solution was extracted with CH_2Cl_2 (3 × 0.5 mL). The combined organic phase was washed with 2 mL of 0.1 N NaOH and 2 mL of H_2O and then dried over Na_2SO_4. The CH_2Cl_2 was evaporated, and the residual product was identified as the diacetate of **1** (0.5 mg) by analysis of MS and ^{1}H NMR data: ^{1}H NMR ($CDCl_3$) δ 1.16 (H_3-7, d, *J* = 6.3), 1.62 (H-5eq, ddd, *J* = 2.6, 11, 14), 1.86 (H-5ax, ddd, *J* = 2.3, 4.0, 14), 1.99 (acetate H_3, s), 2.10 (acetate H_3, s), 3.68 (H-2ax, t, *J* = 11), 3.77 (H-2eq, m), 3.80 (H-6, m), 4.89 (H-3, ddd, *J* = 3.0, 5.4, 11), 5.37 (H-4, m); EIMS (70 eV) *m/z* 157 ([M − $C_2H_3O_2$]$^+$; rel int 11), 156 (7), 115 (36), 113 (37), 97 (43), 96 (88), 85 (100), 71 (51), 69 (71), 57 (68).

Benzoylation of 1−4. A sample of **1** (1 mg) was mixed with 100 μL of benzoyl chloride and 200 μL of pyridine. The mixture was stirred at room temperature for 24 h. Two milliliters of H_2O was added, and the resulting solution was extracted with CH_2Cl_2 (3 × 1 mL). The combined organic phase was then washed successively with 25% aqueous Na_2CO_3 (3 × 1 mL) and H_2O (3 × 1 mL) and dried over Na_2SO_4. The residue obtained upon evaporation was loaded onto a silica gel 60 column (60 × 5 mm) and eluted with hexane−EtOAc (3:2). Four fractions of 1−2 mL each were collected and evaluated by TLC (silica gel 60 F_{254}) using hexane−EtOAc (3:2) as the mobile phase. The first two fractions were subjected to HPLC purification with CH_3CN−H_2O gradient elution at 2 mL/min and UV detection at 230 nm to afford the dibenzoyl derivative of **1** (70% CH_3CN over 20 min, 70%−100% CH_3CN over 1 min, and then 100% CH_3CN over 8 min; t_R = 21 min). This procedure was duplicated for samples of compounds **2**−**4**, leading to the isolation of the corresponding dibenzoates of **2** (t_R = 23 min), **3** (65% CH_3CN over 25 min, 65%−100% CH_3CN over 1 min, and then 100% CH_3CN over 13 min; t_R = 31 min), and **4** (80% CH_3CN over 15 min, 80%−100% CH_3CN over 2 min, and then 100% CH_3CN over 5 min; t_R = 14 min). The products were identified by MS and ^{1}H NMR analysis.

Dibenzoylophiocerin A: UV (CH_3CN) λ_{max} 228 (ϵ 32 000), 272 (ϵ 2300) nm; CD (CH_3CN) $\Delta\epsilon_{max}$ (λ_1 238) −21, $\Delta\epsilon_{max}$ (λ_2 220) +4.6; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.24 (H_3-7, d, *J* = 6.3), 1.82 (H-5eq, ddd, *J* = 2.5, 11, 14), 2.11 (H-5ax, ddd, *J* = 2.1, 3.9, 14), 3.96 (H-2ax, dd, *J* = 11, 11), 3.98 (H-6, ddd, *J* = 2.1, 6.3, 11), 4.04 (H-2eq, ddd, *J* = 1.1, 5.3, 11), 5.25 (H-3, ddd, *J* = 3.0, 5.3, 11), 5.75 (H-4, m), 7.31 (*m*-2H, dd, *J* = 7.5, 8.3), 7.47 (3H, m), 7.59 (*p*-1H, tt, *J* = 1.3, 7.5), 7.86 (*o*-2H, dd *J* = 1.3, 8.4), 8.08 (*o*-2H, dd *J* = 1.3, 8.4); EIMS (70 eV) *m/z* 218 ([M − $C_7H_6O_2$]$^+$; rel int 18), 205 (7), 175 (9), 105 (100), 96 (45), 77 (91), 51 (52); ESIMS *m/z* 363 [M + Na]$^+$.

Dibenzoylophiocerin B: UV (CH_3CN) λ_{max} 230 (ϵ 31 000), 272 (ϵ 2100) nm; CD (CH_3CN) $\Delta\epsilon_{max}$ (λ_1 235) −6.1, $\Delta\epsilon_{max}$ (λ_2

216) −1.9; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (H_3-7, d, J = 6.3), 1.95 (H-5eq, ddd, J = 3.0, 3.0, 15), 2.01 (H-5ax, ddd, J = 3.0, 11, 15), 3.93 (H-6, ddq, J = 3.0, 6.3, 11), 4.08 (H-2eq, dd, J = 1.6, 13), 4.15 (H-2ax, ddd, J = 1.6, 1.6, 13), 5.03 (H-3, ddd, J = 1.6, 1.6, 2.7), 5.39 (H-4, m), 7.47 (*m*-4H, m), 7.58 (*p*-2H, m), 8.07 (*o*-4H, m); EIMS (70 eV) *m/z* 218 ([M − $C_7H_6O_2$]$^+$; rel int 14), 205 (3), 175 (4), 105 (100), 96 (41), 77 (74), 51 (31); ESIMS *m/z* 363 [M + Na]$^+$.

Dibenzoylophiocerin C: UV (CH_3CN) λ_{max} 229 (ϵ 18 000), 272 (ϵ 1400) nm; CD (CH_3CN) $\Delta\epsilon_{max}$ (λ_1 237) +12, $\Delta\epsilon_{max}$ (λ_2 222) −4.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (H_3-7, d, J = 6.1), 1.65 (H-5ax, ddd, J = 11, 11, 13), 2.33 (H-5eq, ddd, J = 1.9, 5.1, 13), 3.43 (H-2ax, dd, J = 10, 11), 3.70 (H-6, ddq J = 1.9, 6.1, 11), 4.25 (H-2eq, dd, J = 5.4, 11), 5.31 (H-3, ddd, J = 5.4, 10, 10), 5.39 (H-4, ddd, J = 5.1, 10, 11), 7.36 (*m*-4H, m), 7.49 (*p*-2H, m), 7.95 (*o*-4H, m); EIMS (70 eV) *m/z* 218 ([M − $C_7H_6O_2$]$^+$; rel int 21), 205 (9), 175 (5), 105 (100), 96 (52), 77 (86), 51 (41); ESIMS *m/z* 363 [M + Na]$^+$.

Dibenzoylophiocerin D: UV (hexane) λ_{max} 227 (ϵ 17 000), 273 (ϵ 1200) nm; CD (hexane) $\Delta\epsilon_{max}$ (λ_1 236) +8.1, $\Delta\epsilon_{max}$ (λ_2 221) −3.9; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (H_3-7, d, J = 6.4), 1.99 (H_3-4′, dd, J = 1.8, 7.3), 3.48 (H-2ax, dd, J = 10, 11), 3.88 (H-6, dq, J = 1.1, 6.4), 4.33 (H-2eq, dd J = 5.4, 11), 5.52 (H-4, dd, J = 3.4, 10), 5.55 (H-5, dd, J = 1.1, 3.4), 5.62 (H-3, ddd, J = 5.4, 10, 10), 5.97 (H-2′, dq, J = 1.8, 11), 6.42 (H-3′, dq, J = 7.3, 11), 7.34 (*m*-4H, m), 7.48 (*p*-2H, m), 7.91 (*o*-4H, m); EIMS (70 eV) *m/z* 311 ([M − $C_6H_9O_2$]$^+$; rel int 5), 216 (55), 105 (100), 77 (58), 69 (81); ESIMS *m/z* 447 [M + Na]$^+$.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for ophiocerins A−D and ophioceric acid (**1**−**5**) and CD spectra for the dibenzoates of **1**−**4** are available free of charge via the Internet at http://pubs.acs.org.

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