Ohioensins: Novel Benzonaphthoxanthenones from *Polytrichum ohioense*

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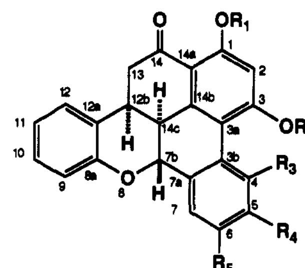
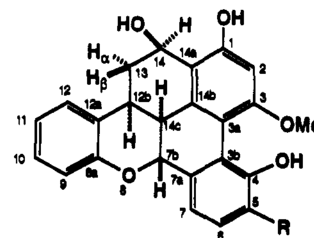
Ohioensins A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), containing the novel polycyclic skeleton of (7bβ,12bα,14cα)-7b,12b,13,14c-tetrahydro-14*H*-benzo[*c*]naphtho[2,1,8-*mna*]xanthen-14-one, have been isolated from the moss *Polytrichum ohioense* (Polytrichaceae) following bioassay-directed fractionation. The structures and relative stereochemistry of ohioensins were established on the basis of spectral analysis (UV, IR, MS, 2D NMR, and CD), chemical correlation, and X-ray diffraction. These compounds showed cytotoxicity against 9PS murine leukemia and the human tumor cell lines A-549 lung carcinoma, MCF-7 breast adenocarcinoma, and HT-29 colon adenocarcinoma. The proposed biogenetic pathway to ohioensins involves *o*-hydroxycinnamate and hydroxylated phenanthrenes as the intermediates.

In the course of our continuing search for novel cytotoxic agents from plants, we found that an extract of the moss *Polytrichum ohioense* Ren. & Card. (Polytrichaceae) collected in Maryland showed cytotoxicity in the 9KB cell culture. This discovery led to an increased interest in collecting and screening mosses by the Developmental Therapeutic Program of the National Cancer Institute.[1,2] Investigations in our research group have resulted in the isolation and structural elucidation of a novel benzonaphthoxanthenone, ohioensin A (**1**), from *P. ohioense*.[3] Ohioensin A contains a novel polycyclic skeleton and exhibits cytotoxicity against 9PS and MCF-7 human breast tumor cells in culture. In this article we report the isolation of the related ohioensins B–E (**2–5**), which possess the same skeleton and relative stereochemistry as ohioensin A.



1	$R_1=R_2=R_3=R_4=H$, $R_5=OH$
1a	$R_1=R_2=Ac$, $R_3=R_4=H$, $R_5=OAc$
2	$R_1=R_4=R_5=H$, $R_2=Me$, $R_3=OH$
2a	$R_1=Ac$, $R_2=Me$, $R_3=OAc$, $R_4=R_5=H$
2b	$R_1=R_2=Me$, $R_3=OMe$, $R_4=R_5=H$
3	$R_1=R_2=R_4=R_5=H$, $R_3=OH$
3a	$R_1=R_2=Ac$, $R_3=OAc$, $R_4=R_5=H$
4	$R_1=R_5=H$, $R_2=Me$, $R_3=R_4=OH$
4a	$R_1=Ac$, $R_2=Me$, $R_3=R_4=OAc$, $R_5=H$
4b	$R_1=R_2=Me$, $R_3=R_4=OMe$, $R_5=H$
5	$R_1=R_5=H$, $R_2=Me$, $R_3=OH$, $R_4=OMe$
5a	$R_1=Ac$, $R_2=Me$, $R_3=OAc$, $R_4=OMe$, $R_5=H$



2c R=H
4c R=OH

Results and Discussion

Ohioensin B (**2**) was isolated as yellow needles. The high-resolution electron impact mass spectroscopy (HREIMS) showed a molecular ion at *m/z* 386.1166 corresponding to the formula of $C_{24}H_{18}O_5$. The IR spectrum indicated the presence of intramolecular hydrogen-bonded hydroxyl (3300–2500 cm^{-1}) and conjugated carbonyl (1630 cm^{-1}) functions. The UV absorptions exhibited bathochromic shifts upon addition of $AlCl_3$, suggesting the presence of a phenolic hydroxyl peri to a carbonyl group.[4]

The ^1H NMR spectrum of **2** (Table I) indicated one methoxyl group at δ 4.09 and two exchangeable singlets at δ 7.35 and 12.06 assigned to a free phenolic hydroxyl and a chelated phenolic hydroxyl, which were further confirmed by the formation of a diacetate (**2a**). The singlet at δ 6.65 was assigned to H-2 based on NOE experiments (Table II). It showed a 33% intensity enhancement when the 3-methoxyl of **2** was irradiated, and the singlet at δ 6.72 of **2a** showed an 11% enhancement when the C-1 acetoxyl was irradiated.

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(3) Zheng, G.-q.; Chang, C.-j.; Stout, T. J.; Clardy, J.; Cassady, J. M. *J. Am. Chem. Soc.* **1989**, *111*, 5500.
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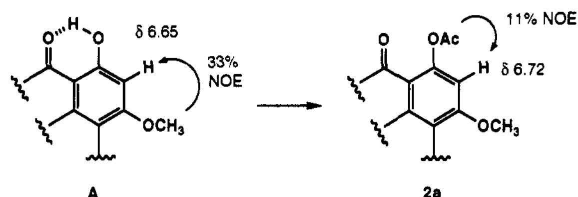
Table I. ^{1}H NMR Data on Ohioensins A–E (1–5)

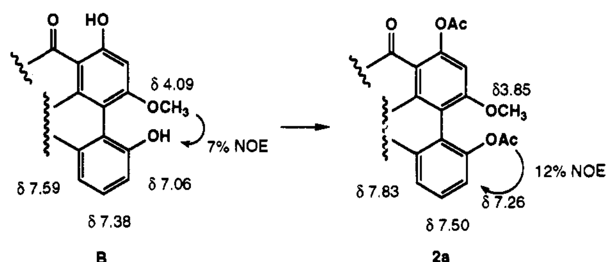
proton	A (1)[a,c]	B (2)[b,c]	C (3)[a,c]	D (4)[b,c]	E (5)[b,c]
	ohioensins: δ (ppm), multiplicity (J, Hz)				
H-2	6.53, s	6.65, s	6.27, s	6.67, s	6.63, s
H-4	8.36, d (8.6)				
H-5	6.84, dd (8.6,2.3)	7.06, dd (8.0,1.2)	6.92, d (7.6)		
H-6		7.38, dd (8.0,7.7)	7.26, t (7.6)	7.06, d (8.5)	6.97, d (8.2)
H-7	7.43, d (2.3)	7.59, dd (7.7,1.2)	7.36, d (7.6)	7.50, d (8.5)	7.49, d (8.2)
H-7b	5.05, d (13.7)	4.90, d (13.3)	5.05, d (13.4)	4.87, d (13.0)	4.85, d (13.1)
H-9	7.08, dd (8.1,1.2)	7.11, dd (8.0,1.4)	7.03, d (7.5)	7.11, dd (8.3,0.8)	7.10, d (8.3)
H-10	7.22, ddd (8.1,7.3,1.2)	7.25, ddd (8.0,6.9,1.2)	7.18, t (7.5)	7.25, ddd (8.3,6.5,0.8)	7.24, dd (8.3,6.5)
H-11	6.99, ddd (8.1,7.3,1.2)	6.99, ddd (8.0,6.9,1.4)	6.94, t (7.5)	7.00, ddd (8.3,6.5,0.8)	6.99, dd (8.3,6.5)
H-12	7.42, dd (8.1,1.2)	7.23, dd (8.0,1.2)	7.32, d (7.5)	7.23, dd (8.3,0.8)	7.24, d (8.3)
H-12b	3.62, ddd (14.8,7.8,4.4)	3.59, ddd (14.7,7.2,4.8)	3.58, ddd (14.9,7.3,4.6)	3.58, ddd (14.7,7.2,4.5)	3.56, ddd (15.0,7.3,4.8)
H-13α	2.73, dd (14.9, 4.4)	2.98, dd (15.4,4.8)	2.75, dd (15.3,4.6)	2.98, dd (15.2,4.5)	2.97, dd (15.5,4.8)
H-13β	2.87, dd (14.9, 14.8)	2.75, dd (15.4,14.7)	2.94, dd (15.3,14.9)	2.74, dd (15.2,14.7)	2.72, dd (15.5,15.0)
H-14c	3.29, dd (13.7,7.8)	3.26, dd (13.3,7.2)	3.12, dd (13.4,7.3)	3.21, dd (13.0,7.2)	3.17, dd (13.1,7.3)
1-OH	12.13, s	12.06, s	12.14, s	12.09, s	12.06, s
3-OH	8.81, s		7.29, s		
4-OH		7.35, s	7.37, s	7.72, s	7.16, s
5-OH				6.08, s	
6-OH	7.43, s				
3-OMe		4.09, s		4.11, s	4.06, s
5-OMe					3.96, s

[a] Recorded at 470 MHz in DMSO-d_6. [b] Recorded at 470 MHz in $CDCl_3$. [c] Solvent effect observed for the chemical shifts of H-10 and H-12 (in DMSO-d_6, $\delta_{H\text{-}12} > \delta_{H\text{-}10}$ as in 1 and 3; in $CDCl_3$, $\delta_{H\text{-}10} \geq \delta_{H\text{-}12}$ as in 2, 4, and 5) and H-13α and H-13β (in DMSO-d_6, $\delta_{H\text{-}13\beta} > \delta_{H\text{-}13\alpha}$ as in 1 and 3; in $CDCl_3$, $\delta_{H\text{-}13\alpha} > \delta_{H\text{-}13\beta}$ as in 2, 4, and 5).

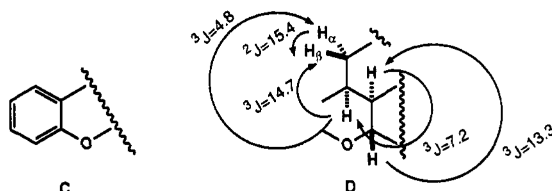
The ^{1}H–^{1}H 2D COSY NMR spectrum indicated a typical AMX pattern for three adjacent aromatic protons. The downfield shifts of these protons in 2a suggested that a free phenolic hydroxyl group was located at this aromatic ring. Irradiation of the acetyl protons resulted in 12% NOE on the signal at δ 7.26 (H-5), which indicated the presence of the hydroxyl group at the 4-position. The



structure for the fragment B was established based on the 7% NOE of this hydroxyl group by irradiating the 3-methoxyl protons.



The four interrelated aromatic signals determined by 2D COSY NMR could be attributed to the proton resonances of the ring without an hydroxyl group (fragment C), which were not affected by acetylation. The remaining aliphatic protons were unambiguously assigned on the basis of 2D COSY analysis, which gave the fragment D.



The complete skeleton of 2 was established by the connection of the above partial structures based on the following evidence. Reduction of the ketone group of 2 with $NaBH_4$ afforded the triol 2c. The ^{1}H NMR spectrum of 2c showed alteration in both chemical shifts and splitting patterns for the 13-CH_2. This led to the conclusion that

Table II. NOE Data of Ohioensins A–E and Their Acetates

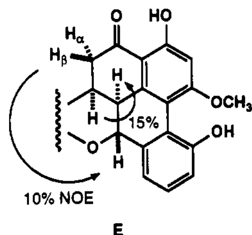
proton irradiated	H-2	H-4	H-5	H-6	H-7	H-7b	H-13β	H-14c	4-OH	5-OH
	% NOE observed on									
ohioensin A (1)										
H-7b					19		7			
acetate (1a)										
3-Ac (δ 2.37)	16	11								
6-Ac (δ 2.41)			9		11					
ohioensin B (2)										
H-12b								15		
H-13β						10				
3-OMe	33								7	
acetate (2a)										
1-Ac (δ 2.25)	11									
4-Ac (δ 2.44)			12							
ohioensin C (3)										
H-7b							13			
4-OH (δ 7.37)			14							
acetate (3a)										
4-Ac (δ 2.16)			36							
ohioensin D (4)										
3-OMe	29								12	
4-OH (δ 7.72)										11
5-OH (δ 6.08)									14	
ohioensin E (5)										
H-13β						24				
3-OMe (δ 4.06)	38									
5-OMe (δ 3.96)				15						

Table III. ^{13}C NMR Data of Ohioensins A–E (1–5)

carbon	A (1)[a]	B (2)[b]	C (3)[a]	D (4)[b]	E (5)[b]
1	157.3	160.7	162.3	160.4	161.0
2	102.8	99.6	103.7	99.9	99.7
3	163.4	162.6	170.7	162.5	162.9
3a	115.9	114.3	116.7	115.8	114.2
3b	130.4[c]	117.8	118.1	126.9	127.0
4	124.6	152.2	155.9	142.3	140.0
5	111.6[d]	119.2	120.2	142.5	147.4
6	152.2	129.3	129.7	113.5	112.3
7	111.9[d]	116.8	113.6	118.8	116.1
7a	140.6	141.1	140.0	130.7	130.6
7b	70.8	69.5	69.5	68.2	69.4
8a	153.6	153.3	152.1	152.5	151.8
9	118.1	117.6	116.8	117.5	117.6
10	128.9	128.5	127.1	128.3	128.5
11	122.1	121.4	120.7	121.2	121.4
12	130.5[c]	129.2	127.1	129.2	129.3
12a	122.8	122.3	127.8	122.2	122.5
12b	28.6	28.8	28.1	29.5	29.7
13	43.2	43.1	42.1	44.8	43.2
14	201.9	201.3	198.9	201.4	201.2
14a	114.4	112.4	107.8	110.6	110.3
14b	140.5	140.8	139.9	141.7	140.0
14c	39.0	37.6	37.2	38.0	38.1
OMe		57.1		56.6	56.8[e]
OMe					56.2[e]

[a] Recorded at 50.3 MHz in DMSO-d_6. [b] Recorded at 50.3 MHz in $CDCl_3$. [c–e] The assignments for these carbons may be interchangeable.

this methylene must link to the carbonyl carbon of the fragment B. In addition, the long-range coupling between H-7 and H-7b was confirmed by both 2D COSY and decoupling experiments. These observations revealed the connectivities between the fragments D and B and led to the fragment E. The remaining two attachment points must thus be connected to the fragment C. The NOE study (Table II) established the relative stereochemistry of 2. Irradiation of H-13β enhanced H-7b (10%), indicating the proximity of the two protons. Irradiation of H-12b enhanced H-14c (15%), suggesting that the six-membered ketone ring and the dihydropyran ring were cis fused.



The ^{13}C NMR spectral data (Table III) of 2 confirmed the presence of a polycyclic skeleton with highly aromatic character. Preliminary assignments were based on the attached proton test (APT) and fully proton-coupled experiments, while the ^{13}C–^{1}H correlation (2D HETCOR) spectrum allowed complete correlation of the protonated carbon resonances with proton signals. The quaternary aromatic carbons were identified with the aid of long-range ^{1}H–^{13}C coupling in the fully ^{1}H-coupled carbon spectrum and by the application of the substituent effect rule.

The EI-MS showed a significant fragment ion at m/z 368, which may result from a rearrangement involving the transfer of H-13 to the C-14 carbonyl oxygen followed by elimination of one molecule of H_2O (Figure 1). Another abundant ion at m/z 293 originated from the charge-induced cleavage of the pyran ether moiety which produced a positively charged ion and a phenolic radical. An

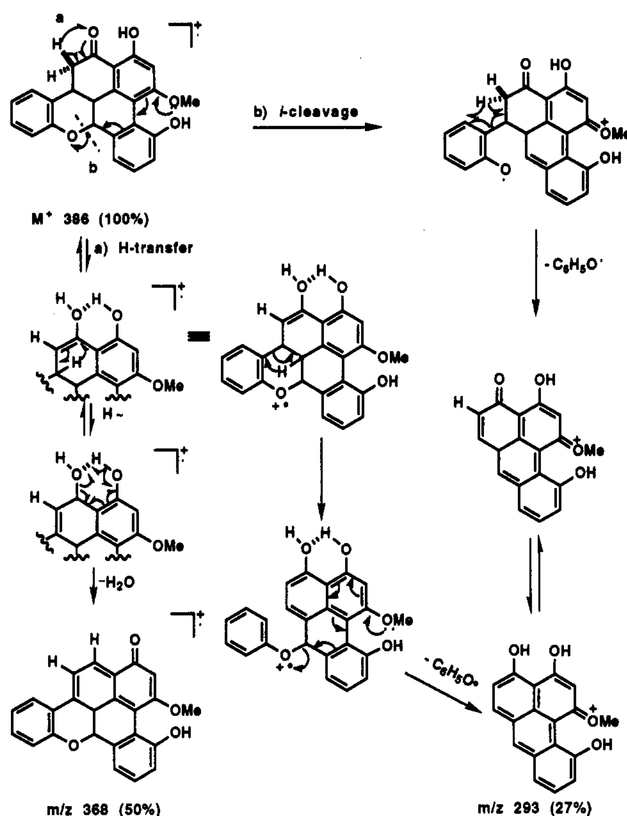


Figure 1. Proposed mechanism of major fragmentation of 2 in EIMS.

alternative mechanism for the loss of the phenolic radical might involve the β-hydrogen rearrangement of the enol followed by charge-site cleavage (Figure 1). These two characteristic ions appeared in all the EI-MS of ohioensins.[5]

Final confirmation of the proposed structure of 2 was achieved by comparison of spectral data of 2 with those of 1, whose structure was already established by X-ray diffraction analysis.[3] With the stereochemistry being relative, 2 was characterized as (7bβ,12bα,14cα)-7b,12b,13,14c-tetrahydro-1,4-dihydroxy-3-methoxy-14H-benzo[c]naphtho[2,1,8-mna]xanthen-14-one.

Ohioensin C (3), yellow crystals, gave the M^+ at m/z 372.0991 corresponding to the formula of $C_{23}H_{16}O_5$. The ^{1}H NMR spectrum (Table I) of 3 indicated the presence of three hydroxyl groups which was confirmed by the formation of a triacetate 3a. The positions of hydroxyl groups were assigned by NOE experiments (Table II) and chemical shift differences upon acetylation. The signals of H-2 and H-5 showed, upon acetylation of 3, downfield shifts of $\Delta\delta$ 0.84 (for two o-hydroxyls) and $\Delta\delta$ 0.25 ppm (for one o-hydroxyl), respectively. A 36% enhancement of the H-5 signal was observed in the NOE experiment when the 4-OAc of 3a was irradiated. Therefore the three hydroxyl groups were assigned to the 1-, 3-, and 4-positions of the biphenyl ring. Thus, compound 3 was identified as the O^3-demethyl analog of 2 or (7bβ,12bα,14cα)-7b,12b,13,14c-tetrahydro-1,3,4-trihydroxy-14H-benzo[c]naphtho[2,1,8-mna]xanthen-14-one. The chemical correlation between 2 and 3 further supported the structural assignment of 3. Permethylation of 2 and 3 gave the same product 2b with identical spectral data and TLC behavior.

(5) Zheng, G.-q. Part 1: Cytotoxic Agents from the Mosses *Polytrichum ohioense* and *Polytrichum pallidisetum*; Part 2: Design and Synthesis of Inhibitors of Myristoyl CoA:Protein N-Myristoyltransferase as Potential Anticancer Drugs. Ph.D. Thesis, Purdue University, West Lafayette, 1990.

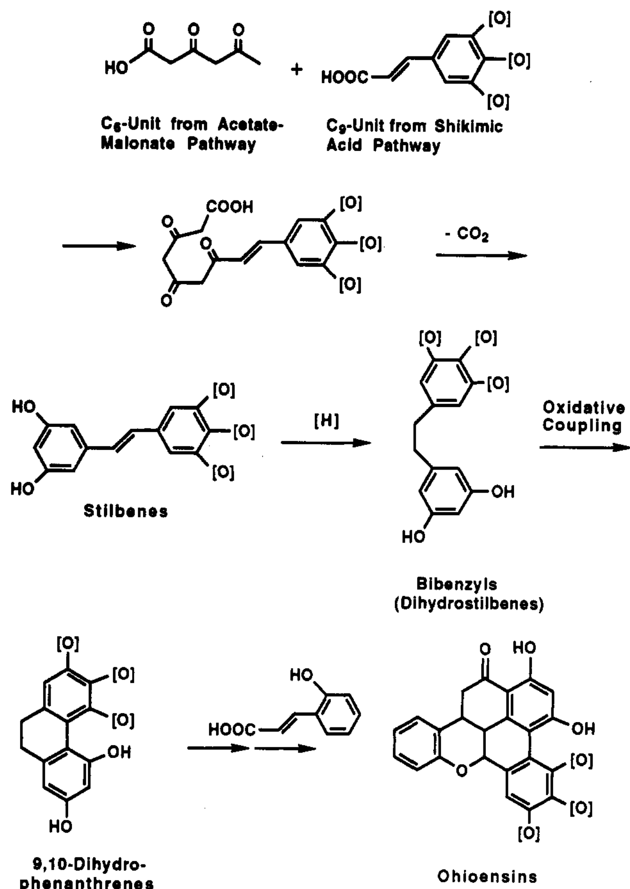


Figure 2. Proposed biogenesis of ohioensins.

Ohioensin D (4) was obtained as yellowish crystals. High-resolution EIMS gave the M^+ at m/z 402.1121 as a base peak for the formula of $C_{24}H_{18}O_6$. The ^1H NMR spectrum of 4 indicated the presence of one methoxyl and three hydroxyl groups which formed a triacetate **4a** upon acetylation. The ^1H NMR and ^{13}C NMR spectral data of 4 were very similar to those of 2 (Tables I and III). Reduction of 4 with sodium borohydride yielded compound **4c**. The 13-CH_2 and the newly-formed 14-Hα were coupled in the ^1H NMR of **4c**. On the basis of these data, compound 4 apparently contained the same skeleton as 2. The locations of substituents were determined on the basis of NOE experiment (Table II) and acetylation. Irradiation of the methoxyl protons enhanced both H-2 (δ 6.67, s) and a hydroxyl proton (δ 7.72, s) by 29% and 12%, respectively, thereby locating this methoxyl at the 3-position and indicating the presence of the hydroxyl group at the 4-position. In addition, irradiation of another hydroxyl proton (δ 6.08, s) also enhanced the 4-hydroxyl signal by 14%, suggesting the presence of this hydroxyl group at the 5-position. Furthermore, the H-6 and H-7 exhibited downfield shifts of $\Delta\delta$ 0.31 and 0.37 ppm upon acetylation, indicating the presence of two hydroxyl groups located at the 4- and 5-positions. Therefore, the structure of 4 was determined as the 5-hydroxyl analog of 2 or (7bβ,12bα,14cα)-7b,12b,13,14c-tetrahydro-1,4,5-trihydroxy-3-methoxy-14*H*-benzo[*c*]naphtho[2,1,8-*mna*]xanthen-14-one.

Ohioensin E (5) was crystallized from MeOH as yellow needles. The HREIMS of 5 showed a stable molecular ion of $C_{25}H_{20}O_6$ which differed from that of 4 by a CH_2 unit. The ^1H NMR spectrum of 5 (Table I) exhibited the signals of two methoxyl and two hydroxyl groups. When the signals of the methoxyls at δ 4.06 and 3.96 were irradiated in the NOE study (Table II), the enhancement of H-2 (38%) and H-6 (15%) indicated the presence of the 3- and 5-methoxyl groups. The H-6 and H-7 signals in the ^1H NMR spectrum of **5a** showed, upon acetylation of 5, downfield shifts of $\Delta\delta$ 0.12 (meta position) and 0.31 (para position), respectively, suggesting the location of a hydroxyl at the 4-position. The signals of ^{13}C NMR spectrum of 5 were assigned by comparison with those of 4 (Table III). The spectral data for both compounds were almost consistent except for an additional methoxyl carbon in the spectrum of 5. The structural correlation between 4 and 5 further supported the assignment. Methylation of both compounds afforded the same product **4b**. The structure of 5 was thus established as the 5-methoxyl analog of 2 or (7bβ,12bα,14cα)-7b,12b,13,14c-tetrahydro-1,4-dihydroxy-3,5-dimethoxy-14*H*-benzo[*c*]naphtho[2,1,8-*mna*]xanthen-14-one.

The single-crystal X-ray diffraction analysis for ohioensin A (1) provided solid evidence for the novel structure and relative stereochemistry.[3] Comparison of CD curves of ohioensins B–E (2–5) with that of ohioensin A indicated that they had the same configurations because they displayed similar Cotton effects.[5]

It has been reported that *o*-hydroxycinnamate and hydroxylated bibenzyls occurred naturally in bryophytes.[6] The biogenetic pathway to the ohioensins in *P. ohioense* might involve the condensation of *o*-hydroxycinnamate with hydroxylated phenanthrenes or 9,10-dihydrophenanthrenes which are less common natural products and could originate from the corresponding bibenzyls (Figure 2).[7-10]

These novel benzonaphthoxanthenones exhibited moderate cytotoxicity against 9PS and certain human tumor cell lines as summarized in Table IV. Compounds 1, 3, 4, and 5 showed cytotoxicity against 9PS and certain human tumor cell lines, while compound 2 was marginally active against HT-29 and MCF-7 cells.

Table IV. Cytotoxicity of Ohioensins A–E (1–5)

compound	ED_{50} (μg/mL)				
	9KB[a]	9PS[b]	A-549[c]	MCF-7[d]	HT-29[e]
ohioensin A (1)	>10	1.0	>10	9.0	>10
ohioensin B (2)	9.7	>10	>10	3.4	4.3
ohioensin C (3)	>10	1.0	8.7	6.7	>10
ohioensin D (4)	>10	1.0	>10	>10	>10
ohioensin E (5)	>10	1.0	6.2	>10	>10

[a] 9KB, human nasopharynxcarcinoma. [b] 9PS, murine P388 leukemia. [c] A-549, human lung carcinoma. [d] MCF-7, human breast adenocarcinoma. [e] HT-29, human colon adenocarcinoma.

Experimental Section

General Experimental Procedures. All melting points were determined on a Thomas-Hoover Uni-Melt or a Fisher-Johns melting point apparatus and were uncorrected. IR spectra were taken in KBr pellet or NaCl cell using Beckman IR-33 and Perkin-Elmer FTIR-1600 spectrometers. UV spectra were obtained in MeOH on a Beckman DU-7 spectrophotometer. Electron impact (EI) and chemical ionization (CI) mass spectra were measured at 70 eV on a Finnigan Model 4023 mass spectrometer. Reagent gases for CIMS were specified. Exact mass measurements were performed on a Kratos MS-50 sector mass spectrometer. ^1H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using TMS as an internal standard on Nicolet 470 MHz and Varian XL-200

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NMR spectrometers. ^{13}C NMR spectra (50 MHz) were recorded in $CDCl_3$ or DMSO-d_6 which was also used as an internal standard on a Chemagnetics A-200 NMR spectrometer. 2D NMR spectra were recorded on a Bruker AM-500 NMR spectrometer. Optical rotation was determined on a Perkin-Elmer 241 digital polarimeter with an 1-dm optical cell at 27 °C. All circular dichroism (CD) spectra were measured in MeOH on a JASCO J-500A spectropolarimeter and reported in molar ellipticity [θ] units. Thin-layer chromatography (TLC) and preparative TLC were carried out on E. Merck or Aldrich 0.25-mm and 2-mm silica gel 60 precoated plates with F-254 fluorescent indicator, Whatman 0.20-mm precoated C-18F reversed-phase plates, and EM Science 0.25-mm precoated RP-2F reversed-phase plates. Silica gel 60 (E. Merck, 230–400 mesh), Florisil (Analtech, 60–100 mesh), and Sephadex LH-20 (Pharmacia) were used for column chromatography. High-pressure liquid chromatography was performed on a Varian A-5000/Vista 54 HPLC instrument with a reversed-phase C_{18} column (4.6 × 150 mm) connected to a precolumn (3 × 8 mm).

Plant Material. The moss *P. ohioense* Ren. & Card. (Polytrichaceae) was collected from Maryland in 1982 and identified by Dr. Richard W. Spjut of the World Botanical Associates, Laurel, MD. The voucher specimens have been deposited in the United States National Herbarium.

Extraction and Isolation. The dried moss material (7 kg) was ground and exhaustively percolated with 95% EtOH. The 95% EtOH solubles were evaporated to dryness in vacuo at 40 °C and yielded the EtOH extract (150 g), which was then partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extract (82.5 g), which showed cytotoxicity against 9KB and 9PS cells in culture at ED_{50} 1.8 and 7.7 μg/mL, was further partitioned between hexane and 90% MeOH, yielding the dark green MeOH extract (30 g) and the hexane extract (50 g), respectively. The biological activity was found to concentrate in the 90% MeOH extract (9KB, ED_{50} 1.6 μg/mL; 9PS, ED_{50} 7.7 μg/mL), which was then subjected to flash chromatography on a silica gel column (1.5 kg of silica gel, 10 × 100-cm column). Gradient elution of the column was started with $CHCl_3$ (3 L), followed by increasing percentage of MeOH in $CHCl_3$ (1%, 2%, 5%, 10%, 20%, 3 L for each composition). Finally, the column was washed with MeOH. Fractions of 250-mL volume were analyzed by silica gel TLC and combined into 18 major fractions (A–R) in descending R_f values.

Ohioensin A (1). Fractions G (1.90 g; 9KB, ED_{50} 3.9 μg/mL) and H (0.91 g; 9KB, ED_{50} 2.0 μg/mL) were combined based on TLC analysis and further chromatographed on a silica gel flash column (60 g, 2 × 70 cm) eluted with gradients of EtOAc in hexane (10%, 15%, 20%, and 30%). Fractions were collected and pooled into 10 fractions (G-1–G-10). Fraction G-7 (26 mg) was further chromatographed on a silica gel column with 2% and 3% MeOH in $CHCl_3$ into five fractions (G-7a–G-7e). Fraction G-7d was triturated with a few milliliters of MeOH to give a yellowish precipitate which was further purified by crystallization in $CHCl_3$/MeOH to yield compound **1** (10.4 mg) as yellow needles: mp 274–275 °C dec; $[\alpha]^{27}_D$ +37° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 361.0 (3.59), 272.5 (4.42), 217.0 (4.48), shifted to 365.5 (3.94), 282.5 (4.52), 220.0 (4.86) nm upon addition of $AlCl_3$ or $AlCl_3$–HCl; IR (KBr) ν_{max} 3500–2500, 1620, 1600, 1570, 1270, 1220, 990, 810, 730 cm^{-1}; HREIMS for $C_{23}H_{16}O_5$ obsd 372.0996 (M^+), calcd 372.0997; EIMS *m/z* (relative intensity) 372 (M^+, 100), 354 ($M^+ - H_2O$, 37), 343 (M^+ − HCO, 4), 337 ($M^+ - H_2O$ − OH, 13), 325 ($M^+ - H_2O$ − HCO, 4), 297 (*m/z* 325 − CO, 5), 279 ($M^+ - C_6H_5O$, 38); ^1H NMR, see Table I; ^{13}C NMR, see Table III; CD (0.027 mM, MeOH) $[\theta]_{395}$ 0°, $[\theta]_{360}$ −5440° (min), $[\theta]_{333}$ 0°, $[\theta]_{298}$ +50 140° (max), $[\theta]_{285}$ +44 880° (sh), $[\theta]_{268}$ 0°, $[\theta]_{255}$ −19 040° (sh), $[\theta]_{228}$ −76 140° (min), $[\theta]_{213}$ 0°, $[\theta]_{211}$ +6800° (max), $[\theta]_{205}$ 0°.

Acetylation of Ohioensin A. A solution of ohioensin A (**1**; 3 mg) in anhydrous pyridine (1 mL) and Ac_2O (1 mL) was stirred overnight. The solution was evaporated in vacuo, and the residual pyridine and Ac_2O were azeotroped with a few drops of toluene. The residue was purified by a small silica gel column with $CHCl_3$ to yield the triacetate (**1a**; 3.5 mg) as yellowish needles crystallized from $CHCl_3$–MeOH (1:1): mp 143–145 °C dec; CIMS (isobutane) *m/z* (relative intensity) 499 (MH^+, 63), 457 ($MH^+ - CH_2CO$, 100), 415 ($MH^+ - 2CH_2CO$, 41), 373 ($MH^+ - 3CH_2CO$, 4), 286 (16), 257 (25); ^1H NMR (470 MHz, DMSO-d_6) δ 2.36 (s, 3 H, 1-OAc), 2.37 (s, 3 H, 3-OAc), 2.41 (s, 3 H, 6-OAc), 2.89 (dd, 1 H, J = 11.8 and 7.8 Hz, H-13α), 3.05 (dd, 1 H, J = 12.6 and 11.8 Hz, H-13β), 3.37 (dd, 1 H, J = 13.2 and 7.3 Hz, H-14c), 3.70 (ddd, 1 H, J = 12.6, 7.8, and 7.3 Hz, H-12b), 5.25 (d, 1 H, J = 13.2 Hz, H-7b), 7.07 (ddd, 1 H, J = 8.1, 7.3, and 1.3 Hz, H-11), 7.13 (dd, 1 H, J = 8.1 and 1.3 Hz, H-9), 7.16 (s, 1 H, H-2), 7.29 (ddd, 1 H, J = 8.1, 7.3, and 1.3 Hz, H-10), 7.31 (dd, 1 H, J = 8.6 and 2.5 Hz, H-5), 7.45 (dd, 1 H, J = 8.1 and 1.3 Hz, H-12), 7.75 (d, 1 H, J = 2.5 Hz, H-7), 8.22 (d, 1 H, J = 8.6 Hz, H-4).

Ohioensin B (2). Fractions C (0.89 g; 9KB, ED_{50} 5.4 μg/mL) and D (0.91 g; 9KB, ED_{50} 17 μg/mL) were combined on the basis of TLC analysis and further chromatographed on a silica gel flash column (60 g, 2.5 × 60 cm) into seven major fractions (C-1 to C-7). Fraction C-3 (126 mg) contained a major component and was further purified by preparative TLC on silica gel (2 mm thickness, 20 × 20) with 1% MeOH in $CHCl_3$. The main band with yellow fluorescence was isolated to give a yellowish solid which was crystallized from MeOH to yield compound **2** (23.4 mg) as yellow needles: mp 246–247 °C dec; $[\alpha]^{27}_D$ −47° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 345.5 (3.72), 264.0 (4.45), 214.0 (4.54), shifted to 388.5 (3.84), 272.5 (4.71), 215.0 (4.89) nm upon addition of $AlCl_3$ or $AlCl_3$–HCl; IR (KBr) ν_{max} 3300–2500, 1630, 1600, 1580, 1230, 1200, 1030, 940, 910, 840, 810, 740 cm^{-1}; HREIMS for $C_{24}H_{18}O_5$ obsd 386.1166 (M^+), calcd 386.1154; EIMS *m/z* (relative intensity) 386 (M^+, 100), 371 (M^+ − Me, 10), 368 ($M^+ - H_2O$, 50), 353 ($M^+ - H_2O$ − Me, 8), 325 (*m/z* 353 − CO, 8), 293 ($M^+ - C_6H_5O$, 27), 278 (*m/z* 293 − Me, 8); ^1H NMR, see Table I; ^1H-decoupled ^{13}C NMR, see Table III; ^1H-coupled ^{13}C NMR (118 MHz, $CDCl_3$) δ 28.83 (d, J = 131.0 Hz, C-14c), 37.61 (dd, J = 130.8 and 6.5 Hz, C-12b), 43.13 (dd, J = 132.0 and 126.3 Hz, C-13), 57.08 (q, J = 147.2 Hz, OMe), 69.47 (d, J = 147.4 Hz, C-7b), 99.60 (dd, J = 161.6 and 7.7 Hz, C-2), 112.37 (d, J = 7.7 Hz, C-14a), 114.34 (d, J = 7.7 Hz, C-3a), 116.81 (dd, J = 164.8 and 8.0 Hz, C-9), 117.60 (dd, J = 160.7 and 7.8 Hz, C-5), 117.79 (m, C-3b), 119.20 (dt, J = 161.2 and 7.1 Hz, C-7), 121.35 (dd, J = 162.6 and 8.0 Hz, C-11), 122.27 (t, J = 8.0 Hz, C-12a), 128.49 (dd, J = 160.8 and 8.5 Hz, C-10), 129.19 (d, J = 159.0 Hz, C-12), 129.26 (d, J = 161.7 Hz, C-6), 140.75 (s, C-14b), 141.08 (d, J = 8.8 Hz, C-7a), 152.21 (d, J = 8.7 Hz, C-4), 153.31 (dd, J = 9.8 and 7.4 Hz, C-8a), 160.65 (s, C-1), 162.60 (s, C-3), 201.27 (t, J = 5.1 Hz, C-14); CD (0.026 mM, MeOH) $[\theta]_{395}$ 0°, $[\theta]_{345}$ −4640° (min), $[\theta]_{325}$ 0°, $[\theta]_{310}$ +3080° (sh), $[\theta]_{284}$ +17 760° (max), $[\theta]_{257}$ 0°, $[\theta]_{248}$ −4300° (sh), $[\theta]_{225}$ −67 160° (min), $[\theta]_{211}$ 0°, $[\theta]_{209}$ +6180° (max), $[\theta]_{203}$ 0°.

Acetylation of Ohioensin B. The diacetate (**2a**; 3.2 mg) was prepared from ohioensin B (**2**; 3 mg) by using the same procedure for the synthesis of **1a** and obtained as yellow crystals from $CHCl_3$–MeOH: mp 212–214 °C dec; CIMS (isobutane) *m/z* (relative intensity) 471 (MH^+, 100); EIMS *m/z* (relative intensity) 470 (M^+, 11), 428 ($M^+ - CH_2CO$, 100), 386 ($M^+ - 2CH_2CO$, 73), 368 ($M^+ - 2CH_2CO - H_2O$, 35), 325 (5), 293 (14), 255 (7); ^1H NMR (470 MHz, $CDCl_3$) δ 2.25 (s, 3 H, 1-OAC), 2.44 (s, 3 H, 4-OAc), 2.69 (dd, 1 H, J = 14.6 and 13.8 Hz, H-13β), 2.85 (dd, 1 H, J = 13.8 and 4.6 Hz, H-13α), 3.24 (dd, 1 H, J = 12.7 and 7.5 Hz, H-14c), 3.61 (ddd, 1 H, J = 14.6, 7.5, and 4.6 Hz, H-12b), 3.85 (s, 3 H, OMe), 4.93 (d, 1 H, J = 12.7 Hz, H-7b), 6.72 (s, 1 H, H-2), 6.99 (ddd, 1 H, J = 8.1, 6.8, and 1.4 Hz, H-11), 7.09 (dd, 1 H, J = 8.4 and 1.4 Hz, H-9), 7.22 (dd, 1 H, J = 8.1 and 1.4 Hz, H-12), 7.23 (ddd, 1 H, J = 8.4, 6.8, and 1.4 Hz, H-10), 7.26 (dd, 1 H, J = 8.2 and 1.2 Hz, H-5), 7.50 (dd, 1 H, J = 8.2 and 7.7 Hz, H-6), 7.83 (dd, 1 H, J = 7.7 and 1.2 Hz, H-7).

Methylation of Ohioensin B. A solution of ohioensin B (**2**; 2 mg) and anhydrous K_2CO_3 (10 mg) in dry acetone (2 mL) was treated with 4 drops of MeI and stirred at room temperature overnight under nitrogen. The reaction mixture was filtered, and the filtrate was evaporated under vacuum. The residue thus obtained was purified by preparative silica gel TLC with 2% MeOH in $CHCl_3$ (R_f 0.53). The product was recrystallized as yellow crystals from MeOH to give 1,4-di-*O*-methylohioensin B (**2b**; 1.4 mg): mp 110 °C dec; IR (KBr) ν_{max} 3010, 2930, 2840, 1680, 1590, 1570, 1460, 1320, 1270, 1240, 1210, 1080, 1030, 970, 750 cm^{-1}; HREIMS for $C_{26}H_{22}O_5$ obsd 414.1419 (M^+), calcd 414.1467; EIMS *m/z* (relative intensity) 414 (M^+, 100), 397 (M^+ − OH, 21), 383 (M^+ − OMe, 9), 365 (M^+ − OH − MeOH, 12), 321 (22), 303 (6), 281 (9), 264 (7), 163 (15), 149 (42), 131 (17), 105 (35), 77 (11), 69 (14), 57 (14), 55 (11), 43 (12); ^1H NMR (270 MHz,

$CDCl_3$) δ 2.72 (dd, 1 H, J = 14.7 and 13.0 Hz, H-13β), 2.88 (dd, 1 H, J = 13.0 and 4.3 Hz, H-13α), 3.19 (dd, 1 H, J = 12.5 and 7.7 Hz, H-14c), 3.61 (ddd, 1 H, J = 14.7, 7.7, and 4.3 Hz, H-12b), 3.93 (s, 3 H, OMe), 3.97 (s, 3 H, OMe), 4.00 (s, 3 H, OMe), 4.86 (d, 1 H, J = 12.5 Hz, H-7b), 6.57 (s, 1 H, H-2), 6.99 (ddd, 1 H, J = 8.5, 7.0, and 1.2 Hz, H-11), 7.03 (dd, 1 H, J = 8.0 and 1.0 Hz, H-5), 7.09 (dd, 1 H, J = 8.2 and 1.2 Hz, H-9), 7.22 (ddd, 1 H, J = 8.2, 7.0, and 1.2 Hz, H-10), 7.30 (dd, 1 H, J = 8.5 and 1.2 Hz, H-12), 7.41 (t, 1 H, J = 8.0 Hz, H-6), 7.57 (dd, 1 H, J = 8.0 and 1.0 Hz, H-7).

Reduction of Ohioensin B. A solution of ohioensin B (**2**; 4 mg) in dry MeOH (2 mL) was treated with $NaBH_4$ (5 mg) and stirred for 5 h at room temperature. After being quenched with water (5 mL), the reaction mixture was acidified to pH 1–2 with 1 N HCl and extracted with $CHCl_3$ (3 × 4 mL). The combined $CHCl_3$ fractions were washed with water, evaporated, and chromatographed on silica gel to give the triol (**2c**; 3.7 mg) as a white solid from $CHCl_3$–MeOH: mp 151–152 °C dec; UV (MeOH) λ_{max} (log ε) 308.0 (4.31), 273.5 (4.45), 213.0 (4.91); IR (KBr) ν_{max} 3300, 1600, 1580, 1490, 1330, 1260, 1200, 1130, 950, 750 cm^{-1}; CIMS (isobutane) m/z (relative intensity) 389 (MH^+, 10), 387 (M^+ – H, 25), 373 (M^+ – Me, 50), 371 (MH^+ – H_2O, 100), 369 (M^+ – H – H_2O, 23), 353 (MH^+ – $2H_2O$, 4); ^1H NMR (200 MHz, $CDCl_3$) δ 1.82 (ddd, 1 H, J = 13.5, 11.9, and 11.5 Hz, H-13β), 2.56 (dt, 1 H, J = 11.9 and 4.0 Hz, H-13α), 2.60 (s, 1 H, exchangeable, 14β-OH), 2.96 (dd, 1 H, J = 12.6 and 7.9 Hz, H-14c), 3.15 (ddd, 1 H, J = 13.5, 7.9, and 4.0 Hz, H-12b), 3.96 (s, 3 H, OMe), 4.63 (dd, 1 H, J = 12.6 and 1.1 Hz, H-7b), 5.33 (dd, 1 H, J = 11.5 and 4.0 Hz, H-14α), 6.64 (s, 1 H, H-2), 6.98 (ddd, 1 H, J = 8.2, 6.7, and 1.2 Hz, H-11), 7.03 (dd, 1 H, J = 7.8 and 1.1 Hz, H-5), 7.07 (dd, 1 H, J = 8.2 and 1.2 Hz, H-9), 7.13 (ddd, 1 H, J = 8.2, 6.7, and 1.2 Hz, H-10), 7.19 (dd, 1 H, J = 8.2 and 1.2 Hz, H-12), 7.28 (s, 1 H, exchangeable, 4-OH), 7.32 (t, 1 H, J = 7.8 Hz, H-6), 7.54 (dt, 1 H, J = 7.8 and 1.1 Hz, H-7), 7.77 (s, 1 H, exchangeable, 1-OH).

Ohioensin C (3). Fraction I (1.35 g; 9KB, ED_{50} 2.4 μg/mL) was combined with the fractions J, K, and M based on the TLC analysis. The combined fractions (6.57 g) were chromatographed on a silica gel flash column (160 g, 5 × 60 cm) with $CHCl_3$, and increasing amounts of MeOH in $CHCl_3$ (5%, 10%, 15%, 20%) to give six major fractions (I-1–I-6). The most active fraction I-2 (0.982 g; 9KB, ED_{50} 1.9 μg/mL) was further chromatographed on a silica gel column (50 g, 2 × 70 cm) with 2%, 4%, 5%, and 10% MeOH in $CHCl_3$ to give five fractions (I-2a–I-2e). The active fraction I-2c (0.187 g) was purified on a silica gel column (6 g, 2 × 30 cm) and eluted with 3%, 5%, and 8% MeOH in $CHCl_3$. Four major fractions (I-2c-1–I-2c-4) were obtained. Fractionation of I-2c-3 (84 mg) was repeated on a silica gel column (3 g, 1 × 30 cm) using 4% MeOH in $CHCl_3$ as solvent to yield three fractions (I-2c-3I–I-2c-3III). The crystallization of fraction I-2c-3II in $CHCl_3$–MeOH afforded compound **3** (4.1 mg) as yellow crystals: mp 230–231 °C dec; $[\alpha]^{27}_D$ –18° (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 355.0 (4.25), 283.0 (4.61), 274.0 (4.62), 216.0 (5.19), shifted to 383.0 (3.84), 377.0 (3.73), 258.5 (4.82), 219.0 (5.16) nm upon addition of $AlCl_3$ or $AlCl_3$–HCl; IR (KBr) ν_{max} 3500–2500, 1630, 1600, 1580, 1320, 1240, 1050, 910, 820, 740 cm^{-1}; HREIMS for $C_{23}H_{16}O_5$ obsd 372.0991 (M^+), calcd 372.0997; CIMS (isobutane) m/z (relative intensity) 373 (MH^+, 100); EIMS m/z (relative intensity) 372 (M^+, 100), 354 (M^+ – H_2O, 23), 343 (M^+ – CHO, 3), 325 (M^+ – H_2O – HCO, 2), 315 (m/z 343 – CO, 3), 279 (M^+ – C_6H_5O, 22); ^1H NMR, see Table I; ^{13}C NMR, see Table III; CD (0.027 mM, MeOH) $[\theta]_{390}$ 0°, $[\theta]_{360}$ –2720° (min), $[\theta]_{325}$ 0°, $[\theta]_{310}$ +11 560° (sh), $[\theta]_{289}$ +34 000° (max), $[\theta]_{260}$ 0°, $[\theta]_{230}$ –87 040° (min), $[\theta]_{210}$ 0°, $[\theta]_{208}$ +31 280° (max), $[\theta]_{203}$ 0°.

Acetylation of Ohioensin C. The triacetate (**3a**; 1.9 mg) was prepared from ohioensin C (**3**; 2 mg) by using the same procedure for the synthesis of **1a** and obtained as a yellow solid from $CHCl_3$–MeOH: mp 87–89 °C dec; CIMS (isobutane) m/z (relative intensity) 499 (MH^+, 70), 457 (MH^+ – CH_2CO, 100), 415 (MH^+ – $2CH_2CO$, 23), 397 (m/z 457 – CH_3CO_2H, 18), 373 (MH^+ – $3CH_2CO$, 4); EIMS m/z (relative intensity) 498 (M^+, 2), 456 (M^+ – CH_2CO, 20), 414 (M^+ – $2CH_2CO$, 22), 372 (M^+ – $3CH_2CO$, 100), 354 (m/z 414 – CH_3CO_2H, 32), 343 (m/z 372 – CHO, 9), 325 (5), 315 (8), 279 (27); ^1H NMR (470 MHz, DMSO-d_6) δ 2.06 (s, 3 H, 1-OAc), 2.16 (s, 3 H, 4-OAc), 2.20 (s, 3 H, 3-OAc), 2.73 (dd, 1 H, J = 15.3 and 4.3 Hz, H-13α), 2.91 (dd, 1 H, J = 15.3 and 14.5 Hz, H-13β), 3.07 (dd, 1 H, J = 12.0 and 7.2 Hz, H-14c), 3.64 (ddd, 1 H, J = 14.5, 7.2, and 4.3 Hz, H-12b), 5.03 (d, 1 H, J = 12.0 Hz, H-7b), 6.90 (dd, 1 H, J = 7.6 and 7.0 Hz, H-11), 6.96 (d, 1 H, J = 8.5 Hz, H-9), 7.01 (s, 1 H, H-2), 7.15 (dd, 1 H, J = 8.5 and 7.0 Hz, H-10), 7.17 (dd, 1 H, J = 7.9 and 1.2 Hz, H-5), 7.30 (d, 1 H, J = 7.6 Hz, H-12), 7.36 (t, 1 H, J = 7.9 Hz, H-6), 7.65 (dd, 1 H, J = 7.9 and 1.2 Hz, H-7).

Methylation of Ohioensin C. 1,3,4-Tri-O-methylohioensin C (**2b**; 1.2 mg) was prepared from ohioensin C (**3**; 1.5 mg) by using the same procedure for the methylation of **2** and obtained as yellow crystals from MeOH: HREIMS for $C_{26}H_{22}O_5$ obsd 414.1408 (M^+), calcd 414.1467. The methylation product had R_f value, mp, EIMS, IR, and ^1H NMR identical with those of **2b** and was thus identified as the same compound.

Ohioensin D (4). Fraction C-5 (202 mg), which originated from the column chromatography of the combined fractions C and D and exhibited two spots in the TLC analysis, was chromatographed on 2-mm-thickness (20- × 20-cm) silica gel preparative TLC plates using 1% MeOH in $CHCl_3$ to yield compounds **4** (lower R_f) and **5** (higher R_f). Compound **4** (9.2 mg) was crystallized from MeOH as yellowish crystals: mp 244–245 °C dec; $[\alpha]^{27}_D$ –59° (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 344.5 (3.57), 315.5 (3.62), 266.0 (4.32), 220.0 (4.40), shifted to 366.5 (3.79), 276.0 (4.35), 226.0 (4.41) nm upon addition of $AlCl_3$ or $AlCl_3$–HCl; IR (KBr) ν_{max} 3400–3000, 1635, 1600, 1580, 1220, 1075, 960, 730 cm^{-1}; HREIMS for $C_{24}H_{18}O_6$ obsd 402.1121 (M^+), calcd 402.1123; CIMS (isobutane) m/z (relative intensity) 403 (MH^+, 100); EIMS m/z (relative intensity) 402 (M^+, 100), 387 (M^+ – Me, 10), 384 (M^+ – H_2O, 36), 370 (M^+ – MeOH, 7), 367 (M^+ – H_2O – OH, 7), 356 (M^+ – H_2O – CO, 4), 309 (M^+ – C_6H_5O, 25), 283 (11), 282 (21), 268 (11), 265 (10); ^1H NMR, see Table I; ^{13}C NMR, see Table III; CD (0.025 mM, MeOH) $[\theta]_{390}$ 0°, $[\theta]_{345}$ –9240° (min), $[\theta]_{320}$ 0°, $[\theta]_{295}$ + 17 680° (sh), $[\theta]_{285}$ +20 900° (max), $[\theta]_{260}$ 0°, $[\theta]_{235}$ –10 450° (sh), $[\theta]_{217}$ –40 200° (min), $[\theta]_{206}$ 0°, $[\theta]_{203}$ +3220° (max), $[\theta]_{201}$ 0°.

Acetylation of Ohioensin D. The triacetate (**4a**; 3.6 mg) was prepared from ohioensin D (**4**; 3 mg) by using the same procedure for the synthesis of **1a** and obtained as a yellow solid from $CHCl_3$–MeOH: mp 115–116 °C dec; CIMS (isobutane) m/z (relative intensity) 529 (MH^+, 100), 501 (MH^+ – H_2O, 28), 487 (MH^+ – CH_2CO, 19), 469 (MH^+ – CH_3CO_2H, 10), 459 (MH^+ – CH_2CO – CO, 19), 455 (MH^+ – CH_2CO – MeOH, 10), 445 (MH^+ – $2CH_2CO$, 5), 409 (MH^+ – $2CH_3CO_2H$, 54); EIMS m/z (relative intensity) 528 (M^+, 4), 486 (M^+ – CH_2CO, 26), 444 (M^+ – $2CH_2CO$, 15), 416 (M^+ – $2CH_2CO$ – CO, 14), 402 (M^+ – $3CH_2CO$, 48), 396 (40), 383 (12), 355 (12), 331 (9), 256 (14); ^1H NMR (470 MHz, $CDCl_3$) δ 2.24 (s, 3 H, 1-OAc), 2.30 (s, 3 H, 5-OAc), 2.44 (s, 3 H, 4-OAc), 2.75 (dd, 1 H, J = 14.3 and 13.0 Hz, H-13β), 2.98 (dd, 1 H, J = 13.0 and 4.1 Hz, H-13α), 3.24 (dd, 1 H, J = 12.3 and 7.5 Hz, H-14c), 3.60 (ddd, 1 H, J = 14.3, 7.5, and 4.1 Hz, H-12b), 3.85 (s, 3 H, OMe), 4.90 (d, 1 H, J = 12.3 Hz, H-7b), 6.72 (s, 1 H, H-2), 7.01 (dd, 1 H, J = 8.5 and 6.8 Hz, H-11), 7.09 (d, 1 H, J = 8.2 Hz, H-9), 7.21 (d, 1 H, J = 8.5 Hz, H-12), 7.24 (dd, 1 H, J = 8.2 and 6.8 Hz, H-10), 7.37 (d, 1 H, J = 6.8 Hz, H-6), 7.87 (d, 1 H, J = 6.8 Hz, H-7).

Methylation of Ohioensin D. 1,4,5-Tri-O-methylohioensin D (**4b**; 0.4 mg) was prepared from ohioensin D (**4**; 1 mg) by using the same procedure for the methylation of **2**. The product was purified by preparative silica gel TLC with 2% MeOH in $CHCl_3$ (R_f 0.44) and obtained as a yellow solid from MeOH: mp 104 °C dec; IR (KBr) ν_{max} 3000, 2920, 2850, 1680, 1590, 1560, 1450, 1320, 1240, 1210, 1110, 1030, 970, 800, 750 cm^{-1}; HREIMS for $C_{27}H_{24}O_6$ obsd 444.1592 (M^+), calcd 414.1573; EIMS m/z (relative intensity) 444 (M^+, 22), 427 (M^+ – OH, 9), 414 (M^+ – CH_2O, 6), 395 (M^+ – OH – MeOH, 3), 351 (9), 325 (16), 293 (3), 279 (10), 219 (54), 167 (13), 149 (62), 127 (13), 111 (20), 99 (21), 85 (47), 83 (38), 71 (73), 69 (53), 57 (100), 43 (58); ^1H NMR (270 MHz, $CDCl_3$) δ 2.72 (dd, 1 H, J = 14.6 and 13.3 Hz, H-13β), 2.88 (dd, 1 H, J = 13.3 and 4.1 Hz, H-13α), 3.15 (dd, 1 H, J = 12.3 and 7.6 Hz, H-14c), 3.60 (ddd, 1 H, J = 14.6, 7.6, and 4.1 Hz, H-12b), 3.70 (s, 3 H, OMe), 3.94 (s, 3 H, OMe), 3.98 (s, 3 H, OMe), 4.00 (s, 3 H, OMe), 4.82 (d, 1 H, J = 12.3 Hz, H-7b), 6.56 (s, 1 H, H-2), 6.99 (ddd, 1 H, J = 8.4, 7.4, and 1.4 Hz, H-11), 6.99 (d, 1 H, J = 8.4 Hz, H-6), 7.08 (dd, 1 H, J = 8.2 and 1.4 Hz, H-9), 7.22 (ddd, 1 H, J = 8.2, 7.4, and 1.4 Hz, H-10), 7.32 (dd, 1 H, J = 8.4 and 1.4 Hz, H-12), 7.59 (d, 1 H, J = 8.4 Hz, H-7).

Reduction of Ohioensin D. The tetrol (**4c**; 1.9 mg) was prepared from ohioensin D (**4**; 3 mg) by using the same procedure for the reduction of **2**. The product was obtained as a light yellow solid from $CHCl_3$–MeOH: mp 176–177 °C dec; CIMS (isobutane) *m/z* (relative intensity) 405 (MH^+, 67), 387 ($MH^+ - H_2O$, 100), 369 ($M^+ - 2H_2O$, 9); ^1H NMR (470 MHz, $CDCl_3$), δ 1.83 (ddd, 1 H, J = 13.8, 11.8, and 11.5 Hz, H-13β), 2.54 (br s, 1 H, exchangeable, 14β-OH), 2.58 (dt, 1 H, J = 11.8 and 4.1 Hz, H-13α), 2.99 (dd, 1 H, J = 12.7 and 8.1 Hz, H-14c), 3.19 (ddd, 1 H, J = 13.8, 8.1, and 4.1 Hz, H-12b), 3.99 (s, 3 H, OMe), 4.69 (d, 1 H, J = 12.7, H-7b), 5.37 (dd, 1 H, J = 11.5 and 4.1 Hz, H-14α), 6.11 (s, 1 H, exchangeable, 5-OH), 6.66 (s, 1 H, H-2), 6.98 (ddd, 1 H, J = 8.2, 6.9, and 1.0 Hz, H-11), 7.01 (dd, 1 H, J = 8.2 and 1.0 Hz, H-9), 7.07 (d, 1 H, J = 8.3 Hz, H-6), 7.21 (ddd, 1 H, J = 8.2, 6.9, and 1.0 Hz, H-10), 7.26 (dd, 1 H, J = 8.2 and 1.0 Hz, H-12), 7.44 (d, 1 H, J = 8.3 Hz, H-7), 8.13 (s, 1 H, exchangeable, 4-OH), 9.17 (s, 1 H, exchangeable, 1-OH).

Ohioensin E (5). Compound **5** (8.5 mg) was crystallized from MeOH as light yellow needles: mp 226–228 °C dec; $[\alpha]^{27}{}_D$ −42° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 344.5 (3.63), 266.5 (4.41), 225.5 (4.47), shifted to 369.0 (3.84), 277.0 (4.42), 227.0 (4.51) nm upon addition of $AlCl_3$ or $AlCl_3$–HCl; IR (KBr) ν_{max} 3500–3000, 1630, 1600, 1580, 1220, 1090, 1070, 965, 800, 735 cm^{-1}; HREIMS for $C_{25}H_{20}O_6$, obsd 416.1262 (M^+), calcd 416.1260; CIMS (isobutane) *m/z* (relative intensity) 417 (MH^+, 100); EIMS *m/z* (relative intensity) 416 (M^+, 100), 401 (M^+ − Me, 12), 398 ($M^+ - H_2O$, 31), 383 ($M^+ - H_2O$ − Me, 8), 373 (M^+ − Me − CO, 5), 355 (*m/z* 383 − CO, 6), 323 ($M^+ - C_6H_5O$, 21), 296 (10), 279 (5), 265 (11); ^1H NMR, see Table I; ^{13}C NMR, see Table III; CD (0.024 mM, MeOH) $[\theta]_{390}$ 0°, $[\theta]_{340}$ −12 480° (min), $[\theta]_{310}$ 0°, $[\theta]_{283}$ +42 640° (max), $[\theta]_{255}$ 0°, $[\theta]_{227}$ −89 440° (min), $[\theta]_{211}$ 0°, $[\theta]_{209}$ +29 120° (max), $[\theta]_{206}$ 0°.

Acetylation of Ohioensin E. The diacetate (**5a**; 3.2 mg) was prepared from ohioensin E (**5**; 3 mg) by using the same procedure for the synthesis of **1a** and obtained as a yellow solid from $CHCl_3$–MeOH: mp 123–124 °C dec; CIMS (isobutane) *m/z* (relative intensity) 501 (MH^+, 100), 483 ($MH^+ - H_2O$, 13), 459 ($MH^+ - CH_2CO$, 60), 417 ($MH^+ - 2CH_2CO$, 13); EIMS *m/z* (relative intensity) 500 (M^+, 15), 458 ($M^+ - CH_2CO$, 46), 416 ($M^+ - 2CH_2CO$, 100), 398 (*m/z* 458 − CH_3CO_2H, 31), 355 (7), 323 (14), 279 (5), 267 (10); ^1H NMR (470 MHz, DMSO-d_6) δ 2.25 (s, 3 H, 1-OAc), 2.44 (s, 3 H, 4-OAc), 2.68 (dd, 1 H, J = 13.6 and 13.1 Hz, H-13β), 2.85 (dd, 1 H, J = 13.6 and 4.8 Hz, H-13α), 3.18 (dd, 1 H, J = 12.6 and 7.3 Hz, H-14c), 3.60 (ddd, 1 H, J = 13.1, 7.3, and 4.8 Hz, H-12b), 3.88 (s, 3 H, 3-OMe), 3.90 (s, 3 H, 5-OMe), 4.88 (d, 1 H, J = 12.6 Hz, H-7b), 6.72 (s, 1 H, H-2), 7.00 (dd, 1 H, J = 8.4 and 6.7 Hz, H-11), 7.09 (d, 1 H, J = 8.4 Hz, H-9), 7.10 (d, 1 H, J = 8.9 Hz, H-6), 7.20 (dd, 1 H, J = 8.4 and 6.7 Hz, H-10), 7.20 (d, 1 H, J = 8.4 Hz, H-12), 7.80 (dd, 1 H, J = 8.9 Hz, H-7).

Methylation of Ohioensin E. 1,4-Di-*O*-methylohioensin E (**4b**; 0.7 mg) was prepared from ohioensin E (**5**; 1 mg) by using the same procedure for the methylation of **2** and obtained as yellow crystals from MeOH: HREIMS for $C_{27}H_{24}O_6$ obsd 444.1552 (M^+), calcd 444.1573. The methylation product showed R_f value, mp, EIMS, IR, and ^1H NMR identical with those of **4b** and was thus identified as the same compound.

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Supplementary Material Available: Details of the X-ray study including structure, tables of atomic coordinates, thermal parameters, interatomic distances, and interatomic angles for **1** as well as NMR spectra of **1**–**5** and their derivatives (23 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.