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OHIOENSINS AND PALLIDISETINS: NOVEL CYTOTOXIC AGENTS FROM THE MOSS *POLYTRICHUM PALLIDISETUM*

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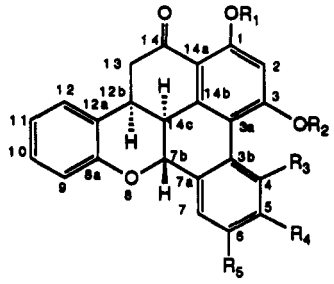
ABSTRACT.—Bioassay-directed fractionation of an EtOH extract of the moss *Polytrichum pallidisetum* (Polytrichaceae) led to the isolation of three novel benzonaphthoxanthenones, 1-*O*-methylohioensin B [**6**], 1-*O*-methyldihydroohioensin B [**7**] and 1,14-di-*O*-methyldihydroohioensin B [**8**], and two novel cinnamoyl bibenzyls, pallidisetin A [**9**] and pallidisetin B [**10**]. Their structures and relative stereochemistry were established by spectral analyses and chemical correlation. Compounds **6–10** exhibited cytotoxic activity against the human tumor cell lines RPMI-7951 melanoma and U-251 glioblastoma multiforme. These two types of compounds could hypothetically be derived from cinnamic acid and bibenzyls through different biogenetic pathways.

As part of the program at the National Cancer Institute to discover antitumor agents from mosses (1,2), two species of the genus *Polytrichum* (Polytrichaceae), *Polytrichum ohioense* Ren. & Card. and *Polytrichum pallidisetum* Funck, were subjected to investigation. Our previous investigation of *P. ohioense* resulted in the isolation and characterization of ohioensins A–E [**1–5**], which are based on the novel benzonaphthoxanthenone skeleton and exhibited cytotoxicity against 9PS and certain human tumor cells in culture (3,4). In the course of our continuing search for antitumor agents from the moss *P. pallidisetum*, five novel compounds [**6–10**] were isolated. They can be classified into benzonaphthoxanthenones and cinnamoyl bibenzyls. These compounds also showed cytotoxic activity against several human tumor cell lines. In this paper, we report the isolation and structural elucidation of **6–10** based on spectral analyses and chemical correlations.

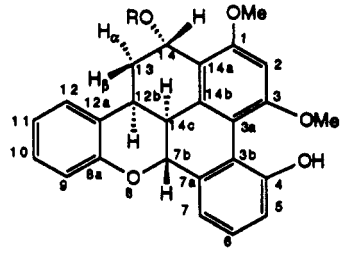
The isolation procedure started with percolation of the ground material of *P. pallidisetum* with 95% EtOH, followed by partitioning of the extract between $CHCl_3$ and H_2O. The active $CHCl_3$ portion was further partitioned between hexane and 90% MeOH. The aqueous MeOH layer, which exhibited significant cytotoxicity against several human tumor cell lines and antitumor activity against murine P-388 lymphocytic leukemia, was fractionated to afford compounds **6–10**.

RESULTS AND DISCUSSION

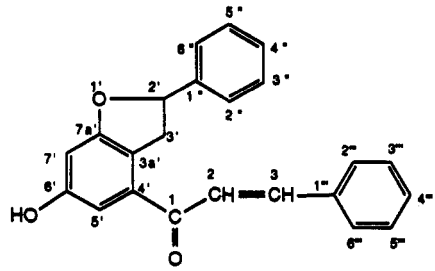
The hreims spectrum of **6** showed an intense molecular ion at *m/z* 400.1330, corresponding to the formula $C_{25}H_{20}O_5$. This molecular formula indicated that **6** contained 16 unsaturations required for the basic skeleton of a benzonaphthoxanthenone (3). The ir spectrum exhibited the characteristic bands for the hydroxyl (3350 cm^{-1}) and conjugated carbonyl (1685 cm^{-1}) functions. In comparison with ohioensin B [**2**]



1 $R_1=R_2=R_3=R_4=H$, $R_5=OH$
2 $R_1=R_4=R_5=H$, $R_2=Me$, $R_3=OH$
3 $R_1=R_2=R_4=R_5=H$, $R_3=OH$
4 $R_1=R_5=H$, $R_2=Me$, $R_3=R_4=OH$
5 $R_1=R_5=H$, $R_2=Me$, $R_3=OH$, $R_4=OMe$
6 $R_1=R_2=Me$, $R_3=OH$, $R_4=R_5=H$



7 R=H
8 R=Me



9 2-(*E*)
10 2-(*Z*)

($C_{24}H_{18}O_5$) (4), **6** showed similar maximum absorptions in the uv spectrum, suggesting the presence of an identical conjugated system.

The ^{1}H-nmr analysis (Table 1) revealed that **6** was a benzonaphthoxanthenone derivative closely related to **2**. The 2D COSY spectrum showed two multi-spin systems and one singlet in the aromatic region. The first system contained signals at δ 7.08 (H-5), 7.39 (H-6) and 7.61 (H-7) and the second system at 7.14 (H-9), 7.23 (H-10), 7.00 (H-11) and 7.29 (H-12). The upfield nature of the singlet at δ 6.67, assigned to H-2, suggested the presence of oxygenated substituents at C-1 and C-3. The assignment of the two methoxyl and the hydroxyl groups to C-1, C-3 and C-4 respectively was supported by nOe and acetylation experiments. Irradiation of the methoxyl at δ 4.11 enhanced H-2 by 17% and 4-OH by 7%. Irradiation of the methoxyl at δ 4.01 enhanced only H-2 by 19%. In addition, irradiation of H-2 resulted in enhancements of the 1-OCH_3 (8%) and the 3-OCH_3 (7%) signals (Figure 1). Therefore, the signal at δ 4.11 was assigned to 3-OCH_3, and that at δ 4.01 to 1-OCH_3. The 4-OH group (δ 7.31, exchangeable with D_2O) was converted to the corresponding acetate. The downfield shift induced at H-5 (Δδ 0.13), H-6 (Δδ 0.07) and H-7 (Δδ 0.22) confirmed the location of the 4-OAc group (Figure 1).

The chemical shifts in the ^{13}C-nmr spectrum of **6** were consistent with the characteristic polycyclic skeleton of benzonaphthoxanthenone and were assigned on the basis of comparison with those of **2**. The carbon signals were nearly identical with those of **2** except for the additional 1-OMe signal at δ 56.4 and the carbonyl signal that shifted upfield by Δδ 5.7. Moreover, the chemical correlation of **6** with **2** confirmed these assignments. Methylation of **6** yielded 1,4-di-*O*-methylohioensin B, identical with

TABLE 1. Comparison of ^{1}H-nmr Data of **6**, **7**, and **8** with **2**.

Proton	δ (ppm), Multiplicity (*J*, Hz)			
	2[a]	**6**[b]	**7**[b]	**8**[b]
H-2	6.65, s	6.67, s	6.63, s	6.63, s
H-5	7.06, dd (8.0, 1.2)	7.08, d (7.7)	7.06, d (7.6)	7.06, d (7.6)
H-6	7.38, dd (8.0, 7.7)	7.39, t (7.7)	7.36, t (7.6)	7.35, t (7.6)
H-7	7.59, dd (7.7, 1.2)	7.61, d (7.7)	7.58, d (7.6)	7.57, d (7.6)
H-7b	4.90, d (13.3)	4.88, d (12.8)	4.65, d (12.9)	4.57, d (12.8)
H-9	7.11, dd (8.0, 1.4)	7.14, dd (7.5, 1.2)	7.07, dd (7.6, 1.3)	7.07, dd (7.5, 1.3)
H-10	7.23, ddd (8.0, 6.9, 1.2)	7.23, td (7.5, 1.5)	7.19, td (7.6, 1.5)	7.19, td (7.5, 1.7)
H-11	6.99, ddd (8.0, 6.9, 1.4)	7.00, td (7.5, 1.2)	6.99, td (7.6, 1.3)	6.98, td (7.5, 1.3)
H-12	7.25, dd (8.0, 1.2)	7.29, dd (7.5, 1.5)	7.36, dd (7.6, 1.5)	7.33, dd (7.5, 1.7)
H-12b	3.59, ddd (14.7, 7.2, 4.8)	3.57, ddd (14.7, 7.6, 4.3)	3.68, ddd (14.0, 7.7, 3.4)	3.66, ddd (14.0, 8.1, 3.6)
H-13α	2.98, dd (15.4, 4.8)	2.89, dd (13.0, 4.3)	2.48, dt (14.0, 3.4)	2.59, dt (14.0, 3.6)
H-13β	2.75, dd (15.4, 14.7)	2.76, dd (14.7, 13.0)	1.69, td (14.0, 2.8)	1.55, td (14.0, 2.5)
H-14β	—	—	5.30, dd (3.4, 2.8)	4.87, dd (3.6, 2.5)
H-14c	3.26, dd (13.3, 7.2)	3.21, dd (12.8, 7.6)	3.05, dd (12.9, 7.7)	3.05, dd (12.8, 8.1)
1-OH	12.06, s	—	—	—
4-OH	7.35, s	7.31, s	7.79, s	7.72, s
14-OH	—	—	3.52, s	—
1-OMe	—	4.01, s	4.01, s	4.01, s
3-OMe	4.09, s	4.11, s	3.98, s	3.96, s
14-OMe	—	—	—	3.52, s

[a]Recorded at 500 MHz in $CDCl_3$.
[b]Recorded at 250 MHz in $CDCl_3$.

material prepared by the methylation of **2** (4). The relative stereochemistry of **6** was determined by analysis of ^{1}H-nmr and cd spectra. Because the methine protons at 7b, 12b, and 14c of **6** were identical in pattern to those of **2**, the relative stereochemistry of **6** was established as trans H-7b/H-14c (J=12.8 Hz) and cis H-14c/H12-b (J=7.6 Hz). Comparison of the cd of **6** with that of **2** indicated that they contained the same configurations as **1**, the relative stereochemistry of which was unequivocally established by single-crystal X-ray diffraction (3,4). Therefore, **6** was characterized as 1-*O*-methylohioensin B or (7bβ,12bα,14cα)-7b,12b,13,14c-tetrahydro-1,3-dimethoxy-4-hydroxy-14*H*-benzo[*c*]naphtho[2,1,8-*mna*]xanthen-14-one.

Compound **7** was isolated as pale yellow crystals. The hreims showed a molecular ion at *m/z* 402.1500 as the base peak, corresponding to the formula $C_{25}H_{22}O_5$, which has two more hydrogen atoms than that of **6**. The eims gave an intense $[M-H_2O]^+$ ion at *m/z* 384 (48%). Other characteristic ions at *m/z* 369 $[M-H_2O-Me]^+$ and 353 $[M-H_2O-MeOH]^+$ indicated the presence of methoxyl groups. The ir showed the

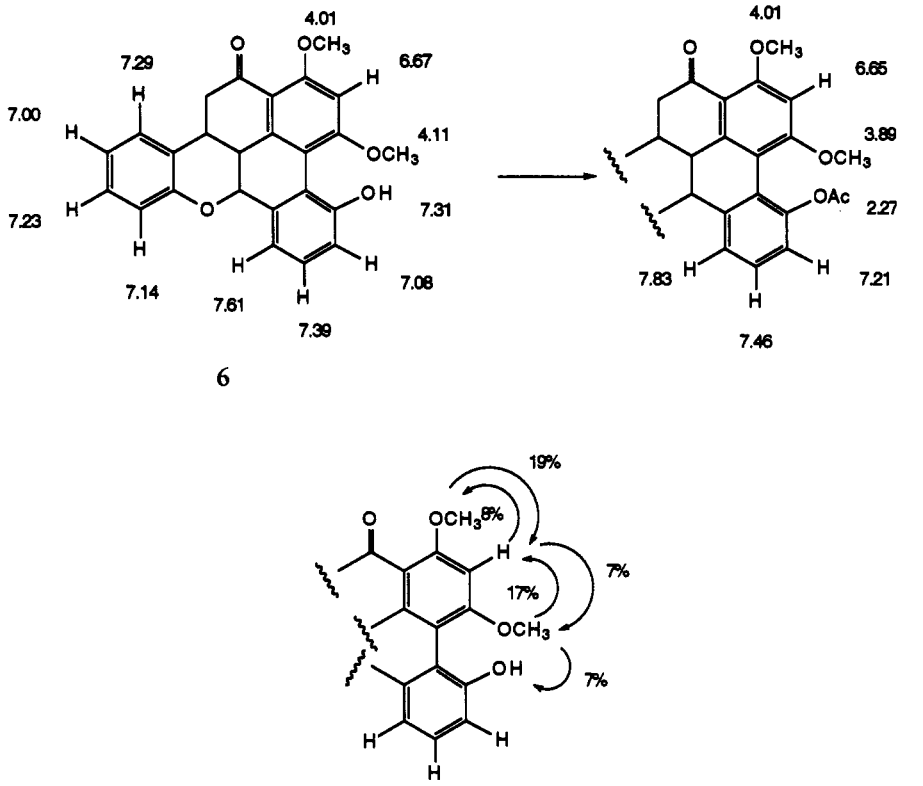
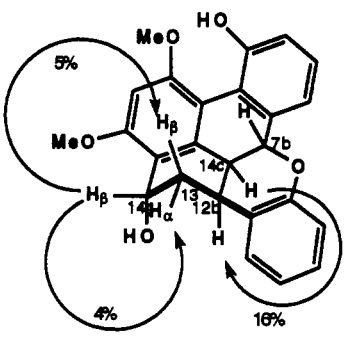


FIGURE 1. Nmr Characteristics of **6** and Its Acetate.

hydroxyl (3330 cm^{-1}), methoxyl (2850), aromatic (1600, 1580), and phenyl C-O (1320, 1240) but no carbonyl absorptions.

The ^{1}H-nmr signals of **7** were very similar to those of **6** (Table 1). The singlet at δ 6.63, assigned to H-2, was not affected by acetylation of **7** indicating that the two methoxyl groups were located at C-1 and C-3. The phenolic hydroxyl at δ 7.79 was located at the C-4 position by spectral analysis after acetylation as in the case of **6**. The coupling patterns of H-7b at δ 4.65 (d) and H-14c at δ 3.05 (dd) as well as H-12b at δ 3.68 (ddd) of the aliphatic region remained similar to those of **6**. However, the two nonequivalent geminal protons, H-13α and H-13β, appeared as a doublet of triplets



7

FIGURE 2. NOe Interactions for **7**.

TABLE 2. ^{1}H- and ^{13}C-Nmr Assignments of Pallidisetin A [**9**] and B [**10**].

Atom	δ (ppm), Multiplicity (*J*, Hz)			
	9		**10**	
	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
1	—	192.46	—	191.43
2	7.00, d (16.2)	131.99	6.52, d (12.2)	132.58
3	8.28, d (16.2)	129.59	6.95 (12.2)	127.50
2′	5.51, dd (H_β) (12.6, 3.1)	79.54	5.53, dd (H_α) (12.5, 3.2)	79.76
3′	2.79, dd (H_β) (16.6, 3.1)	46.07	2.77, dd (H_α) (16.6, 3.2)	45.56
	3.03, dd (H_α) (16.6, 12.6)	—	3.03, dd (H_β) (16.6, 12.5)	—
3a′	—	112.40	—	113.12
4′	—	143.49	—	142.86
5′	6.83, d (2.3)	109.40	6.37, d (2.6)	113.45
6′	—	164.27	—	164.47
7′	6.41, d (2.3)	103.60	6.19, d (2.6)	103.19
7a′	—	165.47	—	165.28
1″	—	140.24	—	140.23
2″/6″	7.52, d (7.4)	127.06	7.06, d (7.4)	127.06
3″/5″	7.41, t (7.4)	129.46	7.12, t (7.4)	129.70
4″	7.35, t (7.4)	129.18	7.10, t (7.4)	129.16
1‴	—	138.54	—	137.86
2‴/6‴	7.55, d (7.4)	127.60	7.53, d (7.3)	128.75
3‴/5‴	7.35, t (7.4)	129.39	7.41, t (7.3)	129.38
4‴	7.25, t (7.4)	128.56	7.35, t (7.3)	129.01
6-OH[c]	5.42, s		5.68, s	

[a]Recorded at 250 MHz in Me_2CO-d_6 and assigned on the basis of 500 MHz 2D COSY.
[b]Recorded at 63 MHz in Me_2CO-d_6 and assigned on the basis of 2D HETCOR.
[c]Observed only in $CDCl_3$.

(*J*=14.0, 3.4 Hz) at δ 2.48 (H-13α) and as a triplet of doublets (*J*=14.0, 2.8 Hz) at δ 1.69 (H-13β), respectively. The spectrum of **7** also exhibited two additional signals at δ 5.30 (1H, dd, *J*=3.4, 2.8 Hz) and 3.52 (1H, br s, D_2O exchangeable) which could be assigned to H-14 and 14-OH, respectively. The relative configuration of 14-OH was determined by nOe studies. Irradiation of H-14β enhanced the signals of H-13α (4% nOe) and H-13β (5% nOe), suggesting that H-14β was gauche to both H-13α (equatorial) and H-13β (axial). Irradiation of H-14c enhanced the signal of H-12b by 16%, indicating that H-14c and H-12b were cis to each other (Figure 2).

Compound **7** was structurally correlated with **6** and ohioensin B [**2**] via chemical reaction. Reduction of **6** with $NaBH_4$ yielded **7**. Except for the lack of carbonyl absorption in the region of 340–360 nm, the cd curve of **7** was very similar in shape to those of **6** and **2**. Therefore, the structure of **7** was established as 1-*O*-

methyldihydroohioensin B or (7bβ,12bα,14cα)-7b,12b,13,14c-tetrahydro-1,3-dimethoxy-4,14α-dihydroxy-14*H*-benzo[*c*]naphtho[2,1,8-*mna*]xanthene.

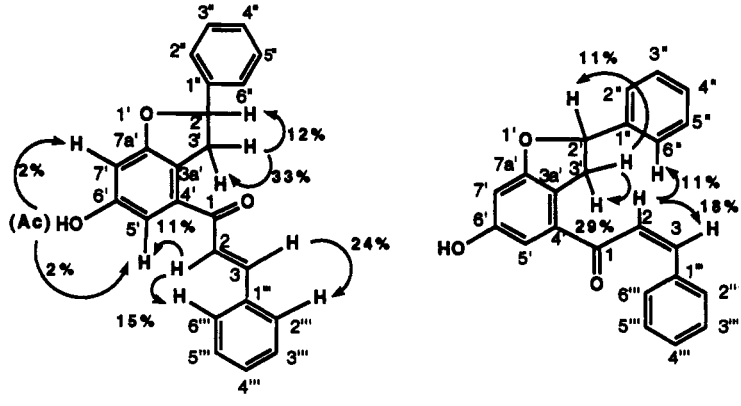
The molecular formula of **8** was determined as $C_{26}H_{24}O_5$ by the hreims, indicating the same degree of unsaturations as that of **7**. This suggested that **8** could be a derivative of **7** with the same skeleton. The absence of carbonyl absorption in the ir spectrum further suggested that **8** was a benzonaphthoxanthenol. The proton spectrum confirmed that **8** contained the same substitution pattern as that of **7** (Table 1). The phenolic hydroxyl was assigned to C-4 with the aid of acetylation. The methoxyl at δ 3.52 was assigned to C-14 by its chemical shift. Furthermore, H-14 shifted upfield to δ 4.87 in **8**, while it appeared at δ 5.30 in **7**. Because the coupling patterns of H-7b, H-12b and H-14c appeared unchanged, **8** should contain the same stereochemistry as **7**. The specific optical rotation and the cd spectrum supported this conclusion. Thus, **8** was identified as 1,14-di-*O*-methyldihydroohioensin B or (7bβ,12bα,14cα)-7b,12b,13,14c-tetrahydro-1,3,14α-trimethoxy-4-hydroxy-14*H*-benzo[*c*]naphtho[2,1,8-*mna*]xanthene.

Compound **9**, named pallidisetin A, gave an $[M]^+$ at *m/z* 342.1277 (hreims) for the formula $C_{23}H_{18}O_3$. The ir spectrum showed hydroxyl (3270 cm^{-1}), conjugated carbonyl (1650), olefinic bond (1620), aromatic ring (1600, 1580), and aromatic C-O (1280, 1190) absorptions. The uv spectrum gave maximum absorptions at 318, 270, and 227 nm typical for a chalcone chromophore (5). The detailed assignment of the ^{1}H-nmr spectrum of **9** is given in Table 3. The singlet at δ 5.42 was recognized as a hydroxyl proton, which disappeared upon the addition of D_2O. Its location was determined by

TABLE 3. Cytotoxicities of Compounds **6–10** (ED_{50}, μg/ml).

Compound	A549[a]	HT-29[b]	RPMI-7951[c]	U-251 MG[d]
6	—	1.0	1.0	2.0
7	—	—	—	0.8
8	1.0	—	1.0	—
9	—	—	1.0	1.0
10	—	—	2.0	2.0

[a]Human lung carcinoma.
[b]Human colon adenocarcinoma.
[c]Human melanoma.
[d]Human glioblastoma multiforme.



9 **10**

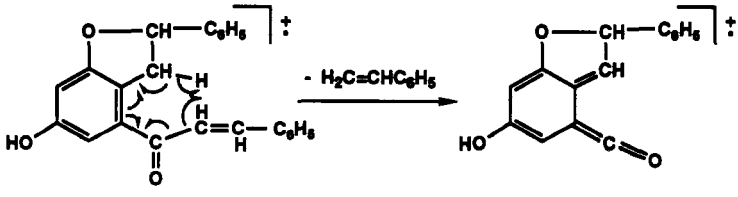
FIGURE 3. NOe Interactions for **9** and **10**.

acetylation and nOe methods. Irradiation of the protons (δ 2.34) of the acetate enhanced two meta-coupled aromatic protons (J=2.4 Hz) at δ 6.80 and 7.05 (Figure 3). There was an ABX system in the aliphatic region that could be interpreted as two nonequivalent geminal protons at δ 2.79 (dd,J=16.6, 3.1 Hz) and 3.03 (dd,J=16.6, 12.6 Hz) and an oxygen-linked methine proton at δ 5.51 (dd,J=12.6, 3.1 Hz). Irradiation of the proton at δ 2.79 enhanced the adjacent methine at δ 5.51 and the geminal proton at δ 3.03 by 12% and 33%, respectively (Figure 3). Two olefinic protons appeared at δ 7.00 and 8.28 as two doublets with a large coupling constant (J=16.2 Hz), suggesting the presence of a trans olefin conjugated with a carbonyl. The β-carbon of the α,β-unsaturated ketone must be linked to an aromatic ring because of the chemical shift of the β-proton (δ 8.28). The remaining six aromatic signals at δ 7.2–7.6 could be completely resolved by the 500 MHz ^{1}H-^{1}H-COSY as two groups of independent monosubstituted C_6H_6 protons (Table 2). This is supported by the observation that in the fully proton-decoupled ^{13}C-nmr spectrum, there were 19 carbon peaks, four carbons less than the formula ($C_{23}H_{18}O_3$) required. Thus, **9** must have the symmetric axes which resulted in 4 pairs of symmetric carbons. Analysis of the 2D HETCOR spectra and application of the substituent effect rule led to the complete assignment of all carbon signals (Table 2).

Two important clues for the establishment of the complete structure of **9** came from the nOe study and hreims fragmentation. Irradiation of the H-2 (δ 7.00) of the α,β-unsaturated ketone moiety enhanced one of the meta-coupled aromatic protons (δ 6.83, H-5′) by 11%, indicating that the α,β-unsaturated ketone system was attached to the ortho-position of H-5′ on the tetrasubstituted benzene ring. The hreims showed a characteristic peak at m/z 238.0680 $[C_{15}H_{10}O_3]^+$ (38%) produced by the loss of C_8H_8 from the cinnamoyl side-chain through a rearrangement assisted by an ortho-substituent (Figure 4). This suggested the presence of a hydrogen-bearing carbon ortho to the cinnamoyl substituent.

Compound **9** may have two cisoid conformers and two transoid conformers (6) arising from the rotation of the cinnamoyl side-chain (C-4′–C-1 and C-1–C-2). The nOe studies indicated that the cisoid conformer shown was the most favorable one. As a novel cinnamoyl bibenzyl, **9** was characterized as 1-(2,3-dihydro-6-hydroxy-2-phenyl-4-benzofuranyl)-3-phenyl-2(*E*)-propen-1-one.

Compound **10**, named pallidisetin B, showed the same molecular formula as **9**. Most of the ir spectrum of **10** was similar to that of **9**. However, the absorptions of the olefinic double bond and carbonyl group of **10** were weaker than those of **9**, implying a weaker conjugated system. This was confirmed by the uv spectrum of **10**, which showed a decrease in the intensity of the corresponding maximum absorption when compared with that of **9**. The conjugated carbonyl absorption of **10** was shifted to 294 nm from 318 nm in **9**. The ^{1}H-^{1}H-COSY nmr data of **10** were compared with those of **9** (Table 2). The nOe result showed that irradiation of the H-3′ signal at δ 2.77 enhanced the adjacent methine (H-2′, δ 5.53) and the geminal proton (δ 3.03) by 11% and 29%



$[M]^+$ 342.1277 m/z 238.0680

FIGURE 4. Partial Ms of **9**.

(Figure 3). The major difference arose from the two olefinic protons of the α,β-unsaturated ketone system, which shifted upfield at δ 6.95 (H-3) and 6.52 (H-2) with the coupling constant (J=12.2 Hz) typical for a cis-olefin. Thus, **10** was identified as the cis-isomer of **9**. The cis-configuration apparently reduced conjugation between the α,β-unsaturated system and the bulky aromatic rings as reflected in the uv, ir and nmr spectra of **10**. The carbon signals of **10** were almost identical with those of **9** as summarized in Table 2. The only difference was that C-3 shifted upfield by about Δδ 2 and C-5′ shifted downfield by Δδ 4. The favorable conformer for **10** was the cisoid shown as determined by the nOe experiment.

The benzonaphthoxanthenones and cinnamoyl bibenzyls might be derived from cinnamic acid and 3,5-dioxohexanoic acid via different pathways (6). The pallidisetins apparently arise from the coupling of bibenzyl with cinnamic acid, while the ohioensins may be derived from the condensation of *o*-hydroxycinnamate with hydroxylated phenanthrenes or 9,10-dihydrophenanthrenes that originate from the corresponding bibenzyls (4). The possible biogenetic connection between the two types of compounds provides a new clue for the chemotaxonomy of *Polytrichum* mosses. The ohioensins [**6–8**] and pallidisetins [**9**, **10**] exhibited cytotoxicity against several human tumor cell lines. The ED_{50} values are summarized in Table 3.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mps were determined on a Fisher-Johns apparatus and are uncorrected. Ir and uv spectra were recorded on a Laser Precision Analytical RFX-40 Ftir and a Beckman UV-5260 spectrophotometer, respectively. Low- and high-resolution mass spectra were determined on a Kratos MS-30 mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on IBM AF 250 and Bruker AM-500 nmr spectrometers using TMS as internal standard. 2D nmr spectra were recorded on a Bruker AM-500 nmr spectrometer. Specific rotations were determined on a Perkin-Elmer 241 digital polarimeter. Cd spectra were measured in MeOH on a Jasco J-500A spectropolarimeter and reported in molar ellipticity [θ] units. Precoated Si gel plates (Merck, 60F-254, 0.25 and 2-mm thick) were used for analytical and prep. tlc, respectively. Compounds were visualized by uv (254 nm), iodine, or vanillin-H_2SO_4 spray reagent.

PLANT MATERIAL.—The moss *Polytrichum pallidisetum* Funck was collected in New Hampshire in 1984, and identified by Dr. Richard W. Spjut of World Botanical Associates, Laurel, Maryland. Voucher specimens have been deposited in the United States National Herbarium.

EXTRACTION AND ISOLATION.—The dried plant material (3 kg) was ground and percolated with 95% EtOH. The EtOH extract (150 g) exhibited cytotoxicity against 9KB and 9PS cells in culture and antitumor activity in P-388 mouse lymphocytic leukemia. This extract was then partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$ extract (42 g) was further partitioned between 90% MeOH and hexane. The aqueous MeOH extract (26 g) showed significant cytotoxicity and was subjected to chromatography over a Sephadex LH-20 column (200 g), eluted with hexane/CH_2Cl_2 and CH_2Cl_2/MeOH to yield 19 fractions (A-S) with decreasing R_f values. The activity was concentrated in fractions C-I, which were eluted with hexane-CH_2Cl_2 (3:1, 1:1, and 1:3).

Fractions C-H were combined (4.16 g) and chromatographed over a Florisil column (200 g) with CH_2Cl_2, followed by increasing percentages of MeOH in CH_2Cl_2 (1%, 2%, 5%, 10%, 20%, and 50%) to give 16 major fractions (C-1 to C-16). Further chromatography of fraction C-4 (41.2 mg) over a Si gel (1.5 g) column with 20% EtOAc in hexane, followed by crystallization with CH_2Cl_2/MeOH afforded **6** (6.7 mg), **7** (2.4 mg), and **8** (1.4 mg).

Chromatography of fraction I (1.69 g) over a Si gel column (60 g) with increasing ratios of MeOH in CH_2Cl_2 (1%, 5%, and 10%) yielded 12 fractions (I-1 to I-12). Crystallization of fraction I-2 (54.2 mg) in CH_2Cl_2/MeOH afforded crude crystals (34 mg), which were further purified on Si gel prep. tlc plates with 20% EtOAc in hexane. The lower-R_f compound was crystallized as colorless plate crystals, named pallidisetin A [**9**] (15.2 mg), and the higher-R_f compound was crystallized as white crystals, named pallidisetin B [**10**] (6.5 mg).

1-O-Methylohioensin B [**6**].—Crystallization from CH_2Cl_2/MeOH gave yellowish needles, mp 275–277° (dec); $[\alpha]^{27}D$ −15° (c=0.1, $CHCl_3$); uv λ max (MeOH) (log ε) 334 (3.72), 303 (4.04), 280 (4.27, sh), 261 (4.41), 226.5 (4.36) nm; ir ν max (KBr) 3350, 2830, 1685, 1617, 1590, 1320, 1280, 1240, 975, 840, 790, 755 cm^{-1}; hreims m/z $[M]^+$ 400.1330 (calcd for $C_{25}H_{20}O_5$, 400.1311); eims m/z $[M]^+$ 400 (100),

$[M-H]^+$ 399 (22), $[M-Me]^+$ 385 (17), $[M-OH]^+$ 383 (27), $[M-H_2O]^+$ 382 (14), [*m/z* 399$-H_2O]^+$ 381 (15), $[M-OMe]^+$ 369 (5), $[M-MeOH]^+$ 368 (10), [*m/z* 399$-MeOH]^+$ 367 (11), $[M-H_2O-MeO]^+$ 351 (12), $[M-C_6H_5O]^+$ 307 (32), [*m/z* 307$-Me]^+$ 292 (11), [*m/z* 307$-C_2H_2]^+$ 281 (22), [*m/z* 307$-C_3H_3]^+$ 268 (9); ^{1}H nmr see Table 1; ^{13}C nmr (63 MHz, $CDCl_3$) δ 30.5 (C-12b), 38.5 (C-14c), 46.3 (C-13), 56.4 (OCH_3), 57.2 (OCH_3), 69.9 (C-7b), 96.5 (C-2), 115.0 (C-3a), 116.6 (C-7), 117.4 (C-9), 117.8 (C-3b), 118.3 (C-14a), 119.0 (C-5), 121.4 (C-11), 122.9 (C-12a), 128.3 (C-10), 129.3 (C-12), 129.4 (C-6), 140.8 (C-14b), 142.3 (C-7a), 152.4 (C-4), 153.3 (C-3), 158.5 (C-1), 159.4 (C-8a), 195.6 (C-14); cd (0.025 mM, MeOH) $[\theta]_{380}$ 0°, $[\theta]_{340}$ −1,500° (min), $[\theta]_{325}$ 0°, $[\theta]_{315}$ +750° (max), $[\theta]_{300}$ 0° (min), $[\theta]_{278}$ +7,350° (max), $[\theta]_{263}$ 0°, $[\theta]_{250}$ −2,850° (min), $[\theta]_{239}$ −600° (max), $[\theta]_{222}$ −27,600° (min), $[\theta]_{207}$ 0°, $[\theta]_{203}$ +14,400° (max), $[\theta]_{202}$ 0°; acetate: white needles crystallized from CH_2Cl_2-MeOH (1:1), mp 232–234° (dec); hreims *m/z* $[M]^+$ 442.1415 (calcd for $C_{27}H_{22}O_6$, 442.1416); eims *m/z* $[M]^+$ 442 (55), $[M-CH_2CO]^+$ 400 (56), $[M-CH_2CO_2H]^+$ 383 (22), [*m/z* 383$-CH_4]^+$ 367 (8), [*m/z* 383$-MeOH]^+$ 351 (9), [*m/z* 351$-C_2H_2]^+$ 325 (6), $[M-CH_2CO-C_6H_5O]^+$ 307 (23), [*m/z* 307$-C_2H_2]^+$ 281 (15), [*m/z* 307$-C_3H_3]^+$ 268 (8); ^{1}H nmr (250 MHz, $CDCl_3$) δ 2.27 (s, 3H, 4-OAc), 2.73 (dd, 1H, *J*=14.8, 13.1 Hz, H-13β), 2.89 (dd, 1H, *J*=13.1, 4.3 Hz, H-13α), 3.20 (dd, 1H, *J*=12.6, 7.6 Hz, H-14c), 3.61 (ddd, 1H, *J*=14.8, 7.6, 4.3 Hz, H-12b), 3.89 (s, 3H, OMe), 4.01 (s, 3H, OMe), 4.89 (d, 1H, *J*=12.6 Hz, H-7b), 6.55 (s, 1H, H-2), 7.00 (t, 1H, *J*=7.4 Hz, H-11), 7.09 (dd, 1H, *J*=7.4 Hz, H-9), 7.21 (d, 1H, *J*=7.7 Hz, H-5), 7.22 (t, 1H, *J*=7.4 Hz, H-10), 7.30 (d, 1H, *J*=7.4 Hz, H-12), 7.46 (t, 1H, *J*=7.7 Hz, H-6), 7.83 (d, 1H, *J*=7.7 Hz, H-7); methyl ether: identical with 1,4-di-*O*-methylohioensin B (4).

Reduction of **6**.—A solution of 1-*O*-methylohioensin B (**6**; 1.5 mg) in dry MeOH (10 drops) and CH_2Cl_2 (10 drops) was treated with $NaBH_4$ (5 mg) and stirred for 3 h at room temperature. H_2O was added to the mixture and the solution was acidified to pH 1–2 with 1N HCl. The product was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried and evaporated. The product showed two spots on a Si gel tlc plate developed with hexane-EtOAc (1:1). The major spot (>80%, lower R_f) was isolated to give **7** as a white solid (0.8 mg), hreims *m/z* 402.1460 (M^+, calcd for $C_{25}H_{22}O_5$, 402.1467), identical with material described below.

1-O-Methyldihydroohioensin B [**7**].—White crystals from MeOH, mp 185–187° (dec); $[\alpha]^{27}_D$ −49° (*c*=0.03, $CHCl_3$); uv λ max (MeOH) (log ε) 305 (3.48), 273 (3.67), 222.5 (4.04) nm; ir ν max (KBr) 3330, 2920, 2850, 1600, 1580, 1320, 1240, 1090, 1040, 970, 780, 760 cm^{-1}; hreims *m/z* $[M]^+$ 402.1500 (calcd for $C_{25}H_{22}O_5$, 402.1467); eims *m/z* $[M]^+$ 402 (100), $[M-H_2O]^+$ 384 (48), $[M-H_2O-Me]^+$ 369 (31), $[M-H_2O-MeO]^+$ 353 (22), [*m/z* 369$-MeOH]^+$ 337 (4), $[M-C_6H_5OH]^+$ 308 (7), $[M-H_2O-C_6H_5O]^+$ 291 (24), $[M-C_8H_7O]^+$ 283 (17), [*m/z* 291$-C_2H_3]^+$ 264 (8); ^{1}H nmr see Table 1; cd (0.025 mM, MeOH) $[\theta]_{330}$ 0°, $[\theta]_{305}$ +4,820° (max), $[\theta]_{290}$ +1,610° (min), $[\theta]_{270}$ +8,040° (max), $[\theta]_{250}$ +3,220° (sh), $[\theta]_{248}$ 0°, $[\theta]_{232}$ −36,980° (min), $[\theta]_{220}$ 0°, $[\theta]_{213}$ +20,000° (max), $[\theta]_{205}$ 0°; acetate: white solid, hreims *m/z* $[M]^+$ 444.1585 (calcd for $C_{27}H_{24}O_6$, 444.1573); eims *m/z* $[M]^+$ 444 (100), $[M-CH_2CO]^+$ 402 (52), $[M-CH_3CO_2H]^+$ 384 (61), $[M-CH_3CO_2H-Me]^+$ 369 (34), $[M-C_6H_5CH_2]^+$ 353 (25), $[M-CH_2CO-C_6H_5OH]^+$ 308 (9), $[M-CH_3CO_2H-C_6H_5O]^+$ 291 (23), $[M-CH_2CO-C_8H_7O]^+$ 283 (16), [*m/z* 291$-C_2H_3]^+$ 264 (12); ^{1}H nmr (250 MHz, $CDCl_3$) δ 1.61 (td, 1H, *J*=14.0, 2.8 Hz, H-13β), 2.26 (s, 3H, 4-OAc), 2.46 (dt, 1H, *J*=14.0, 3.4 Hz, H-13α), 3.05 (dd, 1H, *J*=12.6, 7.4 Hz, H-14c), 3.66 (m, 1H, H-12b), 3.81 (s, 3H, OMe), 3.97 (s, 3H, OMe), 4.63 (d, 1H, *J*=12.6 Hz, H-7b), 5.29 (dd, 1H, *J*=3.4, 2.8 Hz, H-14b), 6.52 (s, 1H, H-2), 6.99 (t, 1H, *J*=7.8 Hz, H-11), 7.06 (d, 1H, *J*=7.8 Hz, H-9), 7.20 (t, 1H, *J*=7.8 Hz, H-10), 7.21 (d, 1H, *J*=7.6 Hz, H-5), 7.37 (d, 1H, *J*=7.8 Hz, H-12), 7.42 (t, 1H, *J*=7.6 Hz, H-6), 7.79 (d, 1H, *J*=7.6 Hz, H-7); diacetate: *m/z* $[M]^+$ 486.1674 (calcd for $C_{29}H_{26}O_7$, 486.1678); eims *m/z* $[M]^+$ 486 (6), $[M-CO]^-$ 458 (54), $[M-CH_2CO]^+$ 444 (39), 428 [*m/z* 458$-CH_2O]^+$ (16), $[M-CH_3CO_2H]^+$ 426 (95), [*m/z* 444$-CO]^+$ 416 (9), $[M-2CH_2CO]^+$ 402 (21), $[M-C_6H_5CH_2]^+$ 395 (12), $[M-CH_3CO_2H-CH_2CO]^+$ 384 (100), [*m/z* 384$-Me]^+$ 369 (44), [*m/z* 384$-MeO]^+$ 353 (38), [*m/z* 353$-C_2H_4]^+$ 325 (10), $[M-2CH_2CO-C_6H_5O]^+$ 309 (7), [*m/z* 384$-C_6H_5O]^+$ 291 (23); ^{1}H nmr (250 MHz, $CDCl_3$) δ 2.25 (s, 3H, 14-OAc), 2.26 (s, 3H, 4-OAc), 3.95 (s, 3H, OMe), 3.97 (s, 3H, OMe).

1,14-Di-O-methyldihydroohioensin B [**8**].—Recrystallization of **8** from MeOH afforded light yellow crystals, mp 135–137° (dec); $[\alpha]^{27}_D$ −37.3° (*c*=0.15, $CHCl_3$); uv λ max (MeOH) (log ε) 306 (3.69), 273 (3.90), 224.5 (4.16) nm; ir ν max (KBr) 3335, 2920, 2850, 1600, 1585, 1320, 1240, 1200, 1090, 970, 780, 750 cm^{-1}; hreims *m/z* $[M]^+$ 416.1625 (calcd for $C_{26}H_{24}O_5$, 416.1624); eims *m/z* $[M]^+$ 416 (4), $[M-MeOH]^+$ 384 (6), [*m/z* 384$-Me]^+$ 369 (3), [*m/z* 384$-MeO]^+$ 353 (3), $[M-C_8H_7O]^+$ 307 (16), 149 (100); ^{1}H nmr see Table 1; cd (0.024 mM, MeOH) $[\theta]_{330}$ 0°, $[\theta]_{305}$ +2,980° (max), $[\theta]_{290}$ +1,320° (min), $[\theta]_{270}$ +7,300° (max), $[\theta]_{250}$ +2,980° (sh), $[\theta]_{247}$ 0°, $[\theta]_{234}$ −31,200° (min), $[\theta]_{220}$ 0°, $[\theta]_{208}$ +17,920° (max), $[\theta]_{203}$ 0°; acetate: white crystals, mp 115–117°; hreims *m/z* $[M]^+$ 458.1763 (calcd for $C_{28}H_{26}O_6$, 458.1729); eims *m/z* $[M]^+$ 458 (5), $[M-MeOH]^+$ 426 (5), $[M-CH_2CO]^+$ 416 (2), $[M-MeOH-CH_2CO]^+$ 384 (8), [*m/z* 384$-MeO]^+$ 353 (3), [*m/z* 353$-MeOH]^+$ 321 (2), 307 (25), 279 (3), 167 (22), 149 (100); ^{1}H nmr (250 MHz, $CDCl_3$) δ 1.53 (td, 1H, *J*=13.9, 2.4 Hz, H-13β), 2.25 (s, 3H, 4-OAc), 2.59 (dt, 1H, *J*=13.9, 3.6

Hz, H-13α), 3.03 (dd, 1H, J=12.8, 8.1 Hz, H-14c), 3.52 (s, 3H, 14-OMe), 3.68 (ddd, 1H, J=13.9, 8.1, 3.6 Hz, H-12b), 3.80 (s, 3H, OMe), 3.95 (s, 3H, OMe), 4.55 (d, 1H, J=12.8 Hz, H-7b), 4.86 (dd, 1H, J=3.6, 2.4 Hz, H-14b), 6.51 (s, 1H, H-2), 6.98 (td, 1H, J=7.5, 1.3 Hz, H-11), 7.06 (dd, 1H, J=7.8, 1.5 Hz, H-9), 7.19 (td, 1H, J=7.5, 1.5 Hz, H-10), 7.21 (d, 1H, J=7.7 Hz, H-5), 7.33 (dd, 1H, J=7.5, 1.3 Hz, H-12), 7.41 (t, 1H, J=7.7 Hz, H-6), 7.78 (d, 1H, J=7.7 Hz, H-7).

Pallidisetin A [**9**].—Compound **9**, with a strong sky-blue fluorescence on tlc plates after uv visualization, was obtained as colorless plate crystals, mp 233° (dec); $[\alpha]^{27}D$ +20.0° (c=0.1, $CHCl_3$); uv λ max (MeOH) (log ε) 318 (4.05), 270 (4.22), 227 (3.92) nm; ir ν max (KBr) 3270, 1650, 1620, 1600, 1580, 1280, 960, 850, 760, 740 cm^{-1}; hreims *m/z* $[M]^+$ 342.1277 ($C_{23}H_{18}O_3$, 100), $[M-C_6H_5]^+$ 265.0891 ($C_{17}H_{13}O_3$, 26), $[M-C_6H_5CH_2]^+$ 251.0751 ($C_{16}H_{11}O_3$, 11), $[M-C_6H_5CH=CH]^+$ 239.0727 ($C_{15}H_{11}O_3$, 11), $[M-C_6H_5CH=CH_2]^+$ 238.0680 ($C_{15}H_{10}O_3$, 38), $[M-C_7H_7-CO]^+$ 223.0794 ($C_{15}H_{11}O_2$, 4), $[M-C_8H_7-CO]^+$ 211.0783 ($C_{14}H_{11}O_2$, 12), $[M-C_8H_8-CO]^+$ 210.0736 ($C_{14}H_{10}O_2$, 47), $[M-C_8H_8-2CO]^+$ 182.0783 ($C_{13}H_{10}O$, 13); ^1H and ^{13}C nmr (in Me_2CO-d_6) see Table 2; cd (0.29 mM, MeOH) $[\theta]_{390}$ 0°, $[\theta]_{355}$ +2,052° (max), $[\theta]_{335}$ 0°, $[\theta]_{316}$ −2,155° (min), $[\theta]_{305}$ −1,778° (sh), $[\theta]_{290}$ −1,368° (max), $[\theta]_{270}$ −2,804° (min), $[\theta]_{247}$ 0°, $[\theta]_{220}$ +3,146° (max), $[\theta]_{205}$ 0°; acetate: white needles, mp 182–183° (dec); hreims *m/z* $[M]^+$ 384.1341 (calcd for $C_{25}H_{20}O_4$, 384.1361); eims *m/z* $[M]^+$ 384 (55), $[M-CH_2CO]^+$ 342 (24), $[M-C_6H_6CH=CH_2]^-$ 280 (15), $[M-CH_2CO-C_6H_5]^+$ 265 (15), $[M-CH_2CO-C_6H_5CH_2]^+$ 251 (11), $[M-CH_2CO-C_6H_5CH=CH_2]^-$ 238 (44), [*m/z* 238−CO]$^+$ 210 (45), [*m/z* 210−CHO]$^-$ 181 (20); ^1H nmr (250 MHz, $CDCl_3$) δ 2.34 (s, 3H, 6-OAc), 2.99 (dd, 1H, J=16.6, 3.1 Hz, H-3′α), 3.13 (dd, 1H, J=16.6, 13.2 Hz, H-3′β), 5.50 (dd, 1H, J=13.2, 3.1 Hz, H-2′β), 6.80 (d, 1H, J=2.2 Hz, H-7′), 6.99 (d, 1H, J=16.2 Hz, H-2), 7.05 (d, 1H, J=2.2 Hz, H-5′), 7.26–7.60 (m, 10H, 2 C_6H_5), 8.29 (d, 1H, J=16.2 Hz, H-3).

Pallidisetin B [**10**].—Compound **10**, with light blue-gray fluorescence on tlc plates with uv visualization, was obtained as colorless needles, mp 194° (dec); $[\alpha]^{27}D$ −29.6° (c=0.1, $CHCl_3$); uv λ max (MeOH) (log ε) 294 (3.19), 270 (3.20), 222 (3.46) nm; ir ν max (KBr) 3150, 1650, 1620, 1600, 1580, 1280, 1260, 860, 780, 770, 690 cm^{-1}; hreims *m/z* $[M]^+$ 342.1236 (calcd for $C_{23}H_{18}O_3$, 342.1256); eims *m/z* $[M]^+$ 342 (100), 265 (36), 251 (17), 239 (14), 238 (48), 211 (15), 210 (77), 182 (21), 181 (33), 180 (24); ^1H and ^{13}C nmr (in Me_2CO-d_6) see Table 2; cd (0.32 mM, MeOH) $[\theta]_{395}$ 0°, $[\theta]_{351}$ +2,301° (max), $[\theta]_{332}$ 0°, $[\theta]_{310}$ −4,011° (min), $[\theta]_{290}$ −3,171° (sh), $[\theta]_{268}$ 0°, $[\theta]_{265}$ +1,990° (max), $[\theta]_{254}$ 0°, $[\theta]_{247}$ −466° (min), $[\theta]_{242}$ 0°, $[\theta]_{235}$ +1,181° (max), $[\theta]_{215}$ 0°; acetate: colorless crystals, mp 167–168° (dec); hreims *m/z* $[M]^+$ 384.1341 (calcd for $C_{25}H_{20}O_4$, 384.1361); eims *m/z* $[M]^+$ 384 (62), $[M-CH_2CO]^+$342 (25), $[M-C_6H_5CH=CH_2]^+$ 280 (18), $[M-CH_2CO-C_6H_5]^-$ 265 (15), $[M-CH_2CO-C_6H_5CH_2]^+$ 251 (17), $[M-CH_2CO-C_6H_5CH=CH_2]^+$ 238 (50), 229 (13), 210 (45); ^1H nmr (250 MHz, $CDCl_3$) δ 2.34 (s, 3H, 6-OAc), 2.91 (dd, 1H, J=16.6, 3.0 Hz, H-3′α), 3.14 (dd, 1H, J=16.6, 13.2 Hz, H-3′β), 5.53 (dd, 1H, J=13.2, 3.0 Hz, H-2′α), 6.56 (d, 1H, J=2.3 Hz, H-7′), 6.67 (d, 1H, J=12.2 Hz, H-2), 6.77 (d, 1H, J=2.3 Hz, H-5′), 7.07 (d, 1H, J=12.2 Hz, H-3), 7.12–7.51 (m, 10H, 2 C_6H_5).

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