Hopane-Type Triterpenes and Binaphthopyrones from the Scale Insect Pathogenic Fungus *Aschersonia paraphysata* BCC 11964

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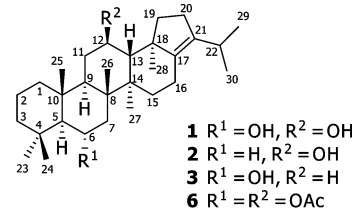
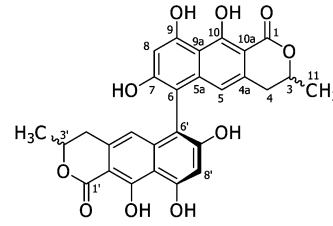
Two new triterpenes, 17(21)-hopene-6α,12β-diol (**1**) and 17(21)-hopen-12β-ol (**2**), the known 17(21)-hopen-6α-ol (zeorinin, **3**), and two new biarylic dihydronaphthopyrones, aschernaphthopyrones A (**4**) and B (**5**), were isolated from the scale insect pathogenic fungus *Aschersonia paraphysata* BCC 11964. Hopene **1** and aschernaphthopyrone A (**4**) exhibited antimalarial activity with IC_{50} values of 15 and 7.3 μM, respectively.

Aschersonia is a genus of entomopathogenic fungi that specifically attacks scale insects. There have been several reports on secondary metabolites from this genus: hopane-3β,15α,22-triol (a triterpene) from *A. aleyrodis*,[1] its 3β-*O*-acetyl derivative from *A. tubulata* BCC 1785,[2] ascherxanthones A and B (tetrahydroxanthone dimers) respectively from *Aschersonia* sp. BCC 8401 and *A. luteola* BCC 8774,[3,4] and destruxins A_4 and A_5 (cyclodepsipeptides) from an *Aschersonia* species.[5] As part of our research program on bioactive compounds from insect pathogenic fungi, we recently conducted a broad survey of scale insect pathogenic *Aschersonia* species and their *Hypocrella sensu lato* teleomorphs for their secondary metabolites. The study resulted in the conclusion that this group of fungi is a hot spot for three hopane triterpenes, zeorin (hopane-6α,22-diol), dustanin (hopane-15α,22-diol), and 3β-acetoxyhopane-15α,22-diol.[6] Recently, we found a mycelial extract from *Aschersonia* strain BCC 11964 that exhibited a unique ^1H NMR spectroscopic profile, suggesting the presence of triterpenes and several aromatic compounds. The ITS rDNA sequence data and the morphological characteristics of this strain indicated that it was identical to another strain (BCC 1467), which is currently described as the rarely collected *Aschersonia paraphysata*.[7] Scale-up fermentation and chemical studies resulted in the isolation of three hopanoids, 17(21)-hopene-6α,12β-diol (**1**), 17(21)-hopene-12β-ol (**2**), and 17(21)-hopene-6α-ol (**3**), and two biaryl dihydronaphthopyrones, aschernaphthopyrones A (**4**) and B (**5**), together with the known compounds 3,4,6-trihydroxymellein,[8−10] 6,8-dihydroxy-3-hydroxymethylisocoumarin,[11−13] and ergosterol. Details of the isolation, structure elucidation, and biological activities of the new compounds are presented here.





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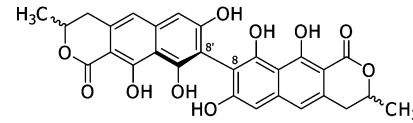

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Results and Discussion

Compound **1** was isolated as a white powder, and the molecular formula was established as $C_{30}H_{50}O_2$ by HRESIMS. The IR spectrum exhibited a broad absorption band at ν_{max} 3423 cm^{-1}, which indicated the presence of hydroxy groups. The ^1H and ^{13}C NMR data in $CDCl_3$ strongly suggested that **1** is a hopane-type triterpene. The presence of two secondary alcohol groups was evident from the ^1H NMR resonances of oxymethines at δ_H 3.98 (1H, dt, J = 3.9, 10.7 Hz) and 3.86 (1H, dt, J = 5.4, 10.9 Hz). The J-values demonstrated that these protons should occupy axial positions. Acetylation of **1** (Ac_2O, pyridine) gave a diacetate derivative (**6**), in which the oxymethine protons were shifted downfield, resonating at δ_H 5.25 (1H, dt, J = 3.8, 10.9 Hz) and 5.08 (1H, dt, J = 5.6, 11.2 Hz). The ^1H and ^{13}C NMR, DEPT135, and HMQC data for **1** allowed the categorization of two sp^2 quaternary carbons (δ_C 139.3 and 137.4, a tetrasubstituted olefin), five sp^3 quaternary carbons, two oxymethines (δ_C 69.3/δ_H 3.98 and δ_C 70.8/δ_H 3.86), four methines, nine methylenes, and eight methyl groups. The planar structure of **1** was deduced by analyses of COSY and HMBC data (Figure 1, Table 1). The key HMBC correlations were those from six methyl groups (H_3-23, H_3-24, H_3-25, H_3-26, H_3-27, and H_3-28) attached to quaternary sp^3 carbons C-4, C-4, C-10, C-8, C-14, and C-18, respectively. An isopropyl group (C-22, C-29, and C-30) was attached to the quaternary sp^2 carbon C-21 (δ_C 137.4). Correlations from H_β-15, H_β-16, H_β-20, and H_3-28 to the other sp^2 quaternary carbon (C-17, δ_C 139.3) indicated the location of the tetrasubstituted olefin as C-17/C-21.

The relative configuration of **1** was addressed on the basis of J-values and NOESY correlations (Figure 2). Key NOESY correlations were those for protons and methyl protons at axial positions. NOESY cross-peaks at the β-face were observed from H_3-25 to H_β-2, H-6, and H_β-11 and from H_3-26 to H-6, H_β-11, and H-13. Important NOESY correlations on the α-face are those from H-12 to H-9, H_3-27, and H_3-28 and from H_3-27 to H_α-7, H-12, and H_α-16. An axial orientation of H-5 was evident from its antiperiplanar relation with H-6 (J = 10.7 Hz). Because of the close chemical shifts of H-5 (δ_H 0.85) and H_α-1 (δ_H 0.86), the NOESY correlations

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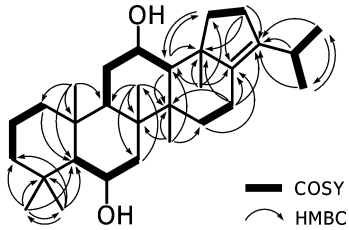


Figure 1. COSY and HMBC correlations for **1**.

from these protons could not be distinguished. However, intense cross-peaks were observed from one or both of these protons to δ_H 1.18 (H_α-3), 1.44 (H-9), and 1.36 (H_α-7). On the basis of these data, **1** was assigned as a new hopane-type triterpene, 17(21)-hopene-6α,12β-diol (Table 1).

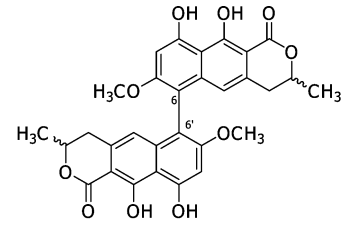
Compound **2** possessed the molecular formula $C_{30}H_{50}O$ (HRESIMS), containing one less oxygen atom than **1**. The ^1H and ^{13}C NMR spectroscopic data were similar to those of **1**. Significant differences were the absence of the ^1H and ^{13}C resonances of an oxymethine group (CH-6 of **1**) instead of the presence of one more aliphatic methylene group, which were indicated on the basis of the ^{13}C NMR, DEPT, and HMQC spectroscopic data. The axial proton H-5 (δ_H 0.72, dd, J = 11.7, 2.2 Hz) displayed intense and weak COSY correlations, respectively, to the methylene protons at δ_H 1.34 (m, H_{ax}-6) and 1.53 (m, H_{eq}-6). Other significant chemical shift differences, when compared with **1**, were the upfield shifts of C-5 (δ_C 56.5; **1**, 61.2), C-7 (δ_C 33.3; **1**, 45.7), and H_3-23 (δ_H 0.84; **1**, 1.16). Therefore, compound **2** was assigned as the 6-deoxy analogue of **1**.

The molecular formula of compound **3** was determined by HRESIMS as $C_{30}H_{50}O$, which was the same as **2**. The comparison of the ^1H and ^{13}C NMR spectroscopic data with those of **1** suggested that **3** was the 12-deoxy analogue **1**. This conclusion was confirmed by the assignments of protons and carbons by analysis of the COSY, HMBC, and NOESY correlations. The notable chemical shift differences, when compared with **1**, were the upfield shifts of C-11 (δ_C 21.4; **1**, 32.0), C-13 (δ_C 48.9; **1**, 54.5), and H_α-19 (δ_H 1.64; **1**, 1.95). The latter data can be explained by the deshielding of this proton by the 12$_\beta$-OH group in **1**, which was absent in **3**. Therefore, compound **3** was assigned as 17(21)-hopen-6α-ol. This compound was previously reported as the dehydration product from zeorin (hopane-6α,22-diol) by heating in HCl/EtOH and was named zeorinin.[14,15] In the present study, **3** was isolated as a secondary metabolite of the fungus BCC 11964. The positive specific rotation ($[\alpha]^{26}_D$ +58, *c* 0.1, $CHCl_3$) was consistent with the literature data for the zeorin-derived sample ($[\alpha]_D$ +59, *c* 0.5, $CHCl_3$).[15]

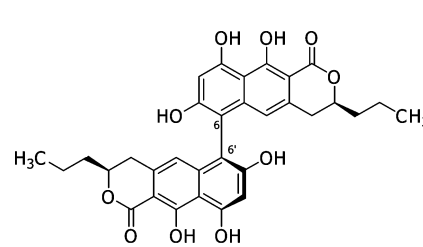
Aschernaphthopyrone A (**4**) was isolated as a greenish powder. The molecular formula of **4** was estimated to be $C_{28}H_{22}O_{10}$ by HRESIMS. The presence of only 14 carbon resonances in the ^{13}C NMR spectrum indicated a symmetric, homodimeric structure. Analysis of the ^1H and ^{13}C NMR, DEPT, and HMQC data revealed that one-half of the molecule, $C_{14}H_{11}O_5$, possessed an ester carbonyl (δ_C 171.3), eight sp^2 quaternary carbons at δ_C 163.0, 159.5, 157.9, 140.3, 133.9, 108.3, 107.8, and 99.0, two sp^2 methines at δ_C 113.5 (δ_H 6.20, s) and 102.3 (δ_H 6.60, s), an oxymethine at δ_C 76.6 (δ_H 4.68, m), a methylene at δ_C 34.4 (δ_H 2.80 and 2.67), and a methyl group at δ_C 20.7 (δ_H 1.32, d, J = 6.3 Hz). Also observed were proton signals of three phenolic OH groups at δ_H 13.47 (br s), 9.81 (br s), and 9.78 (s) (Table 2). COSY correlations revealed that the oxymethine (C-3) was connected to a methyl group (C-11) and a methylene (C-4). The 3,4-dihydro-α-naphthopyrone structure was deduced on the basis of the HMBC correlations. The diastereotopic methylene protons (H_2-4) showed HMBC correlations to the sp^2 methine (δ_C 113.5, C-5) and the quaternary carbons C-4a (δ_C 133.9) and C-10a (δ_C 99.0). The aromatic H-5 (δ_H 6.20) showed HMBC correlations to C-4, C-4a, C-5a, C-6, C-9a, and C-10a. In addition, weak $^4J_{CH}$ correlations from this proton to three oxygenated sp^2 quaternary carbons at δ_C 163.0 (C-10), 159.5 (C-7), and 157.9 (C-9) and a carbonyl carbon at δ_C 171.3 (C-1) were observed. The aromatic H-8 (δ_H 6.60) exhibited intense HMBC correlations to C-6, C-7, C-9, and C-9a. A weak cross-peak to C-10 was also observed. One of the phenolic OH groups at δ_H 9.78 showed HMBC correlations to C-6, C-7, and C-8. These data required dimer formation involving C-6 and C-6′. The upfield shift of H-5 (δ_H 6.20) was consistent with the shielding by the attached aromatic ring.

Aschernaphthopyrone B (**5**) possessed the same molecular formula ($C_{28}H_{22}O_{10}$) as **4**. The ^1H and ^{13}C NMR spectroscopic patterns were similar to those of **4**. The aromatic H-5 (δ_H 6.95, s) was significantly downfield shifted when compared to **4**. This proton showed HMBC correlation to the other sp^2 methine at δ_C 102.0 (δ_H 6.69, s, H-6) as well as to C-4, C-5a, C-9a, and C-10a, but it lacked the correlation to the δ_C 108.8 quaternary carbon. In addition, an intense NOESY correlation was observed between the aromatic protons H-5 and H-6. These data indicated that the δ_H 6.69 (δ_C 102.0) methine should be placed at CH-6. HMBC correlations were observed from H-6 to C-5, C-5a, C-7, C-9a, and the quaternary carbon at δ_C 108.6. Therefore, this upfield quaternary carbon was assigned as C-8, i.e., the location of symmetric dimer formation.

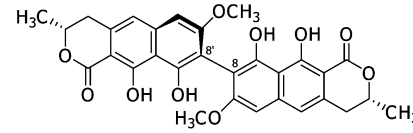
The structure of aschernaphthopyrone A (**4**), a C-6−C-6′ 3,4-dihydro-α-naphthopyrone dimer, is most closely related to pigmentosin A, which possesses a 7,7′-dimethoxy functionality and was isolated from the lichen *Hypotrachyna immaculate*.[16] The chirality of the stereogenic centers C-3/C-3′ and the 6,6′-axis of pigmentosin A has not been determined. Talaroderxines A and B, isolated from the fungus *Talaromyces derxii* NHL 2982, are substituted at C-3/C-3′ with a propyl group.[17] They are atropisomers, and both possess the 3*S*,3′*S* configuration.[17] Viriditoxin also shows structural resemblance to these compounds.[18] Aschernaphthopyrone B (**5**) is closely related to vioxanthin,[19,20] which possesses a 7,7′-dimethoxy functionality. Recently, Müller and coworkers reported the synthesis of vioxanthin and its atropisomer and determined the absolute configuration.[21]



pigmentosin A



talaroderxine B



vioxanthin

The chirality of the 6,6′-axis of aschernaphthopyrone A (**4**) was assigned by the exciton chirality method.[22] The CD spectrum of **4**

Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data for Compounds **1**−**3** in $CDCl_3$

	1		**2**		**3**	
position	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)
1	40.4, CH_2	α 0.86, m; β 1.67, m	40.3, CH_2	α 0.82, m; β 1.68, m	40.5, CH_2	α 0.82, m; β 1.65, m
2	18.5, CH_2	α 1.38, m; β 1.58, m	18.66,[b] CH_2	α 1.39, m; β 1.58, m	18.5, CH_2	α 1.39, m; β 1.56, m
3	43.7, CH_2	α 1.18, m β 1.31, m	42.1, CH_2	α 1.13, dt (4.1, 13.0) β 1.36, m	43.8, CH_2	α 1.19, dt (4.1, 13.7) β 1.35, m
4	33.7, qC		33.26, qC		33.7, qC	
5	61.4, CH	0.85, d (10.7)	56.5, CH	0.72, dd (11.7, 2.2)	61.2, CH	0.84, d (10.6)
6	69.3, CH	3.98, dt (3.9, 10.7)	18.74,[b] CH_2	α 1.53, m; β 1.34, m	69.3, CH	3.95, dt (4.0, 10.6)
7	45.5,[a] CH_2	α 1.36, m; β 1.59, m	33.29, CH_2	α 1.33, m; β 1.28, m	45.7, CH_2	α 1.45, dd (11.9, 10.6) β 1.59, dd (11.9, 4.0)
8	42.4, qC		42.9, qC		41.5, qC	
9	49.1, CH	1.44, m	49.6, CH	1.41, m	50.4, CH	1.32, m
10	39.2, qC		37.4, qC		39.4, qC	
11	32.0, CH_2	α 1.90, ddd (12.5, 5.4, 3.0) β 1.27,[c] m	32.3, CH_2	α 1.89, ddd (12.2, 5.4, 3.0) β 1.30, m	21.4, CH_2	α 1.30, m β 1.57, m
12	70.8, CH	3.86, dt (5.4, 10.9)	71.0, CH	3.85, dt (5.3, 10.9)	23.9, CH_2	α 1.39, m; β 1.34, m
13	54.5, CH	1.47, d (11.1)	54.9, CH	1.47, d (11.1)	48.9, CH	1.41, m
14	43.0, qC		42.0, qC		43.1, qC	
15	32.4, CH_2	α 1.41, m; β 1.27,[c] m	32.0, CH_2	α 1.30, m; β 1.27, m	31.8, CH_2	α 1.31, m; β 1.28, m
16	19.8, CH_2	α 1.93, m β 2.31, dt (14.5, 3.3)	19.8, CH_2	α 1.92, m β 2.28, dt (15.3, 3.5)	19.8, CH_2	α 1.92, m β 2.28, ddd (14.4, 4.0, 2.9)
17	139.3, qC		139.6, qC		139.8, qC	
18	48.7, qC		48.8, qC		49.7, qC	
19	45.4,[a] CH_2	α 1.95, m; β 1.56, m	45.4, CH_2	α 1.95, m; β 1.55, m	41.6, CH_2	α 1.64, m; β 1.30, m
20	28.1, CH_2	α 2.22, m β 2.11, br dd (15.6, 9.1)	27.5, CH_2	α 2.19, m β 2.11, dd (15.4, 9.2)	28.1, CH_2	α 2.23, m β 2.11, br dd (15.4, 9.3)
21	137.4, qC		137.1, qC		136.3, qC	
22	26.4, CH	2.66, m	26.4, CH	2.65, m	26.4, CH	2.64, m
23	36.7, CH_3	1.16, s	33.4, CH_3	0.84,[e] s	36.7, CH_3	1.16, s
24	22.1, CH_3	1.01,[d] s	21.5, CH_3	0.79, s	22.1, CH_3	1.01, s
25	17.5, CH_3	0.90, s	16.1, CH_3	0.84,[e] s	17.5, CH_3	0.87, s
26	17.9, CH_3	1.03, s	16.4, CH_3	0.95, s	17.8, CH_3	1.00, s
27	15.9, CH_3	1.07, s	15.9, CH_3	1.05, s	15.0, CH_3	1.06, s
28	19.3, CH_3	1.01,[d] s	19.3, CH_3	1.02, s	19.0, CH_3	0.83, s
29	21.8, CH_3	0.98, d (6.8)	21.8, CH_3	0.98, d (6.9)	21.9, CH_3	0.97, d (6.9)
30	21.3, CH_3	0.91, d (6.8)	21.2, CH_3	0.91, d (6.8)	21.3, CH_3	0.91, d (6.9)

[a,b] Carbon assignment may be interchanged. [c−e] Proton signals are superimposed.

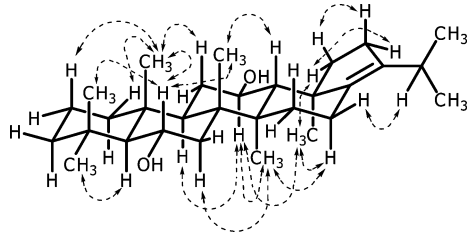


Figure 2. Key NOESY correlations for **1**.

exhibited a strong negative first Cotton effect at 272 nm ($\Delta\varepsilon$ −140.1) and positive second Cotton effect at 252 nm ($\Delta\varepsilon$ +109.0), which indicated the helicity of the chiral axis as *aR*. The CD data were similar to that of talaroderxin B and viriditoxin and were opposite those of talaroderxin A.[17,18] The axial configuration of **5** was assigned as *aR* by comparison of the CD data with those established for synthetic vioxanthin isomers.[21] Thus, the CD spectrum of **5** exhibited a negative first Cotton effect at 272 nm ($\Delta\varepsilon$ −59.5) and a positive second Cotton effect at 252 nm ($\Delta\varepsilon$ +53.5), which was opposite that of the natural vioxanthin, but the same sense as the atropisomer. The absolute configuration of the stereogenic center C-3/C-3′ of **4** and **5** remains unassigned. The symmetry of these molecules indicates that they should possess either a 3*S*,3*S*′ or 3*R*,3*R*′ configuration.

Compound **1** exhibited activity against the malarial parasite *Plasmodium falciparum* K1 with an IC_{50} value of 15 μM, but it was not cytotoxic to NCI-H187 cells (human small-cell lung cancer), MCF-7 cells (human breast cancer), KB cells (oral human carcinoma), and noncancerous Vero cells (African green monkey kidney fibroblasts) at 113 μM (50 μg/mL) (Table 3). Compounds **2** and **3** were inactive in these assays. The 6,6′-binaphthopyrone **4** exhibited moderate antimalarial activity and cytotoxicity against cancer cell lines, but it was not cytotoxic to Vero cells. The 8,8′-dimer **5** showed much weaker activities in these assays. All compounds (**1**−**5**) were inactive in an antituberculosis assay.

Experimental Section

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a GBS Cintra 404 spectrophotometer. CD spectra were recorded on a JASCO J-180 spectropolarimeter. FTIR spectra were taken on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers using the signals of the residual solvent as internal references (δ_H 7.26/δ_C 77.0 for $CDCl_3$ and δ_H 2.50/δ_C 40.0 for DMSO-d_6). ESITOF mass spectra were measured with Micromass LCT and Bruker micrOTOF mass spectrometers.

Fungal Material. *Aschersonia paraphysata* was isolated on January 10, 2001, from a scale insect (Hemiptera) on the underside of a dicotyledonous leaf in Khao Sok National Park, Surat Thani Province, Thailand, by one of the authors (A.L.). The living culture was deposited in the BIOTEC Culture Collection as BCC 11964 on March 9, 2001. The identification of the fungus was based on morphology and sequence data of the ITS rDNA.[7]

Fermentation and Isolation. The fungus BCC 11964 was maintained on potato dextrose agar at 25 °C. The agar was cut into small plugs and inoculated into 2 × 250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB; potato starch 4.0 g, dextrose 20.0 g, per liter). After incubation at 25 °C for 3 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated at 25 °C for 3 days on a rotary shaker (200 rpm). These secondary cultures were transferred into 20 × 1 L Erlenmeyer flasks containing 250 mL of M102 medium (sucrose 30 g, malt extract 20 g,

Table 2. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data for **4** and **5** in DMSO-d_6

	aschernaphthopyrone A (**4**)		aschernaphthopyrone B (**5**)	
position	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)
1, 1′	171.3, qC		171.6, qC	
3, 3′	76.6, CH	4.68, m	76.9, CH	4.81, m
4, 4′	34.4, CH_2	2.80, dd (16.2, 2.9)	34.1, CH_2	3.09, dd (16.1, 2.4)
		2.67, dd (16.2, 11.2)		2.94, dd (16.1, 10.8)
4a, 4a′	133.9, qC		133.6, qC	
5, 5′	113.5, CH	6.20, s	115.4, CH	6.95, s
5a, 5a′	140.3, qC		139.7, qC	
6, 6′	108.3, qC		102.0, CH	6.69, s
7, 7′	159.5, qC		160.5, qC	
8, 8′	102.3, CH	6.60, s	108.6, qC	
9, 9′	157.9, qC		155.9, qC	
9a, 9a′	107.8, qC		107.0, qC	
10, 10′	163.0, qC		162.4, qC	
10a, 10a′	99.0, qC		98.7, qC	
11, 11′	20.7, CH_3	1.32, d (6.3)	20.7, CH_3	1.43, d (6.2)
7-O*H*, 7′-O*H*		9.78, s		10.11, s
9-O*H*, 9′-O*H*		9.81, br s		9.44, br s
10-O*H*, 10′-O*H*		13.47, br s		13.72, br s

Table 3. Antimalarial and Cytotoxic Activities of Compounds **1**−**5**

	antimalaria (IC_{50}, μM)	cytotoxicity (IC_{50}, μM)			
compound	*P. falciparum* K1	NCI-H187	MCF-7	KB	Vero
1	15	>113	>113	>113	>113
2	>23	>118	>118	>118	>118
3	>23	>118	>118	>118	>118
4	7.3	4.5	87	17	>96
5	>19	95	79	>96	52
dihydroartemisinin[a]	0.0046	−[c]	−[c]	−[c]	−[c]
doxorubicin hydrochloride[b]	−[c]	0.086	1.5	0.28	−[c]
ellipticine[b]	−[c]	1.5	−[c]	2.5	3.8

[a] Standard antimalarial drug. [b] Reference compounds for the cytotoxicity assay. [c] Not tested.

Bacto-peptone 2.0 g, yeast extract 1.0 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 0.5 g, per liter), and final fermentation was carried out at 25 °C for 23 days on rotary shakers (200 rpm). The culture was filtered to separate broth (filtrate) and mycelia (residue). The filtrate was extracted with EtOAc (3 × 3.5 L) to give a dark brown gum (1.40 g, extract A). The wet mycelia were macerated in MeOH (2 × 1.5 L, rt, 2 days). To the MeOH solution were added hexanes (2 L) and H_2O (200 mL), and the layers were separated. The hexane layer was concentrated, under reduced pressure, leaving a pale yellow solid (1.50 g, extract B). The aqueous MeOH layer was partially concentrated by evaporation, and the residue was extracted with EtOAc. The EtOAc solution was washed with H_2O and concentrated under reduced pressure to obtain a brown gum (2.08 g, extract C). Extracts A, B, and C were separately subjected to chromatographic fractionation. Extract A was fractionated by column chromatography (CC) on silica gel (3.0 × 15 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 15:85) to obtain 13 pooled fractions: fraction A-1 (86 mg), A-2 (62 mg), A-3 (10 mg), A-4 (53 mg), A-5 (106 mg), A-6 (148 mg), A-7 (88 mg), A-8 (60 mg), A-9 (68 mg), A-10 (155 mg), A-11 (180 mg), A-12 (142 mg), and A-13 (54 mg). Fraction A-1 was subjected to CC on silica gel (CH_2Cl_2/hexanes, step gradient elution from 0:100 to 20:80) to furnish **2** (27 mg) and **3** (53 mg). Fraction A-2 was identified as **1** (62 mg). Fractions A-4−A-9 were combined and triturated in MeOH and then filtered. Compound **5** (222 mg) was furnished as the residual solid. The filtrate was subjected to CC on Sephadex LH-20 (MeOH) to afford **4** (110 mg). Fraction A-10 was triturated in MeOH to obtain **5** (35 mg) as the insoluble solid. The filtrate was purified by CC on Sephadex LH-20 (MeOH) to afford 3,4,6-trihydroxymellein (76 mg). Fractions A-11 and A-12 also mainly contained **5**, which was purified as the insoluble residue (230 mg) by trituration in MeOH. Extract B was fractionated by CC on silica gel (3.0 × 15 cm, CH_2Cl_2/hexanes, step gradient elution from 2:98 to 50:50) to obtain 13 pooled fractions (fraction B-1−B-13). Fraction B-3 (70 mg) was purified by CC on silica gel (CH_2Cl_2/hexanes, step gradient elution from 0:100 to 20:80) to furnish **3** (37 mg). Fractions B-4 (61 mg) and B-5 (202 mg) were mixtures of **2** and **3**, which were separated by repeating the CC on silica gel (CH_2Cl_2/hexanes, step gradient elution from 0:100 to 20:80) to furnish pure compounds **2** (155 mg) and **3** (36 mg). Fractions B-6 (67 mg) and B-7 (41 mg) contained **3**, which was further purified (34 mg) by CC on silica gel. Fraction B-10 was identified as **1** (362 mg). Fractions B-11 (166 mg) and B-12 (23 mg) mainly contained ergosterol. Extract C was fractionated by CC on Sephadex LH-20 (3.8 × 50 cm, MeOH) to obtain 12 pooled fractions. Each fraction was further fractionated by CC on silica gel (MeOH/CH_2Cl_2) to furnish pure compounds **1** (196 mg), **4** (441 mg), **5** (9 mg), and 6,8-dihydroxy-3-hydroxymethylisocoumarin (31 mg).

17(21)-Hopene-6α,12*β*-diol (1): colorless solid; $[\alpha]^{25}_D$ +42 (*c* 0.115, $CHCl_3$); UV (MeOH) end absorption; IR (KBr) ν_{max} 3423, 2956, 1462 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 465.3706 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_2Na$ 465.3703).

17(21)-Hopen-12*β*-ol (2): colorless solid; $[\alpha]^{25}_D$ −44 (*c* 0.10, $CHCl_3$); UV (MeOH) end absorption; IR (KBr) ν_{max} 3318, 2947, 2923, 1460 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 449.3751 $[M + Na]^+$ (calcd for $C_{30}H_{50}ONa$ 449.3754).

17(21)-Hopen-6α-ol (zeorinin, 3): colorless solid; $[\alpha]^{26}_D$ +58 (*c* 0.105, $CHCl_3$); UV (MeOH) end absorption; IR (KBr) ν_{max} 3481, 2953, 2932, 1459 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 449.3751 $[M + Na]^+$ (calcd for $C_{30}H_{50}ONa$ 449.3754).

Aschernaphthopyrone A (4): green powder; $[\alpha]^{26}_D$ −147 (*c* 0.165, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.27), 266 (4.66), 378 (4.15) nm; CD (MeOH) $\Delta\varepsilon$ 272 (−140.1), 252 (+109.0) nm; IR (KBr) ν_{max} 3406, 1636, 1589 cm^{-1};^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 2; HRMS (ESI-TOF) *m*/*z* 519.1280 $[M + H]^+$ (calcd for $C_{28}H_{23}O_{10}$, 519.1286).

Aschernaphthopyrone B (5): green powder; $[\alpha]^{27}_D$ −123 (*c* 0.10, dioxane); UV (MeCN) λ_{max} (log ε) 223 (4.11), 267 (4.57), 368 (4.01), 380 (4.01) nm; CD (MeCN) $\Delta\varepsilon$ 272 (−59.5), 252 (+53.5) nm; IR (KBr) ν_{max} 3475, 3373, 1635, 1583 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 2; HRMS (ESI-TOF) *m*/*z* 519.1287 $[M + H]^+$ (calcd for $C_{28}H_{23}O_{10}$ 519.1286).

Acetylation of 1. Compound **1** (3.0 mg) was treated with Ac_2O (0.15 mL) in pyridine (0.3 mL) at room temperature for 15 h. The mixture was diluted with EtOAc and washed with H_2O. The EtOAc layer was concentrated under reduced pressure, and the residue was purified by CC on silica gel (MeOH/CH_2Cl_2) to afford the diacetate **6** (2.8 mg) as a colorless solid: mp 173−175 °C; IR (KBr) ν_{max} 3442, 2956, 2931, 1734, 1237 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) selected resonances, δ 5.25 (1H, dt, J = 3.8, 10.9 Hz, H-6), 5.08 (1H, dt, J = 5.6, 11.2 Hz, H-12), 2.06 (3H, s, −OCOC*H*$_3$), 2.07 (3H, s, −OCOC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5 (−O*C*OCH$_3$), 170.2 (−O*C*OCH$_3$), 138.7, 137.7, 73.3, 71.7, 58.6, 51.2, 48.6, 48.5, 44.6, 43.3, 43.2, 42.2, 40.7, 40.1, 39.4, 36.2, 33.2, 31.8, 28.0, 27.9, 26.4, 22.1, 22.0 (−OCO*C*H$_3$), 21.9 (−OCO*C*H$_3$), 21.7, 21.2, 19.6, 19.3, 18.2, 17.5, 17.3, 15.7; HRMS (ESI-TOF) *m*/*z* 549.3920 $[M + Na]^+$ (calcd for $C_{34}H_{54}O_4Na$, 549.3920).

Biological Assays. Each sample was dissolved in DMSO to prepare a stock solution (10 mg/mL), which was diluted with H_2O and subjected to biological assays. The assay for activity against *Plasmodium falciparum* (K1, multi-drug-resistant strain) was performed using the microculture radioisotope technique.[23] The anticancer activities against NCI-H187 cells (human small-cell lung cancer), MCF-7 cells (human breast cancer), and KB cells (oral human epidermoid carcinoma) were evaluated using the resazurin microplate assay.[24] Cytotoxicity to Vero cells was performed using the green fluorescent protein microplate assay (GFPMA).[25]

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Supporting Information Available: NMR spectra of compounds **1**−**5** and CD and UV spectra of **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) van Eijk, G. W.; Roeijmans, H. J.; Seykens, D. *Tetrahedron Lett.* **1986**, *27*, 10–16.
(2) Boonphong, S.; Kittakoop, P.; Palittapongarnpim, P.; Jaturapat, A.; Danwisetkanjana, K.; Tanticharoen, M.; Thebtaranonth, Y. *Planta Med.* **2001**, *67*, 279–281.
(3) Isaka, M.; Palasarn, S.; Kocharin, K.; Saenboonrueng, J. *J. Nat. Prod.* **2005**, *68*, 945–946.
(4) Chutrakul, C.; Boonruangprapa, T.; Suvannakad, R.; Isaka, M.; Sirithunya, P.; Toojinda, T.; Kirtikara, K. *J. Appl. Microbiol.* **2009**, *107*, 1624–1631.
(5) Krasnof, S. B.; Gibson, D. M.; Belofsky, G. N.; Gloer, K. B.; Gloer, J. B. *J. Nat. Prod.* **1996**, *59*, 485–489.
(6) Isaka, M.; Hywel-Jones, N. L.; Sappan, M.; Mongkolsamrit, S.; Saidaengkham, S. *Mycol. Res.* **2009**, *113*, 491–497.
(7) Mongkolsamrit, S.; Luangsa-ard, J. J.; Spatafora, J. W.; Sung, G.-H.; Hywel-Jones, N. L. *Mycol. Res.* **2009**, *113*, 684–699.
(8) Claydon, N.; Grove, J. F.; Hosken, M. *Phytochemistry* **1974**, *13*, 2567–2571.
(9) Ayer, W. A.; Attah-Poku, S. K.; Browne, L. M.; Orszanska, H. *Can. J. Chem.* **1987**, *65*, 765–769.
(10) Cui, C.-B.; Ubukata, M.; Kakeya, H.; Onose, R.; Okada, G.; Takahashi, I.; Isono, K.; Osada, H. *J. Antibiot.* **1996**, *49*, 216–219.
(11) McGraw, G. W.; Hemingway, R. W. *Phytochemistry* **1977**, *16*, 1315–1316.
(12) Ayer, W. A.; Browne, L. M.; Feng, M. C.; Orszanska, H.; Saeedi-Ghomi, H. *Can. J. Chem.* **1986**, *64*, 904–909.
(13) Gremaud, G.; Tabacchi, R. *Nat. Prod. Lett.* **1994**, *5*, 95–103.
(14) Asahina, Y.; Yoshioka, I. *Chem. Ber.* **1940**, *73B*, 742–747.
(15) Barton, D. H. R.; Bruun, T. *J. Chem. Soc.* **1952**, 1683–1690.
(16) Elix, J. A.; Wardlaw, J. H. *Aust. J. Chem.* **2004**, *57*, 681–683.
(17) Suzuki, K.; Nozawa, K.; Nakajima, S.; Udagawa, S.; Kawai, K. *Chem. Pharm. Bull.* **1992**, *40*, 1116–1119.
(18) Suzuki, K.; Nozawa, K.; Nakajima, S.; Kawai, K. *Chem. Pharm. Bull.* **1990**, *38*, 3180–3181.
(19) Blank, F.; Ng, A. S.; Just, G. *Can. J. Chem.* **1966**, *44*, 2873–2879.
(20) Zeeck, A.; Russ, P.; Laatsch, H.; Loeffler, W.; Wehrle, H.; Zäfner, H.; Holst, H. *Chem. Ber.* **1979**, *112*, 957–978.
(21) Bode, S. E.; Drochner, D.; Müller, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 5916–5920.
(22) Cai, G.; Bozhkova, N.; Odingo, J.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1993**, *115*, 7192–7198.
(23) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
(24) O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. *Eur. J. Biochem.* **2000**, *267*, 5421–5426.
(25) Changsen, C.; Franzblau, S. G.; Palittapongarnpim, P. *Antimicrob. Agents Chemother.* **2003**, *47*, 3682–3687.

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