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Neolignans and Other Metabolites from *Ocotea cymosa* from the Madagascar Rain Forest and Their Biological Activities[1]

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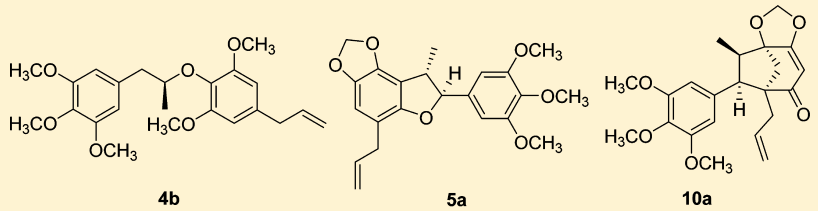
Supporting Information



ABSTRACT: Ten new neolignans including the 6′-oxo-8.1′-lignans cymosalignans A (**1a**), B (**2**), and C (**3**), an 8.O.6′-neolignan (**4a**), ococymosin (**5a**), didymochlaenone C (**6a**), and the bicyclo[3.2.1]octanoids **7**−**10** were isolated along with the known compounds 3,4,5,3′,5′-pentamethoxy-1′-allyl-8.O.4′-neolignan, 3,4,5,3′-tetramethoxy-1′-allyl-8.O.4′-neolignan, didymochlaenone B, virologin B, ocobullenone, and the unusual 2′-oxo-8.1′-lignan sibyllenone from the stems or bark of the Madagascan plant *Ocotea cymosa*. The new 8.O.6′-neolignan **4a**, dihydrobenzofuranoid **5a**, and the bicyclo[3.2.1]octanoid **7a** had in vitro activity against *Aedes aegypti*, while the new compounds **5a**, **7a**, **8**, and **10a** and the known virolongin B (**4b**) and ocobullenone (**10b**) had antiplasmodial activity. We report herein the structure elucidation of the new compounds on the basis of spectroscopic evidence, including 1D and 2D NMR spectra, electronic circular dichroism, and mass spectrometry, and the biological activities of the new and known compounds.

Ocotea (Lauraceae) is a large genus containing about 350 species distributed primarily in the tropical and warm areas of the Americas, with a few in Macronesia, seven in tropical African countries, and 34 in Madagascar.[2,3] *Ocotea cymosa* (Nees) Palacky (vernacular name: varongy) is an endemic medium-sized tree up to 25 m tall widely found throughout the eastern part of Madagascar. Its wood and the wood of other *Ocotea* species growing on the island have been used for furniture, boat building, and making mortars.[4] The leaves, bark, and fruits are aromatic and are used as a condiment or added to locally prepared alcoholic drinks. No medicinal uses of *O. cymosa* have been recorded in Madagascar's pharmacopoeia, although *O. bullata*, a species native to eastern and southern South Africa, has been used to treat headache and male urinary tract infections. Plant species from the genus *Ocotea* are rich sources of neolignans including the bicyclo[3.2.1]octanoid neolignans ocobullenone (**10b**),[5] iso-ocobullenone,[6] sibyllenone (**7b**),[7] ocophyllals A and B,[8] and virolongin-type[9] and benzofuran neolignans.[10] Various biological activities such as insecticidal, antibacterial, antitumor, and antiviral have been reported for the lignans.[11] Aporphine alkaloids[12−15] and flavonoids[16] have also been isolated from plants of this genus.

The search for bioactive compounds and chemical constituents from natural sources with agricultural value has been an ongoing project in the Dow AgroSciences group. An extract of *O. cymosa* stems was selected for investigation for its

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Table 1. ^{1}H NMR Data for Compounds 1a−6a (600 MHz, $CDCl_3$)

position	1a	2	3	4a	5a[a]	6a
2	6.35 s	6.66 d (2.0)	6.35 s	6.44 s	6.62 s	3.13 m
3a						2.86 dd (13.3, 5.0)
3b						1.89 dd (13.3, 13.3)
5		6.77 d (8.0)				
6	6.35 s	6.68 dd (8.0, 2.0)	6.35 s	6.44 s	6.62 s	5.53 s
7					5.08 d (8.4)	
7a	2.99 dd (13.0, 3.0)	2.99 dd (13.1, 2.9)	2.99 brd (13.0)	2.99 dd (13.7, 6.4)		2.69 m
7b	2.09 brt (13.0)	2.09 brt (13.1)	2.06 t (12.4)	2.77 dd (13.7, 6.4)		2.34 m
8	2.25 m	2.23 m	2.23 m	4.39 sextet (6.2)	3.49 m	5.74 m
9	0.67 d (6.6)	0.65 d (6.6)	0.65 d (6.6)	1.27 d (6.2)	1.48 d (6.8)	5.06 m
2′	5.51 s	5.52 s		6.63 s		
3′			5.65 s			6.40 s
5′	5.65 s	5.65 s		6.47 s		6.40 s
6′			5.51 s		6.52 brs	
7′a	2.73 ddt (13.2, 7.4, 1.1)	2.73 dd (13.1, 7.3)	2.74 dd (13.0, 7.2)	3.27 dt (6.6, 1.5)	3.33 ddt (15.5, 6.7, 1.5)	3.36 br dt (6.8, 1.4)
7′b	2.50 ddt (13.2, 7.4, 1.1)	2.51 dd (13.1, 7.3)	2.50 dd (13.0, 7.2)	3.27 dt (6.6, 1.5)	3.28 ddt (15.5, 6.7, 1.5)	
8′	5.58 ddt (17.3, 10.1, 7.4)	5.57 m	5.58 m	5.89 m	5.97 ddt (16.7, 10.1, 6.7)	5.97 m
9′a	5.06 dq (17.3, 1.1)	5.05 brd (17.0)	5.05 brd (17.0)	5.01 m	5.04 dq (10.1, 1.5)	5.13 m
9′b	4.98 ddt (10.1, 2.0, 1.1)	4.98 brd (10.2)	4.98 d (10.1)	5.01 m	5.09 dq (16.7, 1.5)	5.13 m
2′,6′-OCH_3						3.79 s
3-OCH_3	3.83 s	3.87 s	3.87 s	3.83 s	3.86 s	
4-OCH_3	3.82 s	3.85 s		3.82 s	3.84 s	
5-OCH_3	3.83 s		3.87 s	3.83 s	3.86 s	
4-OH			5.37 s			
3′-OCH_3						
5′-OCH_3						
O-CH_2-O	5.84 d (1.5)	5.84 s	5.84 s	5.88 d (1.5)	5.87 d (1.5)	5.62 s; 5.48 s
O-CH_2-O				5.87 d (1.5)	5.91 d (1.5)	

[a]Spectrum obtained at 500 MHz.

Table 2. ^{13}C NMR Data for Compounds 1a−6a (151 MHz, in $CDCl_3$)

carbon	1a	1b[a]	2	3	4a	5a	6a
1	136.2	132.3	133.0	131.5	134.1	136.5	199.9
2	106.2	111.4	112.2	105.7	106.5	102.9	41.6
3	153.0	146.4	148.8	146.9	153.01	153.4	36.7
4	136.3	143.9	147.3	133.0	136.6	137.8	105.2
5	153.0	114.0	111.1	146.9	153.01	153.4	169.0
6	106.2	122.0	121.2	105.7	106.5	102.9	101.3
7	37.5	36.8	36.7	37.3	43.4	92.5	34.7
8	44.7	45.0	44.9	44.9	76.4	44.6	135.8
9	14.4	14.2	14.3	14.3	19.7	17.5	117.1
1′	57.1	57.2	57.2	57.2	122.3	113.0	138.1
2′	106.1	106.2	106.2	106.2	109.5	152.7	154.3
3′	145.1	145.1	145.1	145.2	141.3	112.7	105.4
4′	163.8	163.7	163.8	163.7	146.2	141.7	129.4
5′	100.4	100.3	100.4	100.4	97.7	142.4	105.4
6′	202.8	202.0	202.9	203.1	149.8	107.7	154.3
7′	43.4	43.3	43.4	43.6	34.2	33.6	40.5
8′	132.9	133.0	133.0	133.0	137.3	136.8	136.8
9′	118.1	118.1	118.1	118.1	115.3	115.4	116.4
3-OCH_3	56.1	55.9	55.90	56.2	56.1	56.2	
4-OCH_3	60.9		55.88		60.9	60.9	
5-OCH_3	56.1			56.2	56.1	56.2	
2′-OCH_3							55.9
6′-OCH_3							55.9
OCH_2O	101.4	101.3	101.4	101.4	101.0	101.2	99.9

[a]Data from ref 11.

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bioactivity as an insecticidal and antifungal agent in the Dow AgroSciences screens.

The eradication of malaria still remains one of the world's most important medical goals. In 2010, over three billion people were at risk of malaria. Ninety percent of all malaria-related deaths occurred in sub-Saharan Africa, mainly among children under five years of age.[17] Recently, the Virginia Tech group reported the isolation and structure elucidation of the two phloroglucinols mallotojaponins C and D, with potent activity against both blood stage malaria and gametocytes.[18] In a continuation of this search for antimalarial compounds from Madagascan plants, an extract of *O. cymosa* bark was selected for investigation based on its activity against drug-resistant *Plasmodium falciparum* (Dd2).[19]

Bioguided isolation of ethanol extracts of *O. cymosa* using both antimalarial and insecticidal screens led to the isolation of 10 new metabolites and six known compounds.

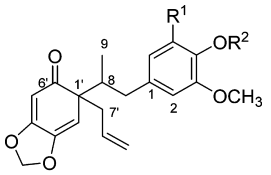
RESULTS AND DISCUSSION

Isolation and Structure Elucidation. Normal-phase chromatography followed by HPLC of the crude ethanol extract of *O. cymosa* stems yielded compounds **1**−**4a**, **6a**, **7a**, and **8**−**10a**. Similar treatment or direct HPLC of the active antiplasmodial hexanes fraction (IC_{50} 1.25 μg/mL) obtained from a liquid−liquid partitioning of the ethanol extract of *O. cymosa* bark yielded the new compounds **5a**, **7a**, **8**, and **10a** and the known virolongin B (**4b**) and ocobullenone (**10b**)[5] as the active antimalarial compounds. The known compounds 3,4,3′,5′-tetramethoxy-8.O.4′-neolignan,[20] 2,3,4,3′,5′-pentamethoxy-8.O.4′-neolignan,[21] didymochlaenone B,[22] virolongin B,[21] and the 6′-oxo-8.1′-lignan sibyllenone[7] were also isolated.

Compound **1a** was isolated as an oil. It had the molecular formula $C_{22}H_{26}O_6$ based on its ^{13}C NMR and HREISMS data, indicating 10 indices of hydrogen deficiency. Its ^1H NMR spectrum in $CDCl_3$ (Table 1) indicated signals of a substituted propyl group including a methyl doublet at δ_H 0.67 (d, J = 6.6 Hz, H-9) bonded to the only aliphatic methine in the molecule at δ_H 2.25 (m) and a methylene unit at δ_H 2.09 (brt, J = 13.0, H-7b) and 2.99 (dd, J = 13.0, 3.0 Hz, H-7a), which coupled with the C-8 methine. This portion of the molecule was confirmed by a COSY experiment. Furthermore, a 2-propenyl group was identified by vinyl resonances at δ_H 5.58 (ddt, J = 17.3, 10.1, 7.4 Hz, H-8′), 5.06 (dq, J = 17.3, 1.1 Hz, H-9′a), and 4.98 (ddt, J = 10.1, 2.0, 1.1 Hz, H-9′b) and aliphatic resonances at δ_H 2.73 (ddt, J = 13.2, 7.4, 1.1 Hz, H-7′a) and 2.50 (ddt, J = 13.2, 7.4, 1.1 Hz, H-7′b). Signals attributed to a methylenedioxy group at δ_H 5.84 (d, J = 1.5 Hz, 2H) and two sp^2 methines at δ_H 5.65 (s, H-5′) and 5.51 (s, H-2′) were also visible. Two singlet resonances of three methoxy groups at δ_H 3.83 (s, 6H) and 3.82 (s, 3H) were observed.

The ^{13}C NMR spectrum of **1a** (Table 2) displayed only 19 signals, and the presence of three pairs of chemically equivalent carbons (C-2 and C-6, C-3 and C-5, and 3-OCH_3 and 5-OCH_3) was readily explained by the presence of a 3,4,5-trimethoxybenzene moiety and confirmed by an HSQC spectrum, which indicated that the two-proton aromatic resonance at δ_H 6.35 (H-2, H-6) was connected to the carbon at δ_C 106.2.

The HMBC spectrum indicated correlations from H-2, H-6, 3-OMe, and 5-OMe to the carbons at δ_C 153.0 (C-3 and C-5). In addition, cross-peaks were observed from H-2, H-6, and 4-OMe to C-4 (δ_C 136.3). The substituted propyl group was determined to be attached to the trimethoxybenzene ring by the long-range correlation of H-2 and H-6 to the methylene carbon at δ_C 37.5 (C-7).



1a $R^1 = OCH_3$, $R^2 = CH_3$
1b $R^1 = R^2 = H$
2 $R^1 = H$, $R^2 = CH_3$
3 $R^1 = OCH_3$, $R^2 = H$

Comparison of the ^{13}C NMR data of **1a** (Table 2) with those of 4-hydroxy-3-methoxy-3′,4′-methylenedioxy-6′-oxo-Δ-$^{2',4',8'}$-8.1′-neolignan (**1b**)[23] indicated that they had identical cyclohexadienone rings. This conclusion was confirmed by analysis of the HMBC spectrum of **1a**, which indicated cross-peaks from the methylenedioxy signal at δ_H 5.84 and the singlets at δ_H 5.65 (H-5′) and 5.51 (H-2′) to C-4′ (δ_C 163.8) and C-3′ (145.1). Furthermore, cross-peaks were observed from H-5′, H-2′, and H-7′ to a quaternary carbon at δ_C 57.1 (C-1′). Additionally, H-5′, H-2′, and H-7′ displayed HMBC correlations to a ketocarbonyl at δ_C 202.8 (C-6′). Compound **1a** was thus identified as 3,4,5-trimethoxy-3′,4′-methylenedioxy-6′-oxo-Δ-$^{2',4',8'}$-8.1′-neolignan and is named cymosalignan A.

The relative and absolute configurations of **1a** were not assigned.

Cymosalignan B (**2**) was also obtained as an oil, and its ^1H NMR spectrum was similar to that of **1a**. An overlay of both spectra indicated that compound **2** contained the same features as **1a** such as the 2-propenyl, the substituted propyl, and the methylenedioxy groups. The only difference was the presence of two methoxy groups in **2** instead of three as in compound **1a**, and the aromatic region indicated an AMX spin system characteristic of a 1,3,4-trisubstituted benzene ring, suggesting that the 5-OMe group present at δ_H 3.83 in **1a** was missing in **2**. The mass spectrum of **2** showed a protonated molecular ion at m/z 357 $[M + H]^+$, and the molecular formula was assigned as $C_{21}H_{24}O_5$ by HRMS, indicating loss of a CH_2O fragment compared to **1a**. This evidence coupled with the NMR spectroscopic data confirmed that **2** is the 5-demethoxy derivative of **1a**, i.e., 3,4-dimethoxy-3′,4′-methylenedioxy-6′-oxo-Δ-$^{2',4',8'}$-8.1′-neolignan, or cymosalignan B.

Cymosalignan C (**3**) was isolated as an oil, for which analysis based on ^{13}C NMR and HRESIMS (m/z = 373.1649 $[M + H]^+$ and 395.1471 $[M + Na]^+$) data, indicated a molecular formula of $C_{21}H_{24}O_6$. The ^1H NMR spectroscopic data of **3** (Table 1) were similar to those of compounds **1a** and **2** and included all the signals of the 2-propenyl, methylenedioxy, and substituted propyl groups. Comparison of the spectroscopic data of **3** with those of **1a** indicated that the only significant difference was the lack of a signal for the 4-methoxy group and the observation of a singlet at δ_C 3.87 integrating for six protons. The Δm of 14 Da between compounds **1a** and **3** and the HMBC correlation of 4-OH (δ_H 5.37, s) and H-2, H-6 protons to C-3 (δ_C 146.9) and C-4 (δ_C 133.0), together with cross-peaks from 4-OH and the 3-OMe protons to C-3, confirmed that compound **3** is the 4-*O*-demethyl derivative of **1a**. It was thus identified as the new neolignan 4-hydroxy-3,4-dimethoxy-3′,4′-methylenedioxy-6′-oxo-Δ-$^{2',4',8'}$,8.1′-neolignan, or cymosalignan C.

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Cymosalignans A (**1a**), B (**2**), and C (**3**) belong to the unusual 6′-oxo-8.1′ group of neolignans and are related to the lignans isolated from *Piper capense*[23] and to piperkadsin B [(7*R*,8*S*)-7-acetoxy-3,3′,4,4′-tetramethoxy-6′-oxo-Δ-$^{2',4',8'}$-8.1′-lignan] isolated from *Piper kadsura*.[24]

Compound **4a** was isolated as an oil. Its ^{1}H NMR spectrum (Table 1) indicated signals of a 1,3,4,5-tetrasubstituted benzene with a side chain bearing an oxymethine at δ_H 4.39 (1H, J = 6.2 Hz, H-8, sextet). The second part of this structure gave signals of an allyl group characterized by olefinic methines at δ_H 5.89 (m, H-8′) and 5.01 (m, H-9′ab) and an aliphatic methylene at δ_H 3.27 (dt, J = 6.6, 1.5 Hz, H-7′ab). In addition signals attributed to a methylenedioxy group at δ_H 5.88 (d, J = 1.5 Hz) and 5.87 (d, J = 1.5 Hz) and three aromatic singlets at δ_H 6.44 (2H), assignable to H-2 and H-6, at δ_H 6.63 for H-2′, and at δ_H 6.47 for H-5′ were observed. From its HMBC spectrum (Figure 1), long-range correlations were observed from the methyl-

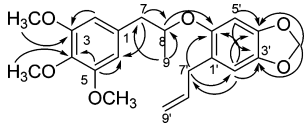
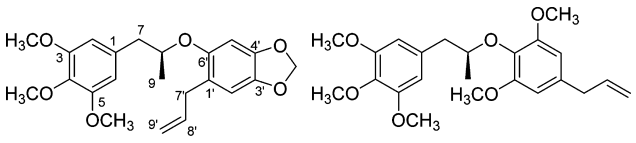


Figure 1. Selected HMBC correlations in **4a**.

enedioxy, H-2′, and H-5′ to C-4′ (δ_C 146.2) and C-3′ (δ_C 141.3). Furthermore, H-2′ (δ_H 6.63) showed a cross-peak to the methylene carbon at δ_C 34.2 (C-7′).

An important HMBC correlation from the methine at δ_H 4.39 (H-8), the methylene at δ_H 3.27 (H_2-7′), and the methine at δ_H 6.63 (H-2′) to the carbon at δ_H 149.8 (C-6′) indicated that **4a** is the new neolignan 3′,4′-methylenedioxy-3,4,5-trimethoxy-Δ$^{8'}$-8.*O*.6′-neolignan. Its configuration was tentatively assigned as *S* based on its positive optical rotation, as for synthetic (*S*)-virolongin B (**4b**), although the magnitude of the rotations differed significantly.[25,26] The isolation of (*S*)-virolongin B from both stem and bark extracts supported this stereochemical assignment.



4a **4b**

Ococymosin (**5a**) had the molecular formula $C_{22}H_{24}O_6$ as determined by ^{13}C NMR spectroscopic data and the positive ion HRESIMS. Its IR spectrum displayed absorption bands characteristic of aromatic ring double-bond methines. Its ^{1}H NMR spectrum (Table 1) exhibited two signals in the aromatic region corresponding to one A_2 (δ_H 6.62, s, 2H) and one A (δ_H 6.52, brs, 1H) spin system; the resonance of a secondary methyl at δ_H 1.48 (d, J = 6.8 Hz, H-9); a set of signals due to the protons of a primary allyl group at δ_H 3.33 (ddt, J = 15.5, 6.7, 1.5 Hz, H-7′a), 3.28 (ddt, J = 15.5, 6.7, 1.5, H-7′b), 5.97 (ddt, J = 16.7, 10.1, 6.7 Hz, H-8′), 5.04 (dq, J = 10.1, 1.5 Hz, H-9′a), and 5.09 (dq, J = 16.7, 1.5 Hz, H-9′b); a methine proton on an oxygenated carbon of a dihydrofuran ring at δ_H 5.08 (d, J = 8.4 Hz, H-7); two signals corresponding to three methoxy groups at δ_H 3.84 (s, 3H, 4-OMe) and 3.86 (s, 6H, 3- and 5-OMe); and signals for two methylenedioxy protons at δ_H 5.87 (d, J = 1.5 Hz, 1H) and 5.91 (d, J = 1.5 Hz, 1H). The ^{13}C NMR spectrum (Table 2) had 22 signals assignable to three methoxy carbons (δ_C 56.0, 56.1, and 56.1), a methylenedioxy carbon (δ_C 101.2), and 18 carbons (2 × C_6–C_3) ascribable to a dihydrobenzofuranoid neolignan skeleton.[11] The ^{1}H and ^{13}C NMR spectroscopic data of **5a** are similar to those of **5b**, a dihydrobenzofuranoid lignan isolated from *Piper capense* (Piperaceae),[11] except for the signals arising from ring A. In the ^{1}H NMR spectrum of **5a**, an A_2 spin system was observed for ring A instead of the AMX system of **5b** (δ_H 6.70, dd, J = 8.1, 1.9 Hz; 6.81, d, J = 1.9 Hz; 6.96, d, J = 8.1 Hz).[11] Comparison of the ^{13}C NMR data of **5a** with those of **5b** confirmed the presence of a 1,3,4,5-tetrasubstituted benzene ring in **5a** instead of the 1,3,4-trisubstituted benzene ring in **5b**. The attachment of the three methoxy groups at C-3, C-4, and C-5, the allyl group at C-1′, and the methylenedioxy group at C-4′ and C-5′ and the presence of a 7.O.2′-8.3′-dihydrobenzofuran ring were substantiated by interpretation of the 1D and 2D NMR spectroscopic data of **5a**, including COSY, HSQC, HMBC, and NOESY experiments. The HMBC correlations observed between the *O*-methyl protons at δ_H 3.86 and the carbons at δ_C 153.4 (C-3 and C-5), and between the *O*-methyl protons at δ_H 3.84 and the carbon at δ_C 137.8 (C-4), indicated that the three methoxy groups present in **5a** were attached to C-3, C-4, and C-5 of ring A. Furthermore, the HMBC long-range correlations (Figure 2) between the allylic methylene

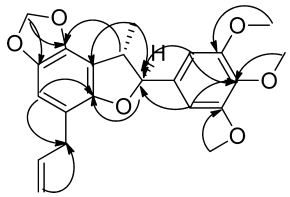


Figure 2. Selected HMBC correlations in **5a**.

protons at δ_H 5.04 and 5.09 and C-7′ (δ_C 33.6) on the one hand and between H_2-7′ protons at δ_H 3.28 and 3.33 and the C-2′ and C-6′ carbons at δ_C 152.7 and 107.7, respectively, on the other hand suggested that the allyl group was located at C-1′. The methylenedioxy group was determined to be attached at C-4′ and C-5′ due to the HMBC cross-peaks observed between the methylene protons at δ_H 5.87 and 5.91 (each a doublet, J = 1.5 Hz) and the C-4′ and C-5′ carbons at δ_C 141.7 and 142.4, respectively. The deshielding of the oxygen-bearing methine carbon (δ_C 92.5, C-7) and the long-range correlations between H-7 proton at δ_H 5.08 and C-2 and C-6 and between the secondary methyl protons at δ_H 1.48 (H_3-9) and C-2′ and C-3′ corroborated the presence of a 2-aryl-3-methyl-2,3-dihydrobenzofuran ring system.

The relative configuration of **5a** was substantiated by the NOESY data, which showed a cross-peak between H-7 and CH_3-9, indicating the *syn* relationship of these groups, and by comparison of its optical rotation with the reported data for **5b**, the structure of which has been confirmed by X-ray diffraction analysis.[11] From the above data, the structure of ococymosin (**5a**) was determined to be *rel*-(7*R*,8*R*)-Δ$^{8'}$-3,4,5-trimethoxy-4′,5′-methylenedioxy-7.O.2′-8.3′-neolignan.

Compound **6a** was isolated as a colorless oil. Its ^{13}C NMR and HRESIMS data indicated the composition $C_{21}H_{24}O_6$, indicative of 10 indices of hydrogen deficiency. The IR spectrum indicated the presence of an α,β-unsaturated carbonyl moiety (1655 cm^{-1}). The combination of the ^{1}H, ^{13}C, and HSQC data indicated the presence of a two-proton aromatic singlet at δ_H 6.40, four methines (one aliphatic and three

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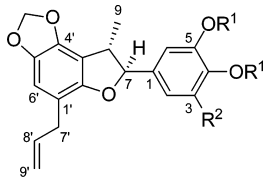


5a $R^1 = CH_3$, $R^2 = OCH_3$
5b $R^1 = -CH_2-$, $R^2 = H$

olefinic), six methylenes (one methylenedioxy, three aliphatic, and two olefinic), a six-proton singlet at δ_H 3.79 attributed to two methoxy groups and seven unprotonated carbons. The 2D NMR COSY and HMBC spectra indicated the presence of two distinct allyl groups. The first one comprised a multiplet of an olefinic methylene between δ_H 5.06 (m, H_2-9), a methine at 5.74 (H-8), and a methylene at δ_H 2.69 and 2.34 (H_2-7); it was connected to a methine (H-2) and methylene (H_2-3) to give the substructure $CH_2{=}CH{-}CH_2{-}CH{-}CH_2$. The HMBC spectrum of **6a** (Figure 3) revealed correlations from H-2, H-3,

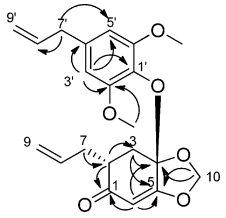
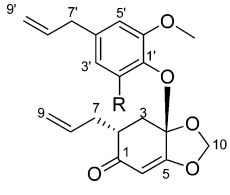


Figure 3. Selected HMBC correlations of **6a**.

H-6, and H-7 to C-1 (δ_C 199.9). In addition, H-3, H-6, and the methylenedioxy (H_2-10) protons exhibited cross-peaks to the unprotonated carbon at δ_C 105.2 (C-4) and 169.0 (C-5). Correlations of the aromatic singlet at δ_H 6.40 (H-3′, H-5′) to the C-7′ methylene carbon (δ_C 40.5) were also observed. Further correlations were seen from the H-3′ and the methoxy singlet at δ_H 3.79 to the carbons at δ_H 154.3 (C-2′, C-6′) (Figure 3).

This evidence led to the assignment of structure **6a** to the new compound. Didymochlaenone A (**6b**), a demethoxy analogue of **6a**, and didymochlaenone B were previously isolated from *Didymochlaena truncatula*, also a plant from the Madagascar rain forest.[22] The name didymochlaenone C is thus proposed for compound **6a**.



6a R = OCH_3 (didymochlaenone C)
6b R = H (didymochlaenone A)

The molecular formula of $C_{20}H_{20}O_5$ for compound **7a** was determined by a combination of ^{13}C NMR and low- and high-resolution MS data. Its ^1H (Table 3) and ^{13}C NMR data (Table 4) were similar to those of the known compound sibyllenone (**7b**)[7] with the signals of two methylenedioxy groups and an allyl group visible at δ_H 5.60 (dddd, J = 16.9, 10.3, 8.9, 5.4 Hz, 1H, H-8′), 4.87 (m, 2H, H_3-9′), 2.39 ddt (J = 13.9, 5.4, 1.5, H-7′a), and 1.30 dd (J = 13.9, 8.9 Hz, H-7′b). The ^1H and COSY data also indicated the C_3 unit was constituted by a methyl doublet at δ_H 1.06 (d, J = 6.9 Hz, 3H, H_3-9) bonded to a methine at δ_H 2.75 (quintet, J = 6.9 Hz, 1H, H-8), which further extended to the methine at δ_H 2.47 (brd, J = 6.9 Hz, 1H, H-7). The $J_{7,8}$ value of 6.9 Hz indicated that these protons had an *anti* orientation, based on comparison with the similar values for the *anti* protons of sibyllenone and iso-ocobullenone and the larger value of 11.9 Hz for the *syn* protons of ocobullenone.[7] The only difference between compounds **7a** and **7b** was the lack of a methoxy group at C-5 in **7a**, and this was confirmed by the presence of three aromatic proton signals at δ_H 6.66−6.78. The analysis of the 2D NMR data from the HSQC and HMBC spectra confirmed the structure of **7a** as a new natural product member of the bicyclo[3.2.1]octanoid neolignan family, identified as demethoxysibyllenone. The relative configuration was determined by the interpretation of the NOESY spectrum with a correlation between H-8 and H-2′. The absolute configurations of **7a**, **7b**, and the related compound **8** are shown as depicted by Zschocke et al.[7] and confirmed by analyses of their physical and spectroscopic data including the ECD spectrum of **8**. Compound **7a** is thus (7*R*,8*S*,1′*S*,3′*S*)-$\Delta^{8'}$-3,4-methylenedioxy-3′,4′-methylenedioxy-1′,2′,3′,6′-tetrahydro-6′-oxo-7.1′-8.3′-neolignan.

The molecular formula of **8** was determined to be $C_{20}H_{20}O_6$ by ^{13}C NMR and HRESIMS data. Its IR spectroscopic data were similar to those of **7a** except for the addition of a hydroxy absorption band at 3420 cm^{-1}. Its ^1H NMR spectroscopic data were also similar to those of **7a** and sibyllenone (**7b**), with signals assignable to two methylenedioxy groups, a secondary methyl group at δ_H 1.06 (d, J = 6.8 Hz), two aromatic protons (δ_H 6.75, brs and 6.76, brs, each 1H), an allyl group, and the α-proton of an α,β-unsaturated carbonyl group (δ_H 5.46, s). These observations suggested that **8** differed from sibyllenone by the replacement of the C-5 methoxy group by a hydroxy group. In confirmation, the ^{13}C NMR chemical shifts of **8** were close to those of sibyllenone,[7] except for the absence of the aromatic methoxy signal at δ_C 56.8 in **8** (Table 4). Its relative configurations at C-7, C-8, and C-1′ were confirmed by the observation of NOESY cross-peaks between CH_3-9 and H-7, between a 2′-proton and H-8, and between H-7 and H-8′. Compound **8** is thus assigned as 5-*O*-demethylsibyllenone. The assignments of all protons and carbons of **8** (Tables 3 and 4) were confirmed by HMBC and NOESY experiments (Figure 4); the coupling constants of the protons of the aromatic ring methylenedioxy groups differ slightly from those reported.[7]

The absolute configuration of **8** was assigned by analysis of its ECD spectrum. The negative Cotton effect for the carbonyl n → π^* transition (305 nm)[27] correlated with the back octant rule applied to a minimized energy (MM2) of **8**. Its structure was thus assigned as (7*R*,8*S*,1′*S*,3′*S*)-$\Delta^{8'}$-5-hydroxy-3,4-methylenedioxy-3′,4′-methylenedioxy-1′,2′,3′,6′-tetrahydro-6′-oxo-7.1′-8.3′-neolignan.

Compound **9** was obtained as a white powder. Its molecular formula was determined as $C_{22}H_{26}O_6$ on the basis of ^{13}C NMR and HRESIMS data. The ^1H NMR spectrum was similar to those of **7a** and sibyllenone (**7b**) (Table 3), and the $J_{7,8}$ value of 7.7 Hz indicated the *anti* orientation of H-7 and H-8. The ^1H NMR spectrum also indicated all signals attributed to the bicylo[3.2.1]octanoid part; the signals of H-2 and H-6 appeared as a broad singlet at δ_H 6.42 (brs, 2H) instead of two separate signals as in **7a**, indicating their symmetrical location. The major difference between the ^1H NMR spectra of **7a** and **9** was the absence in **9** of the signals due to the aryl methylenedioxy groups present in **7a** and **7b** and the presence of signals for

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Table 3. ^{1}H NMR Data for Compounds 7a−10a (600 MHz, $CDCl_3$)

position	7a	7b[a]	8[b]	9	10a[b]
2	6.72 brs	6.31−6.46 (m)	6.75 brs	6.42 brs	6.22 s
5	6.76 d (7.9)				
6	6.66 brd (7.9)	6.31−6.46 (m)	6.76 brs	6.42 brs	6.22 s
7	2.47 brd (6.9) *anti*	2.45 d (7.4) *anti*	2.47 d (7.7) *anti*	2.50 d (7.7) *anti*	3.42 d (12.0) *syn*
8	2.75 quint (6.9)	2.75 q (6.8)	2.74 quint (6.8)	2.84 quint (6.9)	2.92 dq (12, 7.4)
9	1.06 d (6.9)	1.06 d (6.7)	1.06 d (6.8)	1.11 d (6.7)	0.87 d (7.4)
2′	2.15 brd (11.0), 2.32 d (11.0)	2.14 d (10.9), 2.32 d (10.9)	2.14 d (10.7), 2.31 d (10.7)	2.19 d (11.0), 2.36 d (11.0)	2.09 d (10.7), 2.31 d (10.7)
5′	5.47 s	5.47 (s)	5.46 s	5.51 s	5.61
7′a	2.39 ddt (13.9, 5.4, 1.5)	2.40−2.51 (m)	2.39 ddt (13.6, 5.4, 1.7)	2.48 brdd (13.8, 5.5)	2.10 dd (14, 9.0)
7′b	1.30 dd (13.9, 8.9)	1.32 dd (8.7)	1.30 dd (13.6, 9.0)	1.33 dd (13.8, 8.8)	2.60 dd (14, 5.8)
8′	5.60 dddd (16.9, 10.3, 8.9, 5.4)	5.49−5.70	5.60 dddd (15.8, 10.5, 9.0, 5.5)	5.62 dddd (16.3, 10.9, 8.8, 5.5)	5.79 dddd (16.2, 10.4, 9.0, 5.8)
9′	4.87 m	4.84−4.93	4.85 m, 4.88 m	4.89 m	5.09 m
3-OCH_3				3.89 s	3.79 s
4-OCH_3				3.88 s	3.82 s
5-OCH_3		3.90		3.89 s	3.79 s
O-CH_2-O	5.98 d (1.5), 5.97 d (1.5)	5.98 dd (1.5)	5.65 s, 5.88 s		
Alk-O-CH_2-O	5.69 s, 5.66 s	5.68 d (5.0)	5.97 d (1.4), 5.98 d (1.4)	5.73 s, 5.71 s	5.67 s, 5.70 s

[a]Data from ref 7. [b]Spectrum obtained at 500 MHz.

Table 4. ^{13}C NMR Data for Compounds 7a−10a (151 MHz, $CDCl_3$)

carbon	7a	8	9	10a
1	132.8	132.7	134.7	130.9
2	NO[a]	108.2	NO[a]	108.3
3	146.9	147.6	153.1	152.4
4	147.9	138.2	137.4	137.0
5	108.1	140.8	153.1	152.4
6	NO[a]	108.2	NO[a]	108.3
7	55.8	56.0	56.3	54.3
8	48.8	48.9	48.5	44.3
9	15.5	15.6	15.5	14.3
1′	55.9	55.9	55.8	59.7
2′	44.7	44.8	44.7	46.7
3′	89.9	90.0	89.9	91.3
4′	176.1	176.1	176.1	177.7
5′	96.5	96.6	96.6	98.2
6′	201.2	201.1	201.1	200.5
7′	36.5	36.6	36.3	37.7
8′	135.4	135.5	135.3	134.5
9′	117.0	117.0	117.0	118.5
3-OCH_3			56.5	56.0
4-OCH_3			60.9	60.8
5-OCH_3			56.5	56.0
ArOCH_2O	101.4	101.5		
AlkOCH_2O	101.2	101.3	101.4	101.6

[a]Not observed.

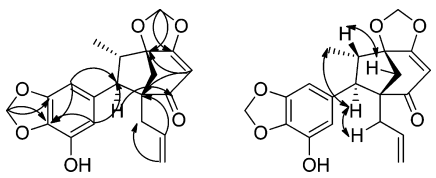
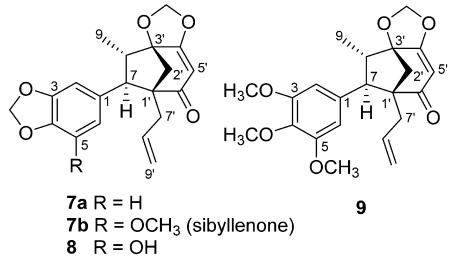
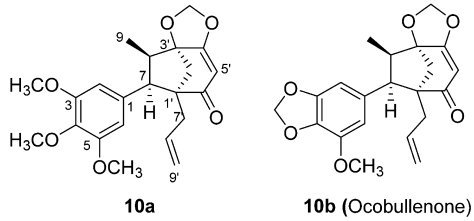


Figure 4. Key HMBC (left) and NOESY (right) correlations for **8**.

three methoxy groups at δ_H 3.89 (6H, 3,5-OCH_3) and 3.88 (3H, 4-OCH_3). The HMBC spectrum of **9** indicated key correlations of the 3,5-OCH_3 protons to the carbons at δ_C 153.1 (C-3,5) and the correlations of the 4-OCH_3 protons to the carbon at δ_C 137.4 (C-4). These facts led to the assignment of the structure of **9** as the new natural product (7*R*,8*S*,1′*S*,3′*S*)-$\Delta^{8'}$-3,4,5-trimethoxy-3′,4′-methylenedioxy-1′,2′,3′,6′-tetrahydro-6′-oxo-7.1′-8.3′-neolignan. The absolute configuration of **9** was assigned based on the comparison of its spectroscopic and physical data with those of **7a**, **7b**, and **8**.





Compound **10a** had the molecular formula $C_{22}H_{26}O_6$ as indicated by ^{13}C NMR and positive ion HRESIMS data. The IR spectrum showed absorption bands suggestive of aromatic and double-bond methine (2985 cm^{-1}) and conjugated ketocarbonyl functions (1641 cm^{-1}). The ^{1}H NMR spectroscopic data of **10a** (Table 3) were similar to those of **7a**, **7b**, and **8**, and the signals for the bicyclooctanoid portion of the molecule closely matched those for ocobullenone (Table 4).[7] In particular, the $J_{7,8}$ value of 12 Hz indicated a *syn* relationship between H-7 and H-8. The ^{13}C NMR spectrum of **10a** exhibited signals due to three aromatic methoxy carbons [δ_C 60.8, 56.0 (×2)] instead of signals for the methylenedioxy and methoxy groups of ocobullenone. Comparison of the ^{1}H and ^{13}C NMR data of

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10a with those of ocobullenone (**10b**)[5,7] confirmed its assignment as an analogue of ocobullenone. HMBC correlations between the methoxy protons at δ_H 3.79 and C-3 and C-5, between the methoxy protons at δ_H 3.82 and C-4, and between H-2/6 (δ_H 6.22) and C-1, C-3, C-4, C-5, and C-7 confirmed the presence of a 3,4,5-trimethoxybenzene moiety at C-7 in **10a** (Figure 5). In addition, the HMBC correlations

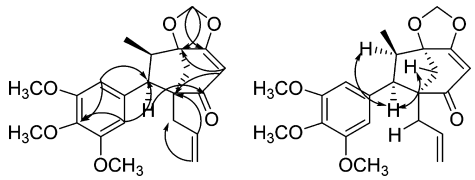

Figure 5. Key HMBC (left) and NOESY (right) correlations for **10a**.

from the protons of the C-9 secondary methyl group to C-7 and the oxygen-bearing tertiary carbon (δ_C 91.3, C-3′), together with the long-range cross-peaks between H-2′ and C-8, C-7, C-7′, and C-4′ and between the olefinic H-5′ and C-1′ and C-3′, confirm the planar structure of **10a**.

The relative and absolute configurations of **10a** were determined by interpretation of the data obtained from NOESY experiments and by its ECD spectrum. NOESY correlations observed between H-7 and H-8, and H-7 and H-2′a, confirmed the relative configurations at C-7, C-8, C-1′, and C-3′ to be as depicted (Figure 5). Interpretation of the negative Cotton effect observed for the n → π^* transition at 305 nm was facilitated by minimizing the energy using a molecular mechanics (MM2) computation of **10a**. In the most stable conformation, the methoxylated aromatic ring made a major contribution to the negative Cotton effect. This ring was situated in front of the carbonyl group and contributed to the negative Cotton effect in the front quadrant of the octant rule.[28,29] From these data, the absolute configuration of **10a** was assigned as (7*R*,8*R*,1′*R*,3′*R*)-$\Delta^{8'}$-3,4,5-trimethoxy-3′,4′-methylenedioxy-1′,2′,3′,6′-tetrahydro-6′-oxo-7.1′-8.3′-neolignan.[30]

Compounds **7a**−**10a** belong to a rare group of bicyclo[3.2.1]octanoid neolignans possessing a 7.1′.8.3′ coupling with unique features such as the deoxygenated C-2′ between the bridge heads and the methylenedioxy group on the cyclohexenone ring. No biological reports have been published for this series of compounds, but their C-2 oxygenated counterparts lacking the methylenedioxy group at C-3′ and C-4′ have been reported to possess potent anti-PAF (platelet-activating factor) activity.[31]

Biological Activities. The biological activities of selected compounds are shown in Table 5. The new 8.O.6′-neolignan **4a**, dihydrobenzofuranoid **5a**, and bicyclo[3.2.1]octanoid (**7a**) all had in vitro activity against *Aedes aegypti*, with ≥80% mortality at 4 mg/mL.

Ococymosin (**5a**) was the most active antiparasitic component among those isolated in the present study, with an IC_{50} value of 0.45 μM against the Dd2 strain of *Plasmodium falciparum*. Virolongin B (**4b**), compound **10a**, and ocobullenone (**10b**) all had IC_{50} values in the single-digit micromolar range, while compounds **7a** and **8** had IC_{50} values in the double-digit micromolar range. Lignans and neolignans have been reported to have a wide range of bioactivities such as antineoplastic,[32] viral reverse transcriptase inhibitor,[33] antimalarial,[33] antileishmanial,[34] and others. The antiplasmodial activity of virolongin B is not surprising since its isomer

Table 5. Antiparasitic and Insecticidal Activities of Isolated Compounds

compound	inhibition of *P. falciparum* Dd2 IC_{50} (μM)	activity against *Aedes aegypti*
4a	NT[a]	active[a]
4b (virolongin B)	3.3 ± 0.6	NT[b]
5a	0.45 ± 0.02	active[a]
7a	14.6 ± 0.7	active[a]
8	~42	NT[b]
10a	7.7 ± 0.5	NT[b]
10b (ocobullenone)	4.1 ± 0.8	NT[b]
artemisinin	0.00082 ± 0.00002	NT[b]

[a]Active: ≥80% mortality at 4 mg/mL. [b]Not tested.

virolongin A has been reported to be active against both a chloroquine-sensitive strain (PoW) and a chloroquine-resistant clone (Dd2) of *Plasmodium falciparum* (IC_{50} values 12.4 and 14.9 μM, respectively).[35] This is the first report on the antiplasmodial activity of 7.O.2′-8.3′-neolignans and dihydrobenzofuranoid neolignans.

None of the isolated compounds significantly inhibited the proliferation of A2780 ovarian cancer cells.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a JASCO P-2000 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. ECD analysis was performed on a JASCO J-810 spectropolarimeter with a 0.1 cm cell in MeOH at room temperature under the following conditions: speed 50 nm/min, time constant 1 s, bandwidth 2.0 nm.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer in $CDCl_3$ with TMS as internal standard. Mass spectra were obtained on a JEOL JMS-HX-110 and an Agilent 6220 LC-TOF-MS. Preparative HPLC was performed using Shimadzu LC-10AT pumps coupled with a semipreparative Varian Dynamax C_{18} column (5 μm, 250 × 10 mm), a Shimadzu SPD M10A diode array detector (DAD), and a SCL-10A system controller.

Insecticidal Bioassay. For in vitro evaluation, the actives are dissolved in dimethyl sulfoxide and tested in a 96-well micotiter plate. For in vivo and in vitro evaluation in mosquitoes, master plates containing 400 mg of a molecule dissolved in 100 mL of DMSO (equivalent to a 4000 ppm solution) are used. A master plate of assembled molecules contains 15 μL per well. To this plate is added 135 μL of a 90:10 water−acetone mixture to each well. This solvent addition is completed shortly before actual run time on the Sagian to minimize any molecule's incompatibility or stability issues. The Sagian robot is programmed to dispense 15 μL aspirations from the master plate into an empty 96-well shallow plate ("daughter" plate). There are 6 reps ("daughter" plates) created per master. The created daughter plates are then immediately infested with YFM larvae (yellow fever mosquito, *Aedes aegypti*).

The day before plates are to be treated, mosquito eggs are placed in Millipore water containing liver powder to begin hatching (4 g into 400 mL). After the daughter plates are created using the Sagian robot, they are infested with 220 μL of the liver powder/larval mosquito mixture (about 1-day-old larvae). After plates are infested with mosquito larvae, a nonevaporative lid is used to cover the plate to reduce drying. Plates are held at room temperature for 3 days prior to grading. After 3 days, each well is observed and scored based on mortality.

For activity against beet armyworm (*Spodoptera exigua*), master plates containing 400 mg of a molecule dissolved in 100 mL of DMSO (equivalent to a 4000 ppm solution) are used. A master plate of assembled molecules contains 30 μL per well. To this plate is added

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270 μL of a 2:1 acetone−water mixture to each well. This solvent addition is completed shortly before actual run time on the Biomek robot to minimize any molecule's incompatibility or stability issues. The Biomek is programmed to dispense 30 μL aspirations from the master plate onto the surface of a 96-well shallow-well plate ("daughter" plate) that has been prefilled approximately half full with a multispecies lep diet. There are 6 reps ("daughter" plates) created per master. The created daughter plates are dried in a fume hood for 5 h and placed in sealed plastic tubs until the following day. The plates are then infested with unhatched beet armyworm (*S. exigua*) eggs using a stainless steel "seeder". After plates are infested with the eggs, a layer of cotton batting is placed over the plate, then sealed with a nonevaporative lid used to reduce drying. Plates are held at 28 °C in a high-humidity chamber for 7 days prior to grading. After 7 days, each well is observed and scored based on mortality.

Antiproliferative Bioassay. The A2780 ovarian cancer cell line assay was performed at Virginia Tech as previously reported.[36,37] The A2780 cell line is a drug-sensitive cell line.[38]

Intraerythrocytic Stages Antimalarial Bioassay. The effect of each fraction and pure compounds on parasite growth of the Dd2 strain was measured in a 72 h growth assay in the presence of drug as described previously with minor modifications.[39,40] Briefly, ring stage parasite cultures (200 μL per well, with 1% hematocrit and 1% parasitaemia) were grown for 72 h in the presence of increasing concentrations of the drug in a 5.05% CO_2, 4.93% O_2, and 90.2% N_2 gas mixture at 37 °C. After 72 h in culture, parasite viability was determined by DNA quantitation using SYBR Green I (50 μL of SYBR Green I in lysis buffer in 0.4 μL of SYBR Green I/mL of lysis buffer).[40] The half-maximum inhibitory concentration (IC_{50}) calculation was performed with GraFit software using a nonlinear regression curve fitting. IC_{50} values are the average of three independent determinations, with each determination in duplicate, and are expressed ± SEM.

Plant Material. Stems and wood of *Ocotea cymosa* (collection: *F. Ratovoson 251*) were collected at an elevation of 1000 m in July 2000 in rainforest near the village of Ambatondrazaka, on the northern edge of Zahamena National Park, 17°28′45″ S, 048°44′10″ E, Madagascar. The sample collected was from a 12 m tree, 15 cm diameter at chest height, with yellow flower buds and open yellow flowers. The plant taxonomy was confirmed by Dr. Henk van der Werff (Missouri Botanical Garden).

Duplicate voucher specimens of each plant were deposited at Centre National d'Application des Recherches Pharmaceutiques (CNARP), the Herbarium of the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar (TAN), the Missouri Botanical Garden, St. Louis, Missouri (MO), and the Museum National d'Histoire Naturelle in Paris, France (P).

Extraction. A ground sample of *O. cymosa* stems (310 g) was extracted with EtOH at room temperature to yield 9.5 g of crude EtOH extract, designated MG 0448. A ground sample of *O. cymosa* wood (137 g) was extracted with EtOH at room temperature to yield 6.0 g of crude EtOH extract, designated MG 0450.

Isolation of Compounds with Insecticidal Activity from *O. cymosa* Stems. The extract MG 0448 exhibited activity against *Aedes aegypti* (AEDSAE) and *Spodoptera exigua* (LAPHEG) in high-throughput screening (HTS). The level 2 screen however indicated weak activity with an MIC of 273 μg/cm^2 for AEDSAE. A total of 1.0 g of the extract MG 0448 was pretreated on polyamide, the resulting extract was dissolved in MeOH, and 50 g of Celite was added. The mixture was dried on a rotary evaporator and then loaded in a 80 g cartridge and chromatographed on a silica gel column on a Combiflash instrument with elution by CH_2Cl_2−MeOH. Twelve fractions were collected and tested for activity against AEDSAE and LAPHEG. From the HTS results it was observed that only fractions 5, 6, 7, and 8 had activity against AEDSAE, with fractions 7 and 8 as the most active with 60% and 73% mortality at 100 μg. Trituration of the weakly active fraction 7 (81.0 mg) afforded a white powder, which was identified as sibyllenone (**7b**, 64.0 mg). Purification of the mother liquor using preparative HPLC gave sibyllenone and the new didymochlaenone C (**6a**) (3.0 mg).

Fraction 8 (600.2 mg) was purified using a combination of Sephadex LH-20 (CH_2Cl_2−50% MeOH), preparative TLC, and HPLC to yield six compounds. The new metabolites cymosalignan A (**1a**, 30.0 mg), B (**2**, 5.0 mg), and C (**3**, 1.2 mg) and compounds **8** (136 mg) and **9** (10 mg) were obtained, together with the known 3,4,5,3′,5′-pentamethoxy-8.O.4′-neolignan (45.5 mg) and sibyllenone (**7b**, 3.2 mg). Purification of fraction F5 on preparative HPLC yielded the three new compounds **8** (31.0 mg), ococymosin (**5a**) (88 mg), and didymochlaenone C (**6a**) (2.0 mg) as well as sibyllenone (4.0 mg). Purification of fraction F6 gave the new compounds **4a** (10 mg) and **7a** (5.0 mg) and the known didymochlaenone B (3.0 mg).

Isolation of Compounds with Antimalarial Activity from *O. cymosa* Wood. A total of 1.8 g of the wood extract was made available to Virginia Tech. To locate the types of metabolites responsible for its activity, 100 mg of the crude EtOH extract was subjected to a liquid−liquid partition using hexanes, EtOAc, and H_2O to afford 18.9 mg of active hexanes fractions (IC_{50} 1.25 μg/mL). HPLC was performed on this fraction on a C_{18} column with a solvent gradient from H_2O−MeOH (syst I): 20:80 to 18:82 for 10 min, to 15:85 from 10 to 15 min, hold at 15:85 for 5 min, to 10:90 from 20 to 25 min, and to 0:100 from 25 to 27 min, ending with 100% MeOH for 36 min. Five compounds were recovered: **3** (t_R: 19.76 min; IC_{50} 42.1 μM, 2.6 mg), virolongin B (**4b**, t_R: 22.88 min; IC_{50} 3.1 μM, 1.1 mg), **8** (t_R: 24.11 min; IC_{50} 28.4 μM, 1.4 mg), **10a** (t_R: 27.76 min; IC_{50} 40.5 μM, 1.4 mg), and **5a** (t_R: 29.27 min; IC_{50} 4.7 μM, 2.1 mg).

*Cymosalignan A (**1a**):* colorless oil; $[\alpha]^{25}_D$ −22 (*c* 0.2, MeOH); UV (λ_{max} from HPLC) 318, 256 nm; IR (ν_{max}, cm^{-1}) 3079, 2916, 1622, 1590, 1509, 1465, 1413, 1387, 1330, 1220, 1198, 1118, 1042, 1009, 949; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 387.1801 $[M + H]^+$ (calcd for $C_{22}H_{27}O_6^+$, 387.1802), 409.1616 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_6Na^+$, 409.1622).

*Cymosalignan B (**2**):* colorless oil; $[\alpha]^{25}_D$ −21.0 (*c* 0.1, MeOH); UV (λ_{max} from HPLC) 320, 258 nm; IR (ν_{max}, cm^{-1}) 3074, 2920, 1625, 1590, 1513, 1463, 1410, 1387, 1261, 1222, 1140, 1026, 943; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS: *m/z* 379.1509 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_5Na^+$, 379.1516).

*Cymosalignan C (**3**):* colorless oil; $[\alpha]^{25}_D$ −16 (*c* 0.1, MeOH); UV (λ_{max} from HPLC) 217, 250, 318 nm; IR (ν_{max}, cm^{-1}) 3396, 2929, 1624, 1517, 1460, 1412, 1389, 1327, 1213, 1114, 1041, 937; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 373.1649 $[M + H]^+$ (calcd for $C_{21}H_{25}O_6^+$, 373.1646) 395.1471 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_6Na^+$, 395.1465), 767.3057 $[2M + Na]^+$ (calcd for $C_{42}H_{48}O_{12}Na^+$, 767.3038).

*Compound **4a**:* colorless oil; $[\alpha]^{25}_D$ +23 (*c* 0.1, MeOH); UV (λ_{max} from HPLC) 300, 256, 234 nm; IR (ν_{max}, cm^{-1}) 2933, 2837, 1645, 1587, 1508, 1457, 1421, 1361, 1326, 1245, 1200, 1124, 1098, 1056, 1005, 922, 837: ^{1}H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 387.1805 $[M + H]^+$ (calcd for $C_{22}H_{27}O_6^+$, 387.1802), 409.1623 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_6Na^+$, 409.1622), 795.3343 $[2M + Na]^+$ (calcd for $C_{44}H_{52}O_{12}Na^+$, 795.3351).

*Ococymosin (**5a**):* colorless oil; $[\alpha]^{25}_D$ +42 (*c* 0.9, MeOH); UV (λ_{max} from HPLC) 300, 240 nm; IR (ν_{max}, cm^{-1}) 2922, 2837, 1657, 1641, 1593, 1501, 1478, 1455, 1414, 1365, 1322, 1269, 1216, 1158, 1039, 996, 954; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 404.1465 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_6Na^+$, 407.1465), 791.3032 $[2M + Na]^+$ (calcd for $C_{44}H_{48}O_{12}Na^+$, 791.3038).

*Didymochlaenone C (**6a**):* colorless oil; $[\alpha]^{25}_D$ −119 (*c* 0.2, MeOH); UV (λ_{max} from HPLC) 246, 280 nm; IR (ν_{max}, cm^{-1}) 3075, 2935, 2840, 1655, 1588, 1496, 1459, 1420, 1333, 1278, 1242, 1180, 1123, 1041, 981, 909; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 373.1864 $[M + H]^+$ (calcd for $C_{21}H_{25}O_6^+$, 373.1651), 395.1452 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_6Na^+$, 395.1465), 767.3008 $[2M + Na]^+$ (calcd for $C_{42}H_{48}O_{12}Na^+$, 767.3038).

*Demethoxysibyllenone (**7a**):* colorless oil; $[\alpha]^{25}_D$ −144 (*c* 0.1, MeOH); UV (λ_{max} from HPLC) 240, 290 nm; IR (ν_{max}, cm^{-1}) 2962, 2906, 1646, 1503, 1488, 1443, 1142, 1361, 1251, 1230, 1199, 1132, 1095, 1037, 928; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; (+)-HRESIMS *m/z* 341.1416 $[M + H]^+$ (calcd for $C_{20}H_{21}O_5^+$,

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341.1384), 363.1172 [M + Na]$^+$ (calcd for $C_{20}H_{20}O_5Na^+$, 363.1203), 703.2492 [2M + Na]$^+$ (calcd for $C_{40}H_{40}O_{10}Na^+$, 703.2514).

*Demethylsibyllenone (**8**):* amorphous powder; $[\alpha]^{25}_D$ +6 (*c* 0.01, MeOH); UV (MeOH) λ_{max} nm (log ε) 205 (3.31) 240 (2.7), 300 (1.9); ECD (*c* 0.02, MeOH) λ_{max} ($\Delta\varepsilon$) 305 (−1.8), 251 (1.3), 225 (−3.1); IR (film) 3420, 1640, 2985, 1512, 1424, 1205, 1043, 920 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; (+)-HRESIMS *m*/*z* 357.1342 [M + H]$^+$ (calcd for $C_{20}H_{21}O_6^+$, 357.1333).

*(7R,8S,1′S,3′S)-Δ$^{8′}$-3,4,5-Trimethoxy-3′,4′-methylenedioxy-1′,2′,3′,6′-tetrahydro-6′-oxo-7.1′-8.3′-neolignan (**9**):* colorless oil; $[\alpha]^{25}_D$ −51 (*c* 0.02, MeOH); UV (λ_{max} from HPLC) 217, 250 nm; IR (ν_{max}, cm^{-1}) 2933, 2837, 1645, 1587, 1508, 1457, 1422, 1362, 1326, 1245, 1200, 1124, 1098, 1057, 1006, 922; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; (+)-HRESIMS *m*/*z* 387.2360 [M + H]$^+$ (calcd for $C_{22}H_{27}O_6^+$, 387.1802), 409.1620 [M + Na]$^+$ (calcd for $C_{22}H_{26}O_6Na^+$, 409.1622), 795.3378 [2M + Na]$^+$ (calcd for $C_{44}H_{52}O_{12}Na^+$, 795.3351).

*(7R,8R,1′R,3′R)-Δ$^{8′}$-3,4,5-Trimethoxy-3′,4′-methylenedioxy-1′,2′,3′,6′-tetrahydro-6′-oxo-7.1′-8.3′-neolignan (**10a**):* colorless oil; $[\alpha]^{25}_D$ +101, (*c* 0.02, MeOH); UV (λ_{max} from HPLC) 217, 250 nm; ECD (*c* 0.1, MeOH) λ_{max} ($\Delta\varepsilon$) 305 (−1.42), 259 (1.5), 240 (−3.2); IR (ν_{max}, cm^{-1}) 3074, 2932, 2837, 1643, 1587, 1509, 1454, 1425, 1381, 1362, 1322, 1248, 1200, 1120, 1057, 1003, 919; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; (+)-HRESIMS *m*/*z* 409.1596 [M + Na]$^+$ (calcd for $C_{22}H_{26}O_6Na^+$, 409.1622), 445.2303 [M + CH_3CN + NH_4]$^+$ (calcd for $C_{24}H_{33}O_6N_2^+$, 445.2333).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}D and 2D NMR spectra of all new compounds (**1**−**10a**) are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

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