

Identification and Biological Evaluation of Secondary Metabolites from the Endolichenic Fungus *Aspergillus versicolor*

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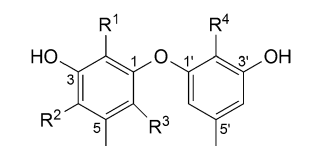
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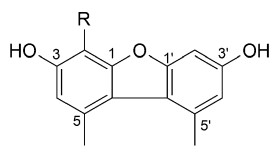
A chemical investigation of the endolichenic fungus *Aspergillus versicolor* (125a), which was found in the lichen *Lobaria quercizans*, resulted in the isolation of four novel diphenyl ethers, named diorcinols F–H (**1–3**, resp.) and 3-methoxyviolaceol-II (**4**), eight new bisabolane sesquiterpenoids, named (–)-(R)-cyclo-hydroxysydonic acid (**5**), (–)-(7S,8R)-8-hydroxysydowic acid (**6**), (–)-(7R,10S)-10-hydroxysydowic acid (**7**), (–)-(7R,10R)-iso-10-hydroxysydowic acid (**8**), (–)-12-acetoxy-1-deoxysydonic acid (**9**), (–)-12-acetoxysydonic acid (**10**), (–)-12-hydroxysydonic acid (**11**), and (–)-(R)-11-dehydro-sydonic acid (**12**), two new tris(pyrogallol ethers), named sydowiols D (**13**) and E (**14**), and fifteen known compounds, **15–29**. All of the structures were determined by spectroscopic analyses, and a number of them were further identified through chemical transformations and electronic circular dichroism (ECD) calculations. Preliminary bioassays of these isolates for the determination of their inhibitory activities against the fungus *Candida albicans*, and their cytotoxicities against the human cancer cell lines PC3, A549, A2780, MDA-MB-231, and HEPG2 were also evaluated.

Introduction. – Among fungi, those that live in close association with other organisms are assumed to be exceptional generators of biologically active compounds [1]. Lichens harbor abundant and diverse fungal communities. Recent studies have demonstrated that endolichenic fungi that live asymptotically in the thalli of lichens are valuable sources of novel bioactive natural products, such as the antibacterial coniothiepinol A [2], a cyclic pentapeptide with synergistic antifungal activity [3], cytotoxic chromone derivatives [4], perylenequinones [5], ophiobolane sesterterpenes [6], diterpenoid geopyxin B [7], a demethoxyviridin that inhibits A β ₄₂ aggregation [8], and antiviral heptaketides [9]. However, chemical investigations of this group of fungi are rather limited.

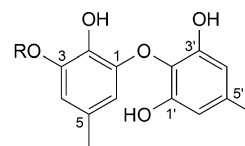
During our search for new cytotoxic natural products from endolichenic fungi [5], the crude extract of *Aspergillus versicolor* (125a), which inhabits the lichen *Lobaria quercizans*, collected in the Laojun Mountain of Yunnan Province in China, exhibited significant cytotoxicity against the human osteosarcoma cancer cell line U2OS with an



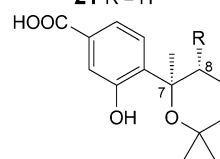
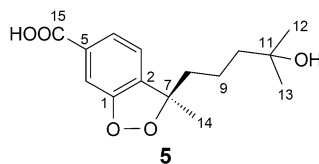
	R ¹	R ²	R ³	R ⁴
1	MeO	H	H	OH
2	prenyl	prenyl	H	H
15	prenyl	H	H	H
16	H	H	H	H
17	H	H	prenyl	H
18	OH	H	H	OH
19	OH	H	H	H



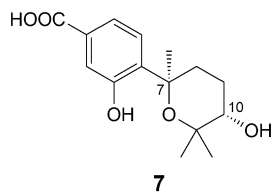
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20 R = H



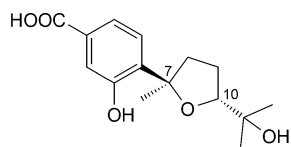
4 R = Me
21 R = H



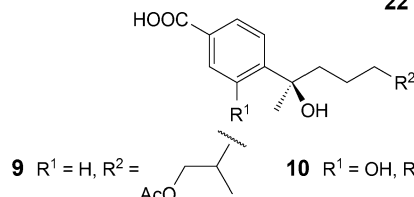
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22 R = H



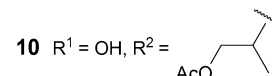
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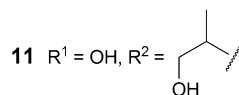
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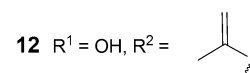
9 R¹ = H, R² =



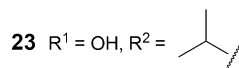
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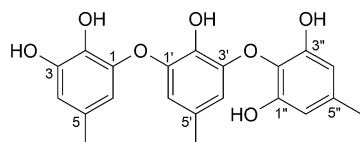
11 R¹ = OH, R² =



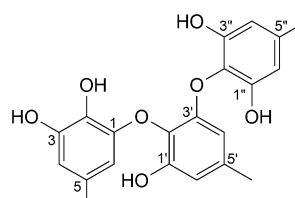
12 R¹ = OH, R² =



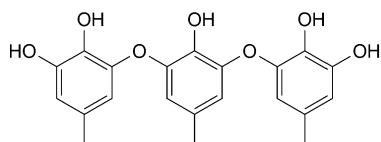
23 R¹ = OH, R² =



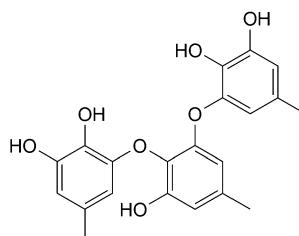
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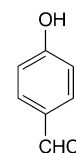
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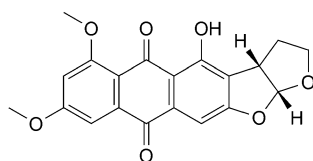
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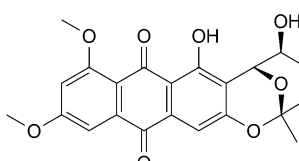
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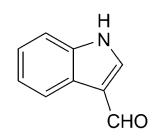
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IC_{50} value of 21.53 $\mu\text{g/ml}$. Fractionation of the AcOEt extract of its solid-substrate fermentation culture afforded 14 new compounds, including four diphenyl ethers, **1**–**4**, eight bisabolane sesquiterpenoids, **5**–**12**, and two pyrogallol ethers, **13** and **14**, together with 15 known compounds, **15**–**29**. Herein, we describe the isolation, and structure elucidation of the isolated compounds, as well as the evaluation of their antifungal and cytotoxic activities.

Results and Discussion. – The AcOEt extract of *A. versicolor* afforded 29 compounds by using a combination of size-exclusion, normal-phase, and reversed-phase chromatography.

Structure Elucidation. Diorcinol F (**1**) was found to have the molecular formula $\text{C}_{15}\text{H}_{16}\text{O}_5$ by a combination of HR-ESI-MS (m/z 277.1072 ($[M+H]^+$; calc. 277.1071)) and ^{13}C -NMR data, accounting for eight degrees of unsaturation. The IR spectrum revealed the presence of OH functions (3355 cm^{-1}) and aromatic moieties (1662 and 1589 cm^{-1}). The ^1H - and ^{13}C -NMR data, together with the HSQC data (Table 1), exhibited signals of two tertiary Me group ($\delta(\text{H})$ 2.07, $\delta(\text{C})$ 20.9, Me–C(5); $\delta(\text{H})$ 2.07, $\delta(\text{C})$ 20.5, Me–C(5')), one MeO group ($\delta(\text{H})$ 3.70, $\delta(\text{C})$ 60.2, MeO–C(2)), four aromatic CH groups ($\delta(\text{H})$ 6.36, $\delta(\text{C})$ 111.5, CH(4); $\delta(\text{H})$ 5.97, $\delta(\text{C})$ 109.3, CH(6); $\delta(\text{H})$ 6.38, $\delta(\text{C})$ 111.9, CH(4'); $\delta(\text{H})$ 6.07, $\delta(\text{C})$ 110.9, CH(6')), three exchangeable H-atoms ($\delta(\text{H})$ 9.16 (2 H); $\delta(\text{H})$ 8.32 (1 H)), and eight aromatic quaternary C-atoms. Based on these data, compound **1** was deduced to be a dimeric ether consisting of 3,4,5-trihydroxytoluene units similar to violaceol I (**18**) [10], with the exception of the presence of MeO–C(2) moiety in **1** instead of HO–C(2) in **18**. This conclusion was also supported by the HMBC from MeO–C(2) to C(2) ($\delta(\text{C})$ 135.8) (Fig. 1). Accordingly, the structure of compound **1** was unambiguously determined as 3-(3-hydroxy-2-methoxy-5-methylphenoxy)-5-methylbenzene-1,2-diol, which is structurally related to the fungal metabolites diorcinols B–E [11]. The trivial name diorcinol F was given to this new compound.

Diorcinol G (**2**) had the molecular formula of $\text{C}_{24}\text{H}_{30}\text{O}_3$, deduced by the same strategy as for **1**, indicating ten degrees of unsaturation. The presence of OH groups and aromatic moieties in **2** was inferred from its IR absorption bands at 3338, 1611, and 1598 cm^{-1} . The 1D-NMR and HSQC data (Table 1) revealed the presence of six Me, two CH_2 , and six aromatic/olefinic CH groups, ten aromatic/olefinic quaternary C-atoms, four of which were oxygenated, and two exchangeable H-atoms. These data suggested that **2** was a derivative of diorcinol (**16**) [12][13], with two additional prenyl (= 3-methylbut-2-en-1-yl) groups. One prenyl group at C(2) was determined by the ^1H , ^1H -COSY correlation H–C(7) ($\delta(\text{H})$ 2.97)/H–C(8) ($\delta(\text{H})$ 5.06), and the HMBCs from Me(10) ($\delta(\text{H})$ 1.54) to C(8) ($\delta(\text{C})$ 122.8) and C(9) ($\delta(\text{C})$ 130.2), from Me(11) ($\delta(\text{H})$ 1.48) to C(8) and C(9), and from H–C(7) to C(1) ($\delta(\text{C})$ 154.1), C(2) ($\delta(\text{C})$ 118.6), and C(3) ($\delta(\text{C})$ 150.5) (Fig. 1). The other prenyl group at C(4) was identified by the ^1H , ^1H -COSY correlation H–C(12) ($\delta(\text{H})$ 2.98)/H–C(13) ($\delta(\text{H})$ 4.88), and the HMBCs from resonance at Me(15) and Me(16) ($\delta(\text{H})$ 1.54) to C(13) ($\delta(\text{C})$ 122.8) and C(14) ($\delta(\text{C})$ 129.9), and from H–C(12) to C(3), C(4) ($\delta(\text{C})$ 123.0), and C(5) ($\delta(\text{C})$ 135.3) (Fig. 1). Thus, the structure of compound **2** was as depicted.

Diorcinol H (**3**) had the molecular formula $\text{C}_{15}\text{H}_{14}\text{O}_4$ based on HR-ESI-MS (m/z 257.0818 ($[M-H]^-$; calc. 257.0808)) and ^{13}C -NMR analyses, which revealed nine

Table 1. ¹H- and ¹³C-NMR Data^{a)} (in (D₆)DMSO) of Compounds **1–4** and **15**. δ in ppm, J in Hz. Atom numbering as indicated in the *Formulae*.

Position	1		2		3		4		15	
	δ (H)	δ (C)	δ (H)	δ (C)	δ (H)	δ (C)	δ (H)	δ (C)	δ (H)	δ (C)
1		150.8		154.1		149.1		146.8		154.2
2		135.8		118.6		130.5		133.1		117.0
3		150.6		150.5		147.2		148.3		156.3
4	6.36 (s)	111.5		123.0	6.60 (s)	115.0	6.39 (s)	106.6	6.45 (s)	111.7
5		132.7		135.3		125.1		126.5		136.3
6	5.97 (s)	109.3	6.54 (s)	114.1		116.9	5.93 (s)	107.4	6.15 (s)	111.5
1'		144.5		159.2		157.3		150.6		158.9
2'		134.4	5.88 (s)	99.1	6.80 (s)	95.8		128.0	6.02 (s)	101.3
3'		146.6		158.3		155.9		150.6		158.4
4'	6.38 (s)	111.9	6.17 (s)	109.2	6.56 (s)	114.2	6.20 (s)	108.2	6.25 (s)	110.2
5'		127.5		139.5		131.7		134.2		139.8
6'	6.07 (s)	110.9	5.97 (s)	106.2		115.7	6.20 (s)	108.2	6.12 (s)	108.5
5-Me	2.07 (s)	20.9	2.14 (s)	19.2	2.69 (s)	24.0	2.06 (s)	21.1	2.13 (s)	20.9
5'-Me	2.07 (s)	20.5	2.12 (s)	21.3	2.73 (s)	24.4	2.14 (s)	21.1	2.15 (s)	21.2
2-MeO	3.70 (s)	60.2			3.87 (s)	60.5				
3-MeO							3.74 (s)	55.9		
7			2.97 (d, $J=6.0$)	23.1					3.10 (d, $J=7.2$)	22.3
8			5.06 (t, $J=6.0$)	122.8					5.12 (t, $J=7.2$)	122.8
9				130.2						130.1
10			1.54 (s)	25.5					1.56 (s)	25.6
11			1.48 (s)	17.5					1.56 (s)	17.6
12			2.98 (d, $J=6.0$)	25.4						
13			4.88 (t, $J=6.0$)	122.8						
14				129.9						
15			1.54 (s)	25.5						
16			1.54 (s)	17.6						

^{a)} Recorded at 600 and 150 MHz, respectively. Assignments were based on ¹H, ¹H-COSY, HMQC, and HMBC experiments.

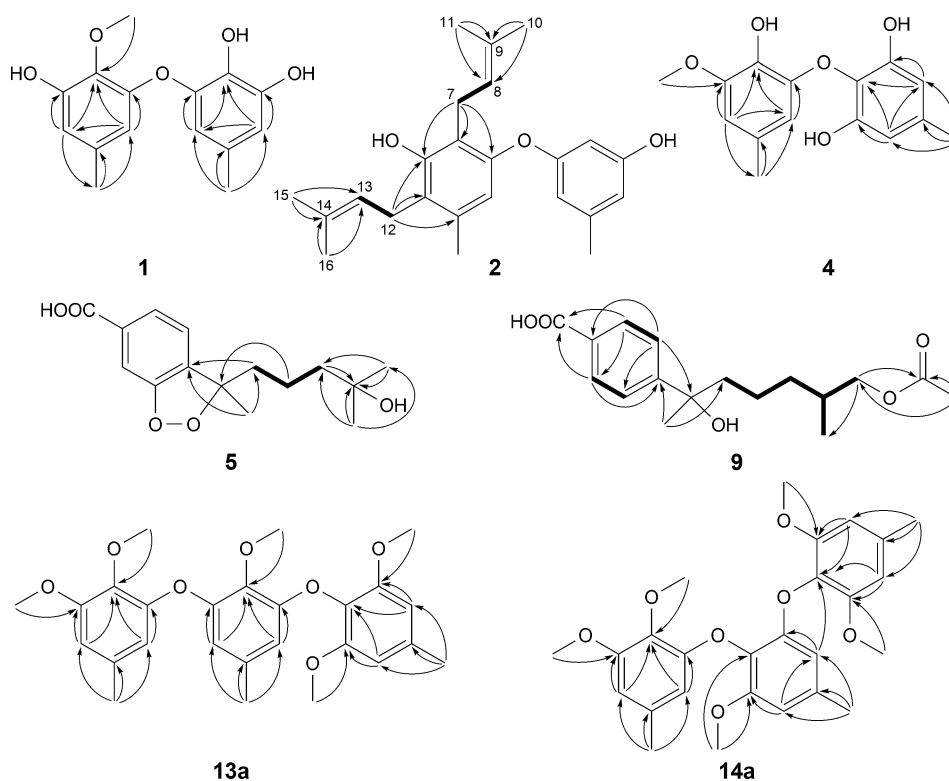


Fig. 1. ^1H , ^1H -COSY (—) and key HMB (H \rightarrow C) correlations of compounds **1**, **2**, **4**, **5**, **9**, **13a**, and **14a**

degrees of unsaturation. Its IR spectrum revealed the presence of OH groups (3351 cm^{-1}) and an aromatic system (1612 and 1584 cm^{-1}). The NMR data of **3** (Table 1) were similar to those of the co-metabolite 3,7-dihydroxy-1,9-dimethyldibenzofuran (**20**) [14] with the noticeable difference of additional resonances ($\delta(\text{H})$ 3.87; $\delta(\text{C})$ 60.5) due to a MeO group at C(2). This finding was supported by the HMBC of MeO–C(2) to C(2) ($\delta(\text{C})$ 130.5). Thus, the structure of **3**, named diorcinol H, was determined as depicted.

3-Methoxyviolaceol-II (**4**) was assigned the molecular formula $\text{C}_{15}\text{H}_{16}\text{O}_5$, which is the same as that of **1**, based on a combination of HR-ESI-MS (m/z 277.1072 ($[M+\text{H}]^+$; calc. 277.1071)) and ^{13}C -NMR data. The ^1H - and ^{13}C -NMR data (Table 1), together with the 2D-NMR data (Fig. 1), of **4** were similar to those of violaceol-II (**21**) [10]. The HMBC of MeO–C(3) ($\delta(\text{H})$ 3.74) with C(3) ($\delta(\text{C})$ 148.3) evidenced that compound **4** was the 3-*O*-Me derivative of **21**, which is consistent with its molecular formula.

(–)-(*R*)-Cyclo-hydroxysydonic acid (**5**) was assigned the molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_5$ by a combination of HR-ESI-MS (m/z 303.1203 ($[M+\text{Na}]^+$; calc. 303.1203)) and ^{13}C -NMR data, indicating six degrees of unsaturation. Its IR spectrum revealed absorption bands at 3390 (OH), 1687 (conjugated C=O group), 1619 and 1605 (aromatic ring) cm^{-1} . The ^1H -NMR data displayed a typical ABX spin system

assignable to a 1,3,4-trisubstituted benzene ring at $\delta(\text{H})$ 6.84 (*d*, $J=7.8$, H–C(3)), 7.35 (*s*, H–C(6)), and 7.61 (*d*, $J=7.8$, H–C(4)). Its ^1H - and ^{13}C -NMR, and HSQC data (Table 2) revealed other resonances including those of three tertiary Me ($\delta(\text{H})$ 1.13, $\delta(\text{C})$ 29.6, Me(12) and Me(13); $\delta(\text{H})$ 1.65, $\delta(\text{C})$ 24.4, Me(14)), three CH_2 ($\delta(\text{H})$ 1.47, $\delta(\text{C})$ 44.3, $\text{CH}_2(10)$; $\delta(\text{H})$ 1.62–1.56, $\delta(\text{C})$ 18.7, $\text{CH}_2(9)$; $\delta(\text{H})$ 1.97, $\delta(\text{C})$ 40.3, $\text{CH}_2(8)$) groups, two sp^3 oxygenated quaternary C-atoms ($\delta(\text{C})$ 69.8 (C(7), C(11)), one conjugated COOH ($\delta(\text{C})$ 167.1, C(15)) group, and six aromatic C-atoms. The ^1H , ^1H -COSY correlations revealed the presence of the spin system $\text{CH}_2(8)\text{--CH}_2(9)\text{--CH}_2(10)$ (Fig. 1). Furthermore, the HMBCs (Fig. 1) from H–C(12) or H–C(13) ($\delta(\text{H})$ 1.13) to C(10), C(11), C(12), and C(13), from H–C(9) to C(7), from H–C(8) to C(2) ($\delta(\text{C})$ 121.8) and C(14), and from H–C(14) to C(2) and C(8) confirmed the presence of an aliphatic side chain at C(2) of the benzene ring. Based on these data, compound **5** was determined as a bisabolane-type sesquiterpenoid similar to hydroxysydonic acid [15], with the exception of the absence of two H-atoms. At present, this compound could be assumed to have the structure of **A** or **B** (Fig. 2). However, further comparisons of the ^{13}C -NMR chemical shifts of C(7) and C(11) in **5** with those of C(1) and C(5) in 1,2-dioxepanes confirmed the structure **A** [16]. The absolute configuration of compound **5** was determined by comparing the experimental and calculated ECD spectra predicted by time-dependent density-functional theory (TDDFT). The absolute configuration of C(7) was assigned as (*R*) based on the finding that the experimental and calculated ECD spectra of **5** were in good agreement (*cf.* Fig. 4,a).

(–)-(7*S*,8*R*)-8-Hydroxysydowic acid (**6**), (–)-(7*R*,10*S*)-10-hydroxysydowic acid (**7**), and (–)-(7*R*,10*R*)-iso-10-hydroxysydowic acid (**8**) were found to be isomers with the same molecular formula of $\text{C}_{15}\text{H}_{20}\text{O}_5$, based on a combination of their HR-ESI-MS and ^{13}C -NMR spectra. Their similar 1D-NMR spectra (Table 2) exhibited signals for three Me and two CH_2 groups, one $\text{CH}\text{--O}$ group, two sp^3 quaternary O-bearing C-atoms, a 1,3,4-trisubstituted benzene ring containing an oxygenated aromatic C-atom, and one conjugated COOH group. These data suggested that compounds **6–8** were hydroxy derivatives of sydowic acid (**22**) [17]. Compounds **6** and **7** were determined as 8-hydroxy- and 10-hydroxysydowic acids, respectively, by the downfield shifts in the resonances of C(8) ($\delta(\text{C})$ 69.1) in **6** and of C(10) ($\delta(\text{C})$ 70.7) in **7**. The differences in

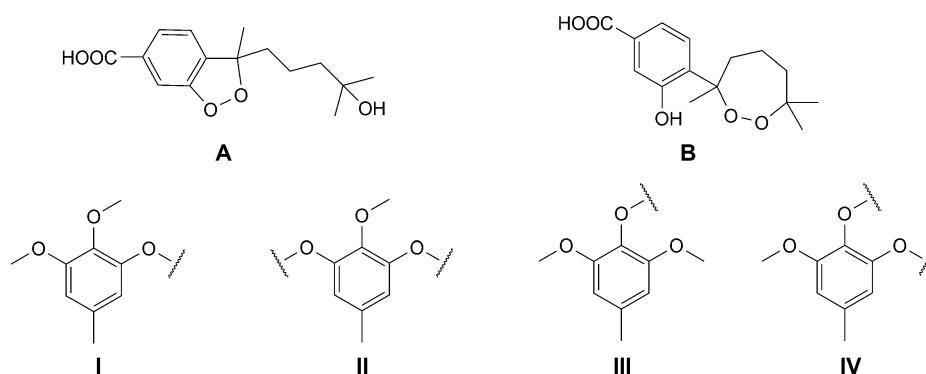


Fig. 2. Structures of **A**, **B**, and the fragments **I**, **II**, **III**, and **IV** of compounds **13a** and **14a**

Table 2. ^1H - and ^{13}C -NMR Data of Compounds **5–9**^a). δ in ppm, J in Hz.

Position	5^b		6^c		7^c		8^c		9^c	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
1		152.5		156.6		156.9		155.4		154.0
2		121.8		136.0		136.7		135.5	7.52 (<i>d</i> , $J=6.6$)	125.1
3	6.84 (<i>d</i> , $J=7.8$)	108.4	7.22 (<i>d</i> , $J=8.4$)	125.5	7.15 (<i>d</i> , $J=8.4$)	124.5	7.08 (<i>d</i> , $J=7.8$)	126.6	8.06 (<i>d</i> , $J=6.6$)	130.3
4	7.61 (<i>d</i> , $J=7.8$)	125.6	7.55 (<i>d</i> , $J=8.4$)	121.4	7.57 (<i>d</i> , $J=8.4$)	121.7	7.55 (<i>d</i> , $J=7.8$)	121.5		127.9
5		148.7		129.8		129.9		129.7	8.06 (<i>d</i> , $J=6.6$)	130.3
6	7.35 (<i>s</i>)	109.7	7.55 (<i>s</i>)	119.4	7.56 (<i>s</i>)	119.3	7.56 (<i>s</i>)	119.3	7.52 (<i>d</i> , $J=6.6$)	125.1
7		69.8		81.3		77.9		88.4		74.9
8	1.97 (<i>t</i> , $J=7.8$)	40.3	4.19 (<i>d</i> , $J=6.6$)	69.1	2.30 (<i>dt</i> , $J=14.0, 3.6, \text{H}_\text{a}$), 2.14 (<i>td</i> , $J=14.0, 3.6, \text{H}_\text{b}$)	27.9	2.49 (<i>dt</i> , $J=12.0, 8.4, \text{H}_\text{a}$), 2.24–2.19 (<i>m</i> , H_b)	38.8	1.85–1.74 (<i>m</i>)	44.3
9	1.62–1.56 (<i>m</i>)	18.7	2.04–2.00 (<i>m</i> , H_a), 1.94–1.88 (<i>m</i> , H_b)	24.5	2.05 (<i>tt</i> , $J=13.2, 3.6, \text{H}_\text{a}$), 1.86–1.82 (<i>m</i> , H_b)	24.1	2.11–2.05 (<i>m</i> , H_a), 1.92–1.86 (<i>m</i> , H_b)	25.5	1.38–1.25 (<i>m</i> , H_a), 1.21–1.14 (<i>m</i> , H_b)	21.3
10	1.47 (<i>t</i> , $J=7.8$)	44.3	1.94–1.88 (<i>m</i> , H_a), 1.60–1.56 (<i>m</i> , H_b)	32.9	3.51–3.48 (<i>m</i>)	70.7	3.89 (<i>t</i> , $J=7.2$)	87.0	1.38–1.25 (<i>m</i> , H_a), 1.11–1.04 (<i>m</i> , H_b)	33.6
11		69.8		75.4		77.9		70.7	1.74–1.67 (<i>m</i>)	32.5
12	1.13 (<i>s</i>)	29.6	1.38 (<i>s</i>)	30.4	1.33 (<i>s</i>)	26.4	1.33 (<i>s</i>)	27.7	3.87 (<i>ddd</i> , $J=10.0, 5.4, \text{H}_\text{a}$), 3.79 (<i>ddd</i> , $J=10.0, 6.6, \text{H}_\text{b}$)	69.5
13	1.13 (<i>s</i>)	29.6	1.15 (<i>s</i>)	28.0	1.00 (<i>s</i>)	25.0	1.19 (<i>s</i>)	24.9	0.84 (<i>d</i> , $J=6.6$)	16.9
14	1.65 (<i>s</i>)	24.4	1.64 (<i>s</i>)	23.4	1.55 (<i>s</i>)	31.1	1.60 (<i>s</i>)	29.6	1.57 (<i>s</i>)	30.3
15		167.1		170.1		170.7		170.2		171.5
1-OH										
12-AcO										171.5
12-AcO									2.02 (<i>s</i>)	21.1

^a) Recorded at 600 and 150 MHz, respectively. Assignments were based on ^1H , ^1H -COSY, HMQC, HMBC and NOESY experiments. ^b) Data were recorded in (D_6)acetone. ^c) Data were recorded in CDCl_3 .

the ^{13}C chemical shifts in the heterocyclic rings of **7** and **8** were consistent with those of linalool oxides [18][19]. The relative configurations of these three compounds were determined by their NOESY correlations (Fig. 3). The NOEs $\text{H}-\text{C}(8)/\text{H}_\text{b}-\text{C}(10)$, $\text{H}_\text{b}-\text{C}(10)/\text{Me}(13)$, and $\text{Me}(14)/\text{Me}(12)$ in **6** indicated that $\text{H}-\text{C}(8)$ and $\text{Me}(14)$ were on different faces. The correlations $\text{H}-\text{C}(10)/\text{H}_\text{a}-\text{C}(9)$, $\text{H}_\text{a}-\text{C}(9)/\text{H}_\text{a}-\text{C}(8)$, $\text{Me}(14)/\text{H}_\text{b}-\text{C}(8)$, and $\text{H}_\text{b}-\text{C}(8)/\text{H}_\text{b}-\text{C}(9)$ in **7** evidenced that $\text{H}-\text{C}(10)$ and $\text{Me}(14)$ were on different faces. Similarly, the NOEs $\text{Me}(14)/\text{H}_\text{a}-\text{C}(9)$ and $\text{H}_\text{b}-\text{C}(9)/\text{H}-\text{C}(10)$ in **8** indicated that $\text{Me}(14)$ and $\text{H}-\text{C}(10)$ were on different faces. The absolute configurations of compounds **6–8** were determined by comparing the experimental and calculated ECD spectra predicted by TDDFT (Fig. 4, *b–4, d*). The experimental and calculated ECD spectra of **6** were in good agreement. The calculated ECD spectra of **7** and of **8** were nearly mirror images of their experimental ECD spectra, respectively.

(–)-12-Acetoxy-1-deoxysydnolic acid (**9**) was found to have the molecular formula $\text{C}_{17}\text{H}_{24}\text{O}_5$ based on its HR-ESI-MS (m/z 331.1520 ($[M+\text{Na}]^+$; calc. 331.1516)) and ^{13}C -NMR data, which revealed six degrees of unsaturation. The 1D-NMR data (Table 2) resembled those of 12-acetoxysydnolic acid [20] with the exception of the A_2B_2 spin system ($\delta(\text{H})$ 7.52 (*d*, $J=6.6$, $\text{H}-\text{C}(2)$ and $\text{H}-\text{C}(6)$) and 8.06 (*d*, $J=6.6$, $\text{H}-\text{C}(3)$ and $\text{H}-\text{C}(5)$)) in **9** rather than the ABX spin system in 12-acetoxysydnolic acid, indicating the absence of a phenolic OH group in **9**. The planar structure of **9** was confirmed by detailed analysis of its 2D-NMR data (Fig. 1).

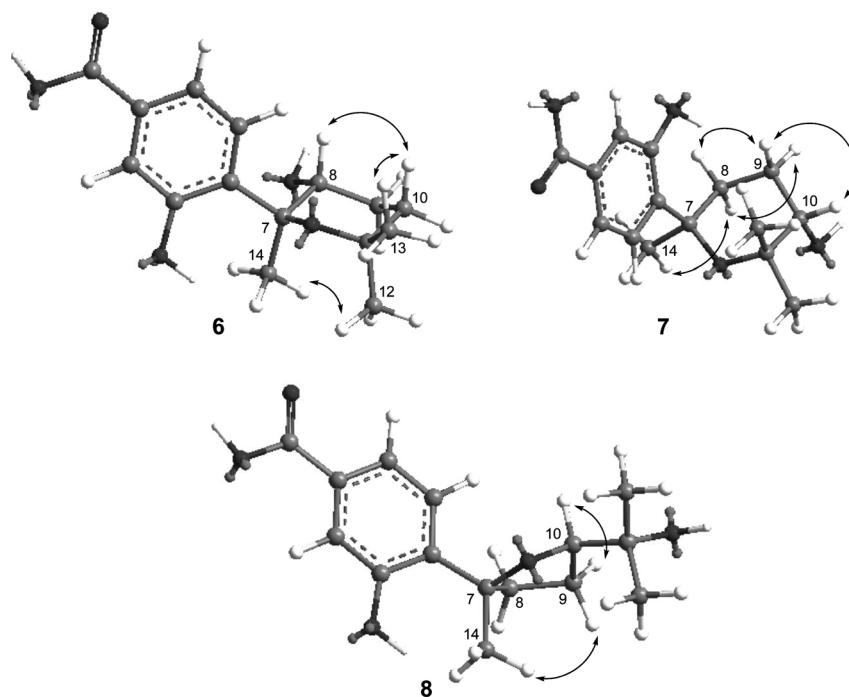


Fig. 3. Selected NOESY correlations ($\text{H} \leftrightarrow \text{H}$) of compounds **6–8**

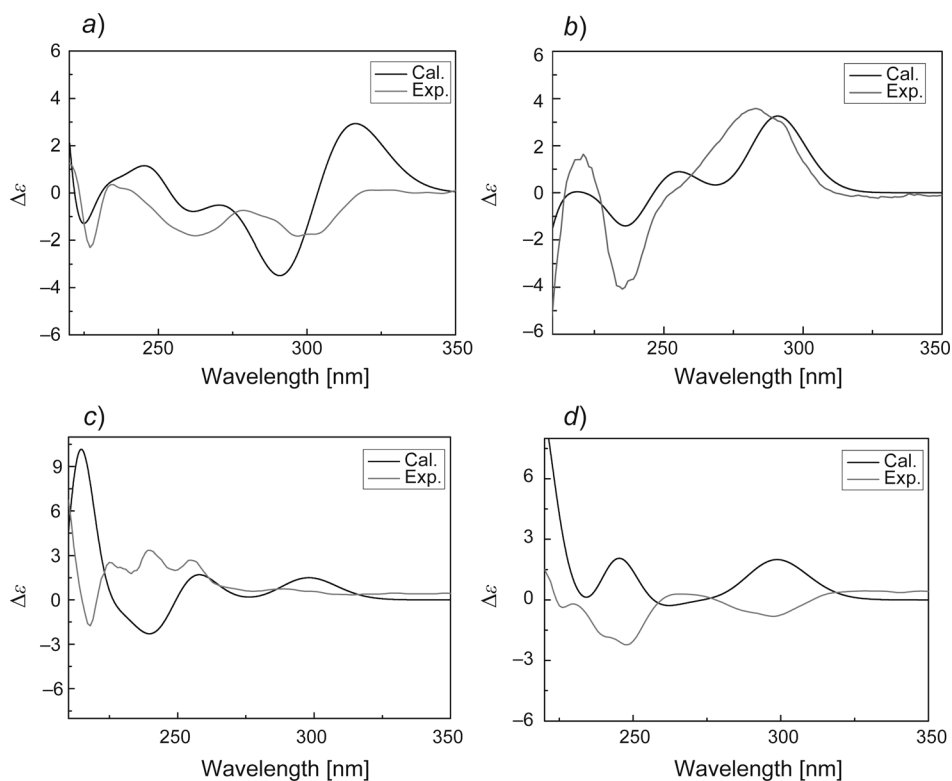
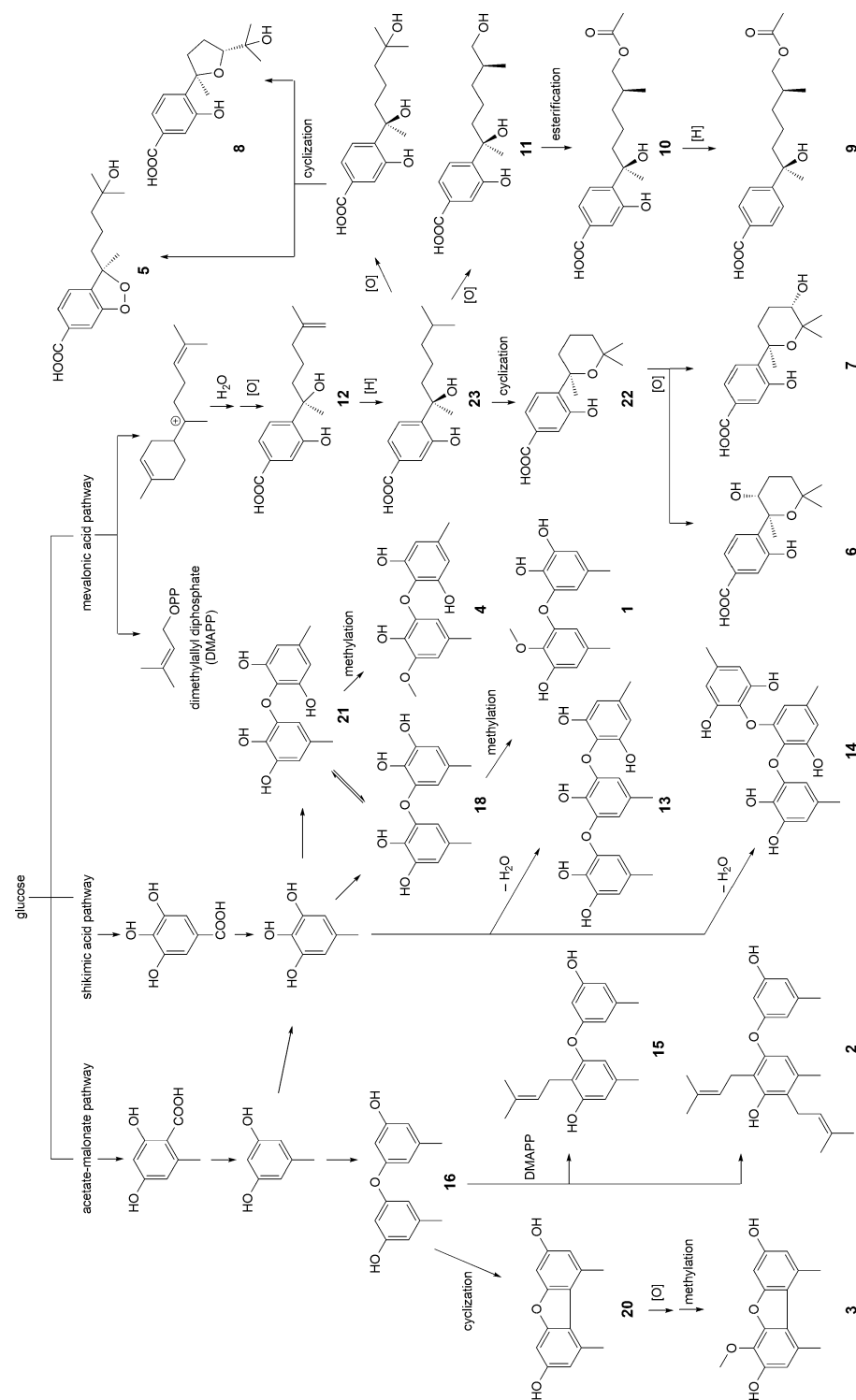


Fig. 4. Experimental and calculated ECD spectra of compound **5** (a), **6** (b), experimental ECD spectrum for **7** and calculated ECD spectrum for (7*S*,10*R*)-**7** (c), and experimental ECD spectrum for compound **8** and calculated ECD spectrum for (7*S*,10*S*)-**8** (d)

Compounds **10**, **11**, and **12** were found to be the 7-epimers of (+)-(7*S*,11*S*)-12-acetoxysydonic acid [20], (+)-(7*S*,11*S*)-12-hydroxysydonic acid [21], and (+)-(*S*)-11-dehydrosydonic acid [20], respectively, based on comparisons of their NMR spectra, MS data, and optical rotation values with those in the literature. Because there was only one stereogenic C-atom, the absolute configuration at C(7) in **12** was assigned as (*R*) by comparing its negative optical rotation value ($[\alpha]_{\text{D}}^{25} = -30.0$ ($c=0.1$, MeOH)) with the positive optical rotation value of (+)-(*S*)-11-dehydrosydonic acid ($[\alpha]_{\text{D}}^{25} = +10.8$ ($c=0.11$, MeOH)) [20]. From biogenetic considerations (*Scheme*), compounds **9–11** can be assumed to have the same absolute configuration, (7*R*), as that of **12**.

The molecular formulae of sydowniols D and E (**13** and **14**, resp.) were both determined as $\text{C}_{21}\text{H}_{20}\text{O}_7$ based on their HR-ESI-MS data (m/z 383.1128 ($[M-H]^-$; calc. 383.1125)). Their ^1H - and ^{13}C -NMR spectroscopic data, which were similar to those of the co-metabolites sydowniols A and B (**24** and **25**, resp.) [22], indicated that both **13** and **14** consist of three ether-linked 3,4,5-trihydroxytoluene moieties. In our study, although compounds **13** and **14** could be separated by HPLC, these were

Scheme 1. Possible Biosynthetic Pathways to Compounds 1–15



observed to be isomerized after standing in MeOH or MeCN. The methylation of a mixture **13/14** (MeI/K₂CO₃) afforded pentamethyl ethers **13a** (C₂₆H₃₀O₇) and **14a** (C₂₆H₃₀O₇), indicating the presence of five OH groups in **13** and **14**.

In the HMBC spectrum of **13a** (Fig. 1), the correlations from H–C(4) (δ (H) 6.49) to C(2) (δ (C) 137.8), attached to a MeO group (δ (H) 3.89, δ (C) 61.2), and C(3) (δ (C) 153.4) attached to a MeO group (δ (H) 3.87, δ (C) 56.2), from Me–C(5) (δ (H) 2.25) to C(4) (δ (C) 108.2), C(5) (δ (C) 133.6), and C(6) (δ (C) 112.6), and from H–C(6) (δ (H) 6.37) to O-bearing C(1) (δ (C) 150.5) and C(2) indicated the partial structure **I** (Fig. 2). The HMBCs from H–C(4') (δ (H) 6.08) to C(2') (δ (C) 137.9), attached to a MeO group (δ (H) 3.99, δ (C) 61.2), and O-bearing C(3') (δ (C) 152.6), from Me–C(5') (δ (H) 2.07) to C(4') (δ (C) 110.1), C(5') (δ (C) 133.2), and C(6') (δ (C) 113.3), and from H–C(6') (δ (H) 6.29) to oxygenated C(1') (δ (C) 150.3) and C(2') indicated moiety **II**, whereas moiety **III** was suggested by the HMBCs from two H-atoms with signals at δ (H) 6.46 (H–C(4'') and H–C(6'')) to C-atoms with signals at δ (C) 153.1 (C(1'') and C(3'')), attached to MeO groups (δ (H) 3.77, δ (C) 56.3), and oxygenated C(2'') (δ (C) 130.3), and from Me(5'') (δ (H) 2.38) to C(4'') (δ (C) 106.3), C(5'') (δ (C) 135.5), and C(6'') (δ (C) 106.3), in addition to symmetry considerations. Due to the presence of a single MeO group, fragment **II** had obviously to be placed between fragments **I** and **III**. The structure of **14a** was deduced by the same strategy that was used for the deduction of **13a**. Three moieties, **I**, **III**, and **IV** (Fig. 2), were indicated by the detailed analysis of the HMBC data of **14a** (Fig. 1), and **III** was connected to **IV** at C(1') (δ (C) 152.4) via an O-atom based on the HMBC between H–C(6') (δ (H) 6.03) and C(2'') (δ (C) 130.3). Thus, **14a** was unambiguously determined, and sydowiols D and E (**13** and **14**, resp.) were identified as shown. The assignments for the ¹H- and ¹³C-NMR data of **13a** and **14a** are compiled in Table 3.

Table 3. ¹H- and ¹³C-NMR Data (in CDCl₃) of Compounds **13a** and **14a**^a. δ in ppm, *J* in Hz.

Position	13a		14a		Position	13a		14a	
	δ (H)	δ (C)	δ (H)	δ (C)		δ (H)	δ (C)	δ (H)	δ (C)
1		150.5		152.2	6'	6.29 (s)	113.3	6.03 (s)	107.3
2		137.8		135.9	2'-MeO	3.99 (s)	61.2		
3		153.4		153.0	3'-MeO			3.74 (s)	56.3
4	6.49 (s)	108.2	6.36 (s)	106.4	5'-Me	2.07 (s)	21.5	2.22 (s)	22.0
5		133.6		132.7	1''		153.1		153.2
6	6.37 (s)	112.6	6.30 (s)	108.6	2''		130.3		130.3
2-MeO	3.89 (s)	61.2	3.99 (s)	61.1	3''		153.1		153.2
3-MeO	3.87 (s)	56.2	3.84 (s)	56.2	4''	6.46 (s)	106.3	6.41 (s)	106.8
5-Me	2.25 (s)	21.7	2.22 (s)	21.9	5''		135.5		135.3
1'		150.3		152.4	6''	6.46 (s)	106.3	6.41 (s)	106.8
2'		137.9		135.6	1''-MeO	3.77 (s)	56.3	3.69 (s)	56.5
3'		152.6		153.3	3''-MeO	3.77 (s)	56.3	3.69 (s)	56.5
4'	6.08 (s)	110.1	6.41 (s)	106.6	5''-Me	2.38 (s)	22.2	2.33 (s)	22.1
5'		133.2		134.9					

^a) Recorded at 600 and 150 MHz, respectively. Assignments were based on HMQC and HMBC experiments.

Diorcinol I (**15**) had the molecular formula of $C_{19}H_{22}O_3$, as established by a combination of HR-ESI-MS (m/z 299.1640 ($[M+H]^+$; calc. 299.1642)) and 1D-NMR data, indicating nine degrees of unsaturation. The UV, IR, and 1D-NMR data of **15** were similar to those of diorcinol G (**2**). Analyses of its 2D-NMR data revealed that the prenyl group at C(4) in **2** was absent in **15**, and this finding was supported by the HMBs from H–C(4) ($\delta(H)$ 6.45) to C(2) ($\delta(C)$ 117.0), C(3) ($\delta(C)$ 156.3), C(5) ($\delta(C)$ 136.3), and C(6) ($\delta(C)$ 111.5). This is the first report on the structure of **15**, which was named diorcinol I.

The known compounds were identified as diorcinol (**16**) [13], diorcinol D (**17**) [11], violaceol I (**18**) [23], cordyol C (**19**) [24], 3,7-dihydroxy-1,9-dimethyldibenzofuran (**20**) [14], violaceol II (**21**) [23], sydownic acid (**22**) [17], sydownic acid (**23**) [25], sydowniol A (**24**) [22], sydowniol B (**25**) [22], aversin (**26**) [26], 6,8-di-*O*-methylnidurufin (**27**) [27], 4-hydroxybenzaldehyde (**28**) [28], and 3-formyl-1*H*-indole (**29**) [29] by comparing their spectroscopic data (MS, $[\alpha]_D$, UV, IR, and 1D-NMR) with those reported previously.

Phenyl ether derivatives which turned out to be the major components in this study were previously obtained from cultured lichen mycobionts [10][14][30], lichens [31][32], sponges [33][34], and the fungi *Aspergillus* sp. [11–13][21–23][35–40], *Penicillium* sp. [20][41], *Hypocrea citrina* [42], *Emericella falconensis* [43], *Emericella violacea* [44], and *Cordyceps* sp. [24]. To date, only one report appeared on the isolation of phenyl ethers from an endolichenic fungus [40]. It is noteworthy that phenyl ethers from natural lichens generally have carboxy groups, such as micareic acid, while phenyl ethers from the endolichenic fungus [40] and the cultured mycobionts [10][14][30] have no such group. In addition, this is the first report on the presence of bisabolane sesquiterpenes in an endolichenic fungus.

Plausible biosynthetic routes to the new phenyl ethers and bisabolane sesquiterpenoids are outlined in the *Scheme*. The phenyl ethers are closely related to orsellinic acid and gallic acid, which are derived from the acetate-malonate pathway [45] and shikimic acid pathway [46], respectively. A series of dehydration, methylation, cyclization, oxidation reactions, and the addition of dimethylallyl diphosphate (DMAPP) unit to **16** lead to the formation of phenyl ethers **1–4** and **13–15**. The bisabolane nucleus is built up *via* the mevalonic acid pathway [21]. Subsequently, **5–12** are formed through a series of oxidation, reduction, cyclization reactions, and esterification of the bisabolane nucleus.

Biological Activities. All of the isolated compounds were evaluated for their cytotoxic activities against the PC3 (human prostate adenocarcinoma), A549 (human lung adenocarcinoma), A2780 (human ovarian carcinoma), MDA-MB-231 (human metastatic breast adenocarcinoma), and HEPG2 (human hepatocellular carcinoma) cancer cell lines by using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay [47]. As shown in *Table 4*, the cytotoxic activity displayed by *A. versicolor* (125a) was attributed to the presence of phenyl ethers. Among these, compounds **2** and **15** exhibited moderate cytotoxicities against all of the tested human cancer cell lines with IC_{50} values ranging from 19.0 to 36.3 μM . Compounds **3**, **20**, and **17** showed weak cytotoxicities against one to three of the tested human cancer cell lines. The results support the conclusion that the prenyl group (in **2** and **15**) enhances cytotoxic activity, whereas an additional MeO group (in **3**) reduces the activity. No cytotoxic activity was observed with the bisabolane sesquiterpenoids. However, it has

Table 4. Cytotoxic and Antifungal Activities of Compounds^{a)}

Compound	IC_{50} [μ M] ^{b)}					MIC (μ g/ml) <i>Candida albicans</i>
	PC3 ^{c)}	A549 ^{d)}	A2780 ^{e)}	MDA-MB-231 ^{f)}	HEPG2 ^{g)}	
3	> 50	> 50	> 50	43.2 \pm 2.7	> 50	> 64
2	24.0 \pm 0.5	23.9 \pm 1.8	31.0 \pm 0.6	19.0 \pm 1.1	24.3 \pm 0.9	> 64
15	24.7 \pm 1.4	26.2 \pm 3.6	36.3 \pm 2.1	23.2 \pm 2.1	27.3 \pm 1.7	32
20	39.4 \pm 1.7	> 50	> 50	44.9 \pm 0.9	45.2 \pm 1.2	64
17	31.6 \pm 0.8	> 50	> 50	> 50	29.8 \pm 1.0	8
18/21	> 50	> 50	> 50	> 50	> 50	8
19	> 50	> 50	> 50	> 50	> 50	8
27	31.9 \pm 2.3	> 50	33.7 \pm 2.5	28.3 \pm 1.8	> 50	> 64
Cisplatin	18.0 \pm 1.5	19.9 \pm 1.7	14.9 \pm 2.3	18.0 \pm 1.6	14.3 \pm 1.5	NT ^{h)}
Fluconazole	NT	NT	NT	NT	NT	2

^{a)} Compounds which are not listed were inactive in all assays. Cisplatin and DMSO were used as the positive and negative controls, respectively, for the cytotoxicity assays; fluconazole and DMSO were used as the positive and negative controls, respectively, for the antifungal activity assay. ^{b)} The IC_{50} values are means \pm standard deviation of three independent replicates. ^{c)} Human prostate adenocarcinoma.

^{d)} Human lung adenocarcinoma. ^{e)} Human ovarian carcinoma. ^{f)} Human metastatic breast adenocarcinoma. ^{g)} Human hepatocellular carcinoma. ^{h)} NT, Not tested.

been reported that the condensation between phenolic bisabolane-type sesquiterpenoids and diphenyl ethers may increase their cytotoxicity [20].

The inhibitory activities of the isolated metabolites were also assessed against the fungus *Candida albicans* by using the microdilution method [48]. The known compounds **17**, the mixture **18/21**, and **19** exhibited significant activities with an MIC value of 8 μ g/ml. Compounds **15** and **20** exhibited weak antifungal activities with MIC values of 32 and 64 μ g/ml, respectively. All of the new compounds were inactive displaying MIC values greater than 64 μ g/ml.

Conclusions. – We reported 14 new compounds, including four diphenyl ethers, **1**–**4**, eight bisabolane sesquiterpenoids, **5**–**12**, and two pyrogallol ethers, **13** and **14**, together with 15 known compounds, **15**–**29**, isolated from the endolichenic fungus *A. versicolor* (125a). Phenyl ethers were confirmed as the major bioactive components in the extract of *A. versicolor* (125a) by evaluation of cytotoxic and antifungal activities. In addition, plausible biogenetic pathways of the new phenyl ethers and bisabolane sesquiterpenoids are also proposed.

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Experimental Part

General. TLC: Pre-coated SiO_2 GF_{254} plates (*Qingdao Marine Chemical Industry*), visualization by using UV (254 nm) light or by spraying with 10% H_2SO_4 /EtOH, followed by heating. Column chromatography (CC): silica gel (SiO_2 , 200–300 mesh; *Qingdao Marine Chemical Industry*), octadecyl

silica gel (ODS, 40–63 μm ; *Fuji Silysia Chemical, Ltd.*), and *Sephadex LH-20* gel (25–100 μm ; *Pharmacia Biotech*). MPLC: *Lisure EZ Purifier* apparatus equipped with a UV/VIS dual-wavelength detector (210 and 254 nm; *Lisure Science Corporation*) and an ODS column (30 \times 130 mm). Semi-prep. HPLC: *Agilent 1100-G1310A* isopump equipped with a *G1322A* degasser, a *G1314A* VWD detector (210 nm), and a *ZORBAX SB-C₁₈* column (9.4 \times 250 mm, 5 μm). Optical rotations: *Perkin-Elmer 241 MC* polarimeter. UV Spectra: *Shimadzu UV-2450* spectrophotometer; λ_{max} (log ϵ) in nm. CD Spectra: *Chirascan* spectropolarimeter; λ ($\Delta\epsilon$) in nm. IR Spectra: *Nicolet iN 10 Micro FTIR* spectrometer; $\tilde{\nu}$ in cm^{-1} . ^1H - and ^{13}C -NMR Spectra: *Bruker Avance-DRX-600* spectrometer; at 600 (^1H) and 150 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, J in Hz; 2D spectra recorded with standard pulse programs and acquisition parameters. MS: *API-4000* triple-stage quadrupole instrument for electrospray ionization (ESI) and *Finnigan LC-Q^{DECA}* mass spectrometer for HR-ESI; in m/z .

Fungal Material. The endolichenic fungus *A. versicolor* (125a) was isolated from the lichen *Lobaria quercizans*, which was collected from Laojun Mountain, Yunnan Province, P. R. China. It was identified by *Xin Zhang* (Shandong Provincial Key Laboratory of Microbial Engineering, Qilu University of Technology, P. R. China) based on its nuclear ITS rDNA sequences (GenBank: GU227343). The strain, which was assigned the Accession No. 125a, was deposited with the lichen laboratory in the College of Life Sciences, Shandong Normal University, Jinan. The fungal strain was cultured in two 500-ml *Erlenmeyer* flasks, each of which contained 100 ml of potato dextrose broth (PDB), at 25° on a rotary shaker (110 rpm) for 8 d to prepare the seed culture. The fermentation was conducted in 20 *Erlenmeyer* flasks (500 ml), each of which contained 80 g of rice. Dist. H_2O (120 ml) was added to each flask, and the contents were soaked overnight before autoclaving at 120° for 30 min. After cooling to r.t., each flask was inoculated with 10 ml of the spore inoculum and incubated under static conditions at r.t. for 60 d.

Extraction and Isolation. The culture medium containing the mycelium was cut into small pieces and extracted using AcOEt (3 \times 8 l). The org. solvent was evaporated under reduced pressure to yield the crude extract (10.9 g), which exhibited cytotoxicity against the human U2OS cell line (IC_{50} 50.35 $\mu\text{g/ml}$). The AcOEt extract was separated into ten fractions, *Frs. A–J*, on a SiO_2 column ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ from 100:0 to 0:100).

Fr. B (49.5 mg) was subjected to gel permeation chromatography (*Sephadex LH-20*; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1) to afford four subfractions, *Frs. B₁–B₄*. *Fr. B₃* (37.5 mg) was purified by HPLC (83% $\text{MeOH}/\text{H}_2\text{O}$, 1.5 ml/min) to yield **2** (t_R 46.0 min; 5.0 mg) and **15** (t_R 18.0 min; 21.4 mg).

Fr. D (1.3 g) was subjected to CC (*Sephadex LH-20*; same solvent system as described above) to afford four subfractions, *Frs. D₁–D₄*. *Fr. D₂* (213.2 mg) was purified by HPLC (71% $\text{MeOH}/\text{H}_2\text{O}$; 1.8 ml/min) to yield **26** (t_R 38.0 min; 7.7 mg) and **27** (t_R 41.0 min; 6.0 mg). *Fr. D₃* (944.0 mg) was separated by MPLC (ODS, $\text{MeOH}/\text{H}_2\text{O}$ from 50:50 to 100:0) to yield six subfractions, *Frs. D_{3A}–D_{3F}*. *Fr. D_{3A}* (328.9 mg) was further purified by HPLC (60% $\text{MeOH}/\text{H}_2\text{O}$; 1.8 ml/min) to afford **28** (t_R 7.0 min; 23.5 mg), **29** (t_R 9.0 min; 5.5 mg), **4** (t_R 14.0 min; 5.2 mg), **1** (t_R 18.0 min; 4.3 mg), and **3** (t_R 26.3 min; 2.1 mg). Further separation of *Fr. D_{3C}* (55.3 mg) by HPLC (71% $\text{MeOH}/\text{H}_2\text{O}$, 1.8 ml/min) yielded **22** (t_R 25.9 min; 18.2 mg). *Fr. D_{3D}* (126.6 mg) was purified by HPLC (76% $\text{MeOH}/\text{H}_2\text{O}$; 1.8 ml/min) to furnish **17** (t_R 24.0 min; 90.7 mg). Compounds **20** (t_R 13.8 min; 8.8 mg) and **16** (t_R 17.3 min; 7.9 mg) were isolated from *Fr. D₄* (20.5 mg) by using HPLC (65% $\text{MeOH}/\text{H}_2\text{O}$; 1.8 ml/min).

Fr. F (4.5 g) was separated by CC (*Sephadex LH-20*; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1) to afford five subfractions, *Frs. F₁–F₅*. Of these, *Fr. F₃* (747.0 mg) was subjected to HPLC (60% aq. MeOH containing 0.2% CF_3COOH) to yield **5** (t_R 30.0 min; 0.5 mg), **9** (t_R 27.0 min; 1.1 mg), **10** (t_R 22.0 min; 67.1 mg), **11** (t_R 24.0 min; 4.3 mg), and **12** (t_R 37.0 min; 7.5 mg). The separation of the residue of *Fr. F₃*, which was performed using a procedure similar to that used for the separation of *Fr. D₃*, afforded **6** (HPLC; 28% $\text{MeCN}/\text{H}_2\text{O}$ (0.2% CF_3COOH); 1.5 ml/min; t_R 39.0 min, 1.1 mg), **7** (HPLC; 25% $\text{MeCN}/\text{H}_2\text{O}$ (0.2% CF_3COOH); 1.8 ml/min; t_R 24.0 min, 0.2 mg), **8** (HPLC; 28% $\text{MeCN}/\text{H}_2\text{O}$ (0.2% CF_3COOH); 1.5 ml/min; t_R 31.0 min, 0.6 mg), and **23** (HPLC; 50% $\text{MeCN}/\text{H}_2\text{O}$ (0.2% CF_3COOH); 1.8 ml/min; t_R 20.5 min, 2.8 mg). *Fr. F₄* (3.4 g) was purified by MPLC (ODS; 50% $\text{MeOH}/\text{H}_2\text{O}$) to yield four subfractions, *Frs. F_{4A}–F_{4D}*. *Fr. F_{4A}* (2.7 g) was further purified by HPLC (47% $\text{MeOH}/\text{H}_2\text{O}$; 1.8 ml/min) to yield **18** (t_R 30.5 min; 652.8 mg) and **21** (t_R 21.0 min; 572.2 mg) as the major compounds. Compounds **18** and **21** were separated as single compounds but isomerized to give a mixture after standing in MeOH , as reported in [10]. *Fr. F_{4B}* (219.8 mg) was purified by HPLC (55% $\text{MeOH}/\text{H}_2\text{O}$; 1.8 ml/min) to yield **19** (t_R 21.0 min;

24.3 mg), whereas the separation of *Fr. F_{4C}* (162.4 mg) by HPLC (40% MeCN/H₂O; 1.8 ml/min) afforded **13** (*t_R* 23.6 min; 19.5 mg), **14** (*t_R* 18.9 min; 14.5 mg), **24** (*t_R* 27.8 min; 13.9 mg), and **25** (*t_R* 21.4 min; 20.9 mg). Interconversions of **13** and **14**, and of **24** and **25** were observed similarly to those of **18** and **21**.

Diorcinol F (= 3-(3-Hydroxy-2-methoxy-5-methylphenoxy)-5-methylbenzene-1,2-diol; **1**). Red amorphous solid. UV (MeOH): 278 (5.33), 227 (6.31). IR: 3355, 2933, 1662, 1589, 1510, 1459, 1342, 1320, 1229, 1193, 1138, 1076, 1022, 990, 826. ¹H- and ¹³C-NMR: Table 1. HR-ESI-MS: 277.1072 ([*M* + H]⁺, C₁₅H₁₇O₅⁺; calc. 277.1071).

Diorcinol G (= 3-(3-Hydroxy-5-methylphenoxy)-5-methyl-2,6-bis(3-methylbut-2-en-1-yl)phenol; **2**). Brown oil. UV (MeOH): 281 (3.65). IR: 3338, 2965, 2922, 2856, 1611, 1598, 1449, 1321, 1154, 1136, 1075, 1054, 1023, 991, 835. ¹H- and ¹³C-NMR: Table 1. HR-ESI-MS: 367.2267 ([*M* + H]⁺, C₂₄H₃₁O₅⁺; calc. 367.2268).

Diorcinol H (= 4-Methoxy-1,9-dimethyldibenzo[b,d]furan-3,7-diol; **3**). Brown amorphous solid. UV (MeOH): 231 (4.10). IR: 3351, 2971, 2927, 2851, 1612, 1584, 1519, 1450, 1407, 1383, 1269, 1241, 1200, 1149, 1135, 1079, 1000, 838. ¹H- and ¹³C-NMR: Table 1. HR-ESI-MS: 257.0818 ([*M* – H][–], C₁₅H₁₃O₄[–]; calc. 257.0808).

3-Methoxyviolaceol-II (= 2-(2-Hydroxy-3-methoxy-5-methylphenoxy)-5-methylbenzene-1,3-diol; **4**). Red amorphous solid. UV (MeOH): 278 (3.78), 228 (4.68). IR: 3278, 2940, 2861, 1604, 1515, 1463, 1427, 1361, 1319, 1206, 1143, 1083, 1063, 1022, 984, 820. ¹H- and ¹³C-NMR: Table 1. HR-ESI-MS: 277.1072 ([*M* + H]⁺, C₁₅H₁₇O₅⁺; calc. 277.1071).

(–)-(R)-Cyclo-hydroxysydonic Acid (= (3R)-3-(4-Hydroxy-4-methylpentyl)-3-methyl-3H-1,2-benzodioxole-6-carboxylic Acid; **5**). Colorless amorphous solid. [*α*]_D²⁵ = –40.0 (*c* = 0.1, MeOH). UV (MeOH): 294 (3.83), 258 (3.85). CD (MeOH): 297 (–1.83), 262 (–1.81), 227 (–2.30). IR: 3390, 3077, 2968, 1687, 1619, 1605, 1497, 1452, 1381, 1263, 1184, 1118, 1022, 941, 905, 829, 766. ¹H- and ¹³C-NMR: Table 2. HR-ESI-MS: 303.1203 ([*M* + Na]⁺, C₁₅H₂₀NaO₅⁺; calc. 303.1203).

(–)-(7S,8R)-8-Hydroxysydowic Acid (= 3-Hydroxy-4-[(2S,3R)-3-hydroxy-2,6,6-trimethyltetrahydro-2H-pyran-2-yl]benzoic Acid; **6**). Colorless amorphous solid. [*α*]_D²⁵ = –30.0 (*c* = 0.1, MeOH). UV (MeOH): 281 (3.73), 228 (4.48). CD (MeOH): 298 (+3.59), 250 (–4.08). IR: 3242, 2972, 2937, 1699, 1572, 1511, 1438, 1370, 1261, 1220, 1130, 1103, 1065, 1046, 984, 956, 892, 771, 747. ¹H- and ¹³C-NMR: Table 2. HR-ESI-MS: 279.1232 ([*M* – H][–], C₁₅H₁₉O₅[–]; calc. 279.1227).

(–)-(7R,10S)-10-Hydroxysydowic Acid (= 3-Hydroxy-4-[(2R,5S)-5-hydroxy-2,6,6-trimethyltetrahydro-2H-pyran-2-yl]benzoic Acid; **7**). Colorless amorphous solid. [*α*]_D²⁵ = –20.0 (*c* = 0.1, MeOH). UV (MeOH): 278 (4.47), 228 (5.26). CD (MeCN): 255 (+2.69), 240 (+3.33), 225 (+2.53), 218 (–1.75). IR: 3263, 2972, 1694, 1573, 1511, 1436, 1378, 1267, 1221, 1180, 1108, 1068, 1038, 963, 833, 771, 749. ¹H- and ¹³C-NMR: Table 2. HR-ESI-MS: 279.1232 ([*M* – H][–], C₁₅H₁₉O₅[–]; calc. 279.1227).

(–)-(7R,10R)-Iso-10-hydroxysydowic Acid (= 3-Hydroxy-4-[(2R,5R)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl]benzoic Acid; **8**). Colorless amorphous solid. [*α*]_D²⁵ = –30.0 (*c* = 0.1, MeOH). UV (MeOH): 283 (3.69), 228 (4.40). CD (MeCN): 297 (–0.82), 248 (–2.23). IR: 3241, 2974, 2930, 1694, 1574, 1510, 1417, 1375, 1289, 1212, 1182, 1095, 1062, 1022, 948, 892, 772, 749. ¹H- and ¹³C-NMR: Table 2. HR-ESI-MS: 303.1205 ([*M* + Na]⁺, C₁₅H₂₀NaO₅⁺; calc. 303.1203).

(–)-12-Acetoxy-1-deoxysydonic Acid (= 4-[(2R,6S)-7-(Acetyloxy)-2-hydroxy-6-methylheptan-2-yl]benzoic Acid; **9**). Colorless oil. [*α*]_D²⁵ = –60.0 (*c* = 0.1, MeOH). UV (MeOH): 227 (3.87). CD (MeCN): 245 (–3.21), 233 (–5.13). IR: 3436, 2941, 1715, 1611, 1461, 1409, 1392, 1373, 1249, 1183, 1124, 1036, 1019, 862, 780, 709. ¹H- and ¹³C-NMR: Table 2. HR-ESI-MS: 331.1520 ([*M* + Na]⁺, C₁₇H₂₄NaO₅⁺; calc. 331.1516).

Sydowiol D (= 3-[3-(2,6-Dihydroxy-4-methylphenoxy)-2-hydroxy-5-methylphenoxy]-5-methylbenzene-1,2-diol; **13**)/*Sydowiol E* (= 3-[2-(2,6-Dihydroxy-4-methylphenoxy)-6-hydroxy-4-methylphenoxy]-5-methylbenzene-1,2-diol; **14**). Red amorphous solid. UV (MeOH): 277 (3.42). IR: 3388, 2923, 1703, 1598, 1509, 1461, 1312, 1189, 1065, 827. HR-ESI-MS: 383.1128 ([*M* – H][–], C₂₁H₁₉O₇[–]; calc. 383.1125).

Methylation of 13 and 14. MeI (0.2 ml) and K₂CO₃ (62.3 mg) were added to a mixture **13/14** (5.8 mg) in MeCN (7 ml), and the suspension was stirred at 40° for 50 h. At this time, TLC indicated the disappearance of **13** and **14**. The mixture was cooled to r.t., and the reaction was quenched with 2M HCl soln. (22 ml). The mixture was diluted with H₂O and extracted with CH₂Cl₂ (30 ml × 3). The org. layers were combined, washed with H₂O, saturated with NaCl, dried (MgSO₄), filtered, and concentrated *in*

vacuo. The residue was purified by HPLC (75% MeCN/H₂O; 1.8 ml/min) to yield **13a** (0.6 mg) and **14a** (1.3 mg).

2,3,2',1'',3''-Penta-O-methylsydowiol D (= 1-(2,3-Dimethoxy-5-methylphenoxy)-3-(2,6-dimethoxy-4-methylphenoxy)-2-methoxy-5-methylbenzene; **13a**). Colorless amorphous solid. UV (MeOH): 270 (3.53), 206 (5.26). IR: 2937, 2836, 1596, 1578, 1503, 1462, 1415, 1323, 1223, 1130, 1104, 1042, 1005, 967, 816, 775. ¹H- and ¹³C-NMR: Table 3. HR-ESI-MS: 455.2068 ([M + H]⁺, C₂₆H₃₁O₇⁺; calc. 455.2064).

2,3,1',1'',3''-Penta-O-methylsydowiol E (= 2-(2,3-Dimethoxy-5-methylphenoxy)-1-(2,6-dimethoxy-4-methylphenoxy)-3-methoxy-5-methylbenzene; **14a**). Colorless amorphous solid. UV (MeOH): 269 (3.01), 207 (4.92). IR: 2917, 2850, 1596, 1504, 1465, 1415, 1324, 1222, 1129, 1094, 1005, 811, 717. ¹H- and ¹³C-NMR: Table 3. HR-ESI-MS: 477.1879 ([M + Na]⁺, C₂₆H₃₀NaO₇⁺; calc. 477.1884).

Diorcinol I (= 3-(3-Hydroxy-5-methylphenoxy)-5-methyl-2-(3-methylbut-2-en-1-yl)phenol; **15**). Brown oil. UV (MeOH): 281 (3.30). IR: 3324, 2965, 2920, 2857, 1587, 1496, 1453, 1420, 1325, 1211, 1143, 1054, 1023, 989, 834. ¹H- and ¹³C-NMR: Table 1. HR-ESI-MS: 299.1640 ([M + H]⁺, C₁₉H₂₃O₃⁺; calc. 299.1642).

Biological Assays. Cytotoxicities of the compounds against the PC3, A549, A2780, MDA-MB-231, and HEPG2 cell lines were evaluated using the MTT assay [45], with cisplatin as positive control. Growth inhibitory activity against the fungus *Candida albicans* was assessed by the microdilution method [46], with fluconazole as positive control. *Candida albicans* was obtained from Affiliated Qianfoshan Hospital of Shandong University, P. R. China.

Theory and Calculation Details. The calculations were performed with the Gaussian 09 program package [49]. Conformational searches were performed by MD simulations based on the COMPASS force field [50] and by scanning the potential energy surface (PES) on the main chain dihedral angles using the semiempirical AM1. All ground-state geometries were optimized at the B3LYP/6-31G* level at 298 K, followed by calculations of their harmonic frequency analysis to confirm these minima and then calculations of *r.t.* free energies. Electronic excitation energies and rotational strengths in gas phase and in MeOH/MeCN were calculated using TDDFT [51] at the same level in velocity formalism for the first 60 states. The solvent effect of MeOH/MeCN has been modeled by a conductor-like screening model for real solvents (COSMO) [52][53]. The rotatory strengths were summed and energetically weighted following the Boltzmann statistics, and the ECD curves were simulated by using the Gaussian function [54]:

$$\Delta\epsilon(E) = \frac{1}{2.296 \times 10^{-39}} \frac{1}{\sigma\sqrt{\pi}} \times \sum_i \Delta E_i R_i e^{-[(E-\Delta E_i)/\sigma]^2}$$

where σ is half the band width at 1/e height, and ΔE_i and R_i are the excitation energies and rotatory strengths for transition i , resp. A σ value of 0.4 eV was applied for calculations.

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