Tetranorditerpene Lactones, Potent Antifungal Antibiotics for Human Pathogenic Yeasts, from a Unique Species of *Oidiodendron*

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The culture filtrate of a fungus isolated from decaying *Picea glauca* wood and tentatively identified as *Oidiodendron* cf. *truncatum* showed strong antibiotic activity against the pathogenic yeast, *Candida albicans*. Four new tetranorditerpenoids, oidiodendrolides A (3), B (4), and C (5) and oidiodendronic acid (7) were isolated along with three known tetranorditerpenoids, LL-Z1271α (=PR1387) (1), PR1388 (2), and acrostalidic acid (6), from rice fermented by the above fungus. The structures of oidiodendrolides A (3), B (4), and C (5) and oidiodendronic acid (7) were established on the basis of spectroscopic and chemical investigations. The antifungal activity of the above tetranorditerpenoids against the pathogenic yeasts, *Candida albicans* and *Cryptococcus neoformans* is discussed.

Key words *Oidiodendron* cf. *truncatum*; tetranorditerpene lactone; anti-yeast antibiotic; oidiodendrolide; oidiodendronic acid

The incidence of life-threatening fungal infections has steadily increased in immunocompromised hosts such as human immunodeficiency virus (HIV) infected persons and cancer patients.[1] It is well known that *Candida albicans* (Robin) Berkhout is the major opportunistic pathogen in such cases and its resistance to the orally active azoles, which are the most widely used antifungals today, is attracting much attention. Therefore, novel antifungal agents having unique activity against *C. albicans* are required to overcome these infections.

In the course of searching for antifungal substances, we recently reported the isolation of new 18,22-cycloergostane steroids, Mer-NF8054X[2] and emesterones A and B,[3] from the culture filtrates of *Emericella heterothallica* (Kwon, Fennell *et* Raper) Malloch *et* Cain, strains ATCC 16847 (mating type A) and/or ATCC 16824 (mating type a). These compounds had antifungal activity against the pathogenic yeast, *C. albicans*.[3,4]

Screening for further antifungal substances from fungal sources, filtrates from cultures of 24 strains cultivated in potato-dextrose (PD) broth belonging to the genus *Oidiodendron* were tested against *C. albicans*, *Aspergillus fumigatus* Pidoplichko *et* Kirilenko, and *Aspergillus niger* Van Tieghem, and against the bacteria *Staphylococcus aureus* Rosenbach and *Escherichia coli* (Migula) Castellani *et* Chalmers. Five strains possessed antibacterial activity against *S. aureus*, but only the broth of *Oidiodendron* cf. *truncatum* (UAMH 9473) showed especially strong antifungal activity against the pathogenic yeast, *C. albicans* (Table 1). The above results prompted us to investigate the active metabolites of this unique fungus.

Oidiodendron cf. *truncatum* UAMH 9473 was isolated from the wood of a decayed log of white spruce [*Picea glauca* (Moeuch) Voss] collected in Elk Island National Park, Alberta, Canada. This strain resembled *O. truncatum* Barron but differs in cultural morphology and in the size. It is possibly a new species of *Oidiodendron*, but requires further investigation before a name can be applied.

In order to get a large amount of extract, the above fungus was cultivated in various media. Strong antifungal activity against *C. albicans* was also observed in the chloroform extract from fermented rice. The separation and purification of this extract gave four new tetranorditerpenoids, oidiodendrolides A (**3**), B (**4**), and C (**5**) and oidiodendronic acid (**7**), along with LL-Z1271α (=PR 1387) (**1**), PR 1388 (**2**), and acrostalidic acid (**6**). Compounds **1**[5] and **6**[6] were first isolated from the fungus of *Acrostalagmus* (=*Verticillium*) sp. Compounds **1** and **2** were also isolated from *Oidiodendron truncatum* Barron as PR 1387 and PR 1388, respectively.[7] The above extract from *Oidiodendron* cf. *truncatum* UAMH 9473 also gave three degraded anthraquinone derivatives, bisdechlorogeodin (**8**), asterric acid (**9**), and sulochrin (**10**).

The structural elucidation of **3**, **4**, **5**, and **7** and the antifungal activity of **1**—**5** against the pathogenic yeasts are described in this paper.

Structure of 3 The molecular formula of **3** was confirmed to be $C_{16}H_{18}O_6$ by high-resolution electron-impact ionization mass spectrometry (EI-MS). The ^{1}H-NMR spectrum of **3** was similar to that of **2** (Table 2), except for the absence of a methoxy group (δ 3.68 in **2**) in **3**. Therefore, **3** was assumed to be a demethyl derivative of **2**. In order to confirm the structure, **2** was moderately hydrolysed with potassium carbonate to give **3**, which was identical with naturally occurring oidiodendrolide A, based on a comparison of spectroscopic data, including circular dichroism (CD) curves. The structure of oidiodendrolide A was consequently confirmed and is portrayed in **3**. The assignments of the ^{1}H-NMR signals of **3** were determined as shown in Table 2 by analysis of homonuclear ^{1}H–^{1}H shift correlation (^{1}H–^{1}H COSY) spectra.

Structure of 4 The molecular formula of **4** was confirmed to be $C_{17}H_{22}O_7$ by high-resolution EI-MS. The ^{1}H-NMR spectrum of **4** was similar to that of **2** (Table 2), except for the absence of a vinyl proton (δ 6.05 in **2**) in **4** and the presence of methylene protons (δ 2.97, 3.03) and a hydroxy group (δ 2.55) in **4**. The ^{13}C-NMR signals at δ 118.4 and 157.0 assigned to double bond carbons in **2** shifted upfield to δ 38.5, assigned as a methylene carbon, and δ 69.7 assigned

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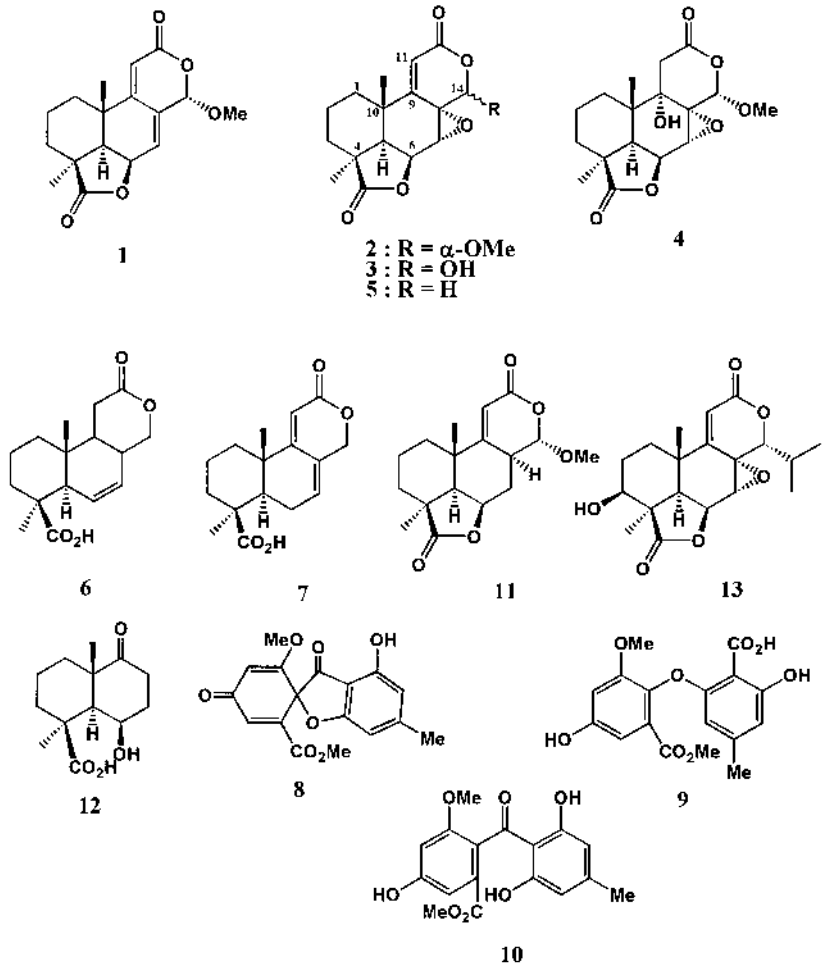

Chart 1

Table 1. Antifungal Screening of the Extracts of *Oidiodendron* spp.

Fungal Sources	Zones of inhibition (mm)				
	C. albicans	*A. fumigatus*	*A. niger*	*S. aureus*	*E. coli*
O. flavum UAMH 1524	—	—	—	10[a)]	—
O. griseum TL 6	—	—	—	13	—
O. griseum TL 10	—	—	—	9[a)]	—
O. cf. *truncatum* UAMH 9473	22	9[a)]	11[a)]	11[a)]	—
O. truncatum UAMH 1399	—	—	—	16	—

The minus (—) means no inhibition. *a*) The inhibition zone was not clear.

to a carbon bearing a tertiary hydroxy group in **4**. From the above results and comparison of the molecular formula to **2**, **4** assumed to be a hydrate of **2**. The planar structure of **4** was determined from detailed analyses of the ^{1}H–^{1}H COSY, ^{1}H–detected heteronuclear multiple-quantum coherence *via* direct bond (HMQC), and heteronuclear multiple bond connectivity by 2D multiple quantum NMR (HMBC) spectra (Fig. 1).

The stereochemistry of **4** was determined by analysis of the difference nuclear Overhauser enhancement (NOE) spectra (Fig. 2) and the 2D NOE spectroscopy (NOESY) spectrum. When the methyl group at C-10 (δ 0.89) was irradiated, 0.3% NOE's were observed on both 7-H (δ 3.87) and 14-H (δ 4.88), along with enhancements on 3β-H (δ 2.08) and 11β-H (δ 2.97). Consequently, these protons are on the same side of the decalin ring, and the epoxide and methoxy group are on the opposite side, relative to the methyl group on the decalin ring. On the other hand, 0.8% and 1.5% NOE's were observed for 5-H (δ 2.09) and 6-H (δ 4.85), respectively, by irradiation of the C-4 methyl residue (δ 1.30). Therefore, the configurations of 5-H and 6-H were determined to be on the same side as the methyl group at C-4. These results confirmed the stereochemistry of oidiodendrolide B (**4**), except for the configuration of the hydroxy group. The stereochemistry of the hydroxy group was determined from the NOE (7.8%) on 5-H (δ 2.09) by irradiation of the hydroxy proton (δ 2.55). The relative structure of oidiodendrolide B was consequently confirmed as shown in **4**. The assignments of the ^{1}H- and ^{13}C-NMR signals of **4** are summarized in Table 2.

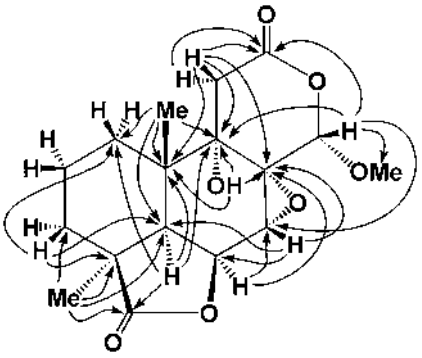

Fig. 1. Correlations in the HMBC Spectrum of **4**

Arrow indicates the HMBC correlation from proton HA to carbon CB: HA→CB.

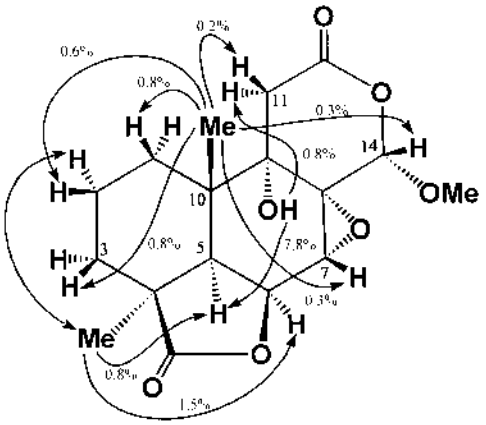

Fig. 2. Differential NOE Observations of **4**

Arrow indicates the NOE on proton HB irradiated at proton HA: HA→HB.

Structure of 5 The molecular formula of **5** was confirmed to be $C_{16}H_{18}O_5$ from analysis of the quasi-molecular ion peak observed at *m/z* 291.1262 in the high-resolution chemical ionization mass spectrum (CI-MS). The ^{1}H-NMR spectrum of **5** was similar to that of **2** (Table 2), except for the absence of methoxy group (δ 3.68 in **2**) and an acetal proton (δ 5.44 in **2**), and the appearance of a methylene protons bearing an oxygen function [δ 4.06 (d), 4.63 (d)] in **5**. The ^{13}C-NMR signal at δ 57.7 assigned to a methoxy carbon in **2** disappeared in **5**, and that at δ 99.4 assigned to an acetal carbon in **2** changed to δ 72.1, assigned to an ether carbon in **5**. Therefore, **5** was assumed to be a demethoxy derivative of **2**. The planar structure of **5** was determined from detailed analyses of the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra (Fig. 3).

The stereochemistry of **5** was determined from the difference NOE experiments (Fig. 4). NOE (0.2%) was observed on 7-H (δ 3.83) along with the enhancements (0.5 and 0.3%, respectively) on 3β-H (δ 2.17) and 14β-H (δ 4.64) when the methyl group at C-10 (δ 1.10) was irradiated, whereas 0.6, 1.0, and 1.5% NOE's were observed on 3α-H (δ 1.44), 5-H (δ 1.81), and 6-H (δ 4.92), respectively, upon irradiation of the other methyl group at C-4 (δ 1.23). Therefore, 7-H is on the same side of the decalin ring as the methyl group at C-10 and the other methyl group at C-4; 5-H, and 6-H were opposite with respect to the above methyl residue. These results confirmed the stereochemistry of oidiodendrolide C (**5**). The relative structure of oidiodendrolide C was consequently confirmed to be as shown in **5**. The assignments of the ^{1}H- and ^{13}C-NMR signals of **5** are summarized in Table 2.

Structure of 7 The molecular formula of **7** was confirmed to be $C_{16}H_{20}O_4$ from the molecular ion peak observed at *m/z* 276.1343 in the EI-MS. The planar structure of **7** was determined from detailed analyses of the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra (Fig. 5). Correlation peaks were observed for 6β-H (δ 2.90) between the methyl group at C-10 (δ 1.01) in the NOESY spectrum (Fig. 6) of **7**, whereas the correlation peaks for 5-H (δ 1.65) and 6α-H (δ 2.54) were observed with the methyl group at C-4 (δ 1.27). These results showed that two methyl groups were opposite to each other in the *trans*-decalin ring, and the proton at C-5 was on the same side as the methyl group at C-4. The relative structure of oidiodendronic acid was consequently confirmed as shown in **7** in Chart 1. The assignments of the ^{1}H- and ^{13}C-NMR signals of **7** are summarized in Table 2. This is the first report of **7** being isolated as a natural product, although this compound has been reported as a synthetic intermediate to **1**.[8]

Absolute Configurations of 2—5 The absolute configuration of **1** was determined as shown in Chart 1 from the Cotton effect at 291—293 nm in the CD spectrum of the cyclohexanone derivative (**12**) derived from the dihydro-LL-Z1271α (**11**) by G. A. Ellestad *et al*.[5] This reference also mentions that the CD curve of **11** showed a negative Cotton effect at 255 nm ($\Delta\varepsilon$ −6.7) for the α,β-unsaturated δ-lactone chromophore. The absolute structure of **2** was not clearly determined in the previous paper.[7] Since **2**, **3**, and **5** had negative Cotton effects at 260—263 nm ($\Delta\varepsilon$ −5.3, −3.4, and −7.9, respectively) in the same way as **11** causing the enelactone systems, the absolute structures of **2**, **3** and **5** were confirmed as being the same as **1** as shown in Chart 1. Since **7** had an $\alpha,\beta,\gamma,\delta$-unsaturated δ-lactone chromophore, showing a negative Cotton effect at 270 nm ($\Delta\varepsilon$ −30.1) in the CD spectrum, the absolute structure of **7** was assumed to be the same as **1**. The absolute stereochemistry of **4** could not be determined because of the absence of an α,β-unsaturated δ-lactone chromophore, although a weak negative Cotton effect at 267 nm ($\Delta\varepsilon$ −0.14) was observed in the CD spectrum. Compound **4** was assumed to have the same absolute configuration as **1**, **2**, **3**, and **5** because they occurred together.

Biological Activity Compound **1** was first reported as an antifungal substance effective against *C. albicans* but no data were provided.[5] Barrero *et al*.[8] determined the minimum inhibition concentrations (MIC) of **1** as 3.12, 3.12, and 3.12 μg/ml for *Saccharomyces cerevisiae* MEYEN *et* HANSEN, *C. albicans*, and *Cryptococcus neoformans* (SANFELICE) VUILLEMIN, respectively. We determined the MIC of **1**—**5** against 11 strains of pathogenic yeast and 4 strains of filamentous fungal pathogens (Table 3).

The antifungal activity against the pathogenic yeast, *C. albicans*, showed MIC's of 8, 16, and 32 μg/ml for **1**, **2**, and **5**, respectively, whereas **3** and **4** showed no antifungal activity up to a concentration of 64 μg/ml. This trend was the same in other pathogenic yeasts, although the activities differed (2—32 μg/ml for **1**, 2—64 μg/ml for **2** and 16—>64 μg/ml for **5**). In contrast, the above compounds (**1**—**5**) had almost no activity against the filamentous pathogens.

Table 2. ^{1}H- and ^{13}C-NMR Chemical Shifts and Coupling Constants (J in Hz) of Tetranorditerpene Lactones (**2**—**7**) in $CDCl_3$

Carbon No.	**2** δ_C	**2** δ_H	**3**[a] δ_H	**4** δ_C	**4** δ_H
1	29.5 t	1.42 m	1.47 m	26.8 t	1.34 m
		1.75 m	1.68 m		1.64 m
2	17.5 t	1.67 m	1.61 m	18.0 t	1.51 m
		1.74 m	1.70 m		1.66 m
3	28.4 t	1.47 m	1.42 m	28.8 t	1.46 m
		2.23 m	2.16 ddd (15.0, 5.8, 5.3)		2.08 m
4	41.8 s			42.2 s	
4α-Me	24.1 q	1.30 s	1.23 s	24.5 q	1.30 s
4β-CO	180.0 s			180.7 s	
5	43.8 d	1.89 d (5.0)	1.80 d (4.2)	41.4 d	2.09 d (4.8)
6	71.9 d	4.95 dd (5.0, 4.7)	4.90 dd (4.2, 1.2)	71.9 d	4.85 br d (4.8)
7	53.0 d	4.02 d (4.7)	3.91 br s	58.0 d	3.87 br s
8	56.2 s			62.1 s	
9	157.0 s			69.7 s	
9α-OH					2.55 br s
10	35.9 s			39.4 s	
10-Me	25.3 q	1.15 s	1.08 s	19.6 q	0.89 s
11	118.4 d	6.05 s	6.00 br s	38.5 t	2.97 d (16.0)
					3.03 d (16.0)
12	162.1 s			168.7 s	
14	99.4 d	5.44 s	5.21 br	101.4 d	4.88 s
14-OMe	57.7 q	3.68 s		58.0 q	3.61 s

Carbon No.	**5** δ_C	**5** δ_H	**6** δ_H	**7** δ_C	**7** δ_H
1	29.6 t	1.47 m	1.01 m	35.9 t	1.46 ddd (12.5, 12.5, 4.0)
		1.68 m	1.53—1.67		1.92 m
2	17.7 t	1.59 m	1.53—1.67	19.2 t	1.65 m
		1.72 m	1.53—1.67		1.92 m
3	28.6 t	1.44 m	1.00 m	37.6 t	1.08 ddd (14.2, 13.6, 1.5)
		2.17 ddd (15.0, 5.8, 5.8)	2.32—2.55		2.23 br d (14,2)
4	42.1 s			43.9 s	
4α-Me	24.3 q	1.23 s	1.33 s	28.3 q	1.27 s
4β-CO	180.4 s			181.9 s	
5	43.9 d	1.81 d (4.3)	2.05 br s	48.9 d	1.65 m
6	72.4 d	4.92 d (4.3)	6.24 br d (10.5)	24.7 t	2.54 ddd (14.7, 5.2, 4.0)
					2.90 br dd (14.7, 11.5)
7	53.4 d	3.83 s	5.40 br d (10.5)	130.9 d	6.09 d
8	55.8 s		2.32—2.55	125.3 s	
9	157.8 s		1.88 m	162.7 s	
10	36.1 s			37.5 s	
10-Me	26.0 q	1.10 s	0.77 s	19.4 q	1.01 s
11	117.8 d	5.95 s	2.64 dd (18.0, 5.0)	109.9 d	5.75 s
			2.23 dd (18.0, 12.5)		
12	162.7 s			165.6 s	
14	72.1 t	4.06 d (12.7)	3.89 dd (11.5, 10.5)	69.6 t	4.76 br d (13.6)
		4.63 d (12.7)	4.46 dd (10.5, 5.0)		4.82 dd (13.6, 1.5)
14-OMe					

a) This spectrum was measured in CD_3OD.

Compound **1** shows the strongest activity against human pathogenic yeasts when compared to **2** and **5**, whereas **3** and **4** showed almost no activity. Compounds **1**, **2**, and **5** have the α,β-unsaturated δ-lactone moiety in the molecule. However, the ene-lactone system of **3** is in equilibrium with an aldehyde–carboxylic acid system and **4** has the non-conjugated δ-lactone system. From the above facts, the planar 6-membered lactone is probably essential for the antifungal activity of this series of compounds, and the activity is decreased when the lactone ring is open and/or non-planar. The γ-lactone moiety in the molecule may be necessary for antifungal activity, but results are inconclusive.

It is interesting that these compounds (**1**, **2**, and **5**) were as active against fluconazole-resistant *C. albicans* strain ATCC 90029 as other strains of this pathogen. With regards to the agents of non-albicans candidiasis, **1** and **2** were especially effective against *C. tropicalis* (Castellani) Berkhout, *C. parapsilosis* (Ashford) Langeron *et* Talice, *C. dubliniensis* D. J. Sullivan, *C. kefyr* (Beijerinck) Van Uden *et* Buckley, *C. guilliermondii* (Castellani) Langeron *et* Guerra, and

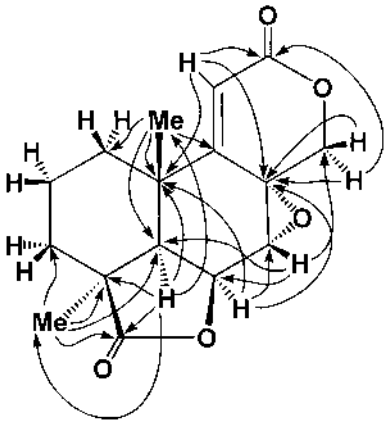

Fig. 3. Correlations in the HMBC Spectrum of **5**

Arrow indicates the HMBC correlation from proton HA to carbon CB: HA→CB.

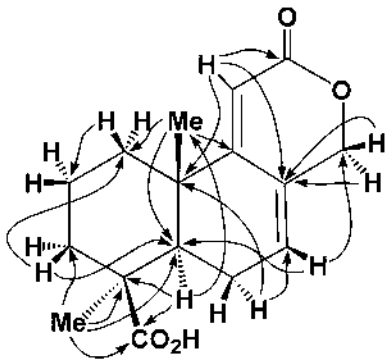

Fig. 5. Correlations in the HMBC Spectrum of **7**

Arrow indicates the HMBC correlation from proton HA to carbon CB: HA→CB.

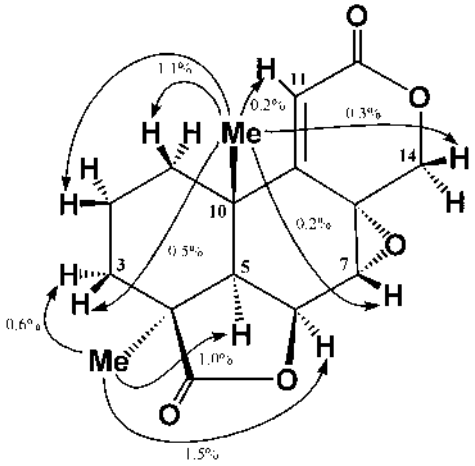

Fig. 4. Differential NOE Observations of **5**

Arrow indicates the NOE on proton HB irradiated at proton HA: HA→HB.

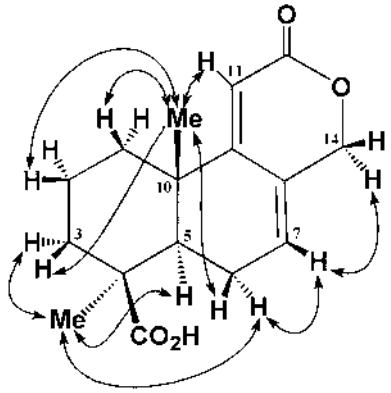

Fig. 6. Correlations in the NOESY Spectrum of **7**

Arrow indicates the NOE correlation between proton HA and proton HB: HA↔HB.

Table 3. Antifungal Activity of Tetranorditerpenoids Isolated from *Oidiodendron* cf. *truncatum*, UAMH9473 (**1**—**5**)

Test organism	Strain	MIC (μg/ml)				
		1	**2**	**3**	**4**	**5**
Candida albicans	ATCC 90028	8	16	>64	>64	32
C. albicans	ATCC 90029	8	16	>64	>64	32
C. albicans	1463D	8	16	>64	>64	32
C. tropicalis	IFM 46816	32	64	>64	>64	64
C. parapsilosis	IFM 46863	8	16	>64	>64	32
C. dubliniensis	CBS 7987	8	8	>64	>64	32
C. kefyr	IFM 46921	2	2	>64	>64	16
C. guilliermondii	IFM 46823	8	32	>64	>64	32
Pichia anomala	IFM 47182	2	4	>64	>64	16
Cryptococcus neoformans	ATCC 90112	4	8	>64	>64	16
Exophiala dermatitidis	Yeast type	16	32	>64	>64	>64
Trichophyton mentagrophytes var. *mentagrophytes*	KCH 1155	32	64	>64	>64	>64
Aspergillus fumigatus	IFM 41243	>64	>64	>64	>64	>64
A. flavus	IFM 41934	64	64	>64	>64	>64
A. niger	H7160B	64	64	>64	>64	>64

Pichia anomala (HANSEN) KURTZMAN, the teleomorph of *C. pelliculosa* REDAELLI. These results were the same as those for *C. albicans*. Notably, a MIC of 2 μg/ml was noted against *C. kefyr* for **1** and **2** and against *Pichia anomala* for **1**. Compounds **1** and **2** were also effective against *C. neoformans*, the causal agent of cryptococcosis, and the yeast form of the

dimorphic dematiaceous fungus, *Exophiala dermatitidis* (KANO) DE HOOG.

Histoplasma capsulatum DARLING [teleomorph: *Ajellomyces capsulatus* (KWON-CHUNG) MCGINNIS *et* KATZ] is a dimorphic fungus that causes histoplasmosis, one of the most severe mycoses.[9] Compound **2** showed a clear inhibition zone against the yeast phase of *H. capsulatum*, although the MIC was not determined. This fact is noteworthy because the causal agents of deep mycoses, *e.g.* coccidioidomycosis caused by *Coccidioides immitis* RIXFORD *et* GILCHRIST, paracoccidioidomycosis caused by *Paracoccidioides brasiliensis* (SPLENDORE) ALMEIDA, and blastomycosis by *Blastomyces dermatitidis* GILCHRIST *et* STOKES (teleomorph: *Ajellomyces dermatitidis* MCDONOUGH *et* LEWIS) are all dimorphic with an infective yeast phase proliferating in the patient.

Nagilactones, *e.g.*, nagilactone E (**13**), are norditerpene dilactones originally isolated from *Podocarpus* sp. (Podocarpaceae),[10] and have basically the same configuration as the oidiodendrolides. These nagilactones had weak antifungal activity against *C. albicans*. The MIC of **13** was 800 μg/ml, but this compound enhanced the antifungal activity of naturally occurring phenylpropanoids.[9]

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. EI- and CI-MS were taken with a JEOL JMS-MS600W spectrometer. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL Lambda-500 (^{1}H, 500.00 MHz; ^{13}C, 125.43 MHz) spectrometer, using tetramethylsilane as an internal standard. CD curves were determined on a JASCO J-600 spectropolarimeter. Column chromatography was performed using Kieselgel 60 (Art. 7734, Merck). Low-pressure liquid chromatography (LPLC) was performed with a Chemco Low-Prep 81-M-2 pump and glass column (200×10 mm) packed with Silica gel CQ-3 (30—50 μm, Wako). TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected by UV light at 254 nm or by spraying with phosphomolybdate reagent and then heating.

Antifungal Screening of the Extracts from *Oidiodendron* spp. Twenty four strains belonging to various species of the genus *Oidiodendron* were cultivated on a rotatory shaker (100 rpm) at 25 °C for 7 to 20 d in 500 ml Erlenmeyer flasks each containing 200 ml of PD broth. The following species and strains were used: *O. cerealis* (VON THUMEN) BARRON UAMH 1522, *O. chlamidosporicum* MORRALL UAMH 6520, *O. citrinum* BARRON UAMH 1525, *O. echinulatum* BARRON UAMH 8467, *O. flavum* SZILVINYI UAMH 1524, *O. griseum* ROBAK UAMH 1403, SH316, TL 6 and TL-10, *O. maius* BARRON UAMH 1540, SH 10Aa, SH 84Aa and TL 7, *O. periconioides* MORRALL UAMH 8527 and TL12, *O. pilicola* KOBAYASI UAMH 7526, *O. rhodogenum* ROBAK UAMH 1405, *O. scytaloides* GAMS *et* SODERSTROM UAMH 6521 and UAMH 8509, *O. setiferum* UDAGAWA *et* TOYAZAKI UAMH 5715, *O. tenuissimum* (PECK) HUGHES UAMH 8511, *O. truncatum* BARRON UAMH 1399, and *O*. cf. *truncatum* UAMH 9473 and TL13. Antifungal and antibacterial activities were determined by paper disc assay with *C. albicans* 7N, *A. fumigatus* 167, *A. niger* H7160B, *S. aureus* 2014, and *E. coli* NIHJ JL-2 as test organisms. The filtered culture broth of *Oidiodendron* sp. was used to soak the paper discs (8 mm diameter) which were placed on the assay plates. *C. albicans, A. fumigatus*, and *A. niger* were cultivated in Sabouraud's glucose (SB) agar at 25 °C, whereas *S. aureus* and *E. coli* were cultivated in brain heart infusion (BHI) agar supplemented with 2% glucose at 37 °C. After 48—72 h incubation for fungi and 24 h incubation for bacteria, zones of inhibition (mm in diameter) were recorded. The results are summarized in Table 1. Fungi which showed no inhibition for any microorganisms tested are not shown.

Isolation of Biologically Active Compounds from *Oidiodendron* cf. *truncatum* *O*. cf. *truncatum* UAMH 9473 was cultivated at 25 °C for 21 d in 36 Roux flasks containing 150 g each of soaked rice in each flask. The fermentated rice was extracted with hexane, $CHCl_3$, and acetone, in turn. Each organic layer was dried over Na_2SO_4 and then evaporated *in vacuo*. The $CHCl_3$ extract (10.5 g) was chromatographed on silica gel with hexane–acetone (2 : 1) to obtain three fractions. From the least polar fractions, LL-Z1271α=PR1387 (**1**) (4 mg) was obtained by recrystallization from hexane-acetone. The more polar fraction was purified by LPLC [hexane–acetone (4 : 1)] to give acrostalidic acid (**6**) (22 mg), and repeated purification using chloroform–acetone (50 : 1) gave PR1388 (**2**) (192 mg) and oidiodendrolide C (**5**) (76 mg). The most polar fraction was purified by LPLC [chloroform–acetone (40 : 1)] to give bisdechlorogeodin (**8**) (3 mg), oidiodendrolide B (**4**) (2 mg) and sulochrin (**10**) (30 mg), and repeated purification using chloroform–acetone (50 : 1) gave oidiodendronic acid (**7**) (2 mg), oidiodendrolide A (**3**) (12 mg), and asterric acid (**9**) (16 mg).

Oidiodendrolide A (**3**): Colorless needles, mp 240—242 °C (from chloroform–acetone). EI-MS *m/z* (%): 306.1119 (M^+, 306.1103 for $C_{16}H_{18}O_6$, 5), 288 ($M-H_2O$, 10), 277 (5), 261 (30), 232 (60), 217 (27), 204 (100). CI-MS *m/z* (%): 307 (M+1, 86), 265 (100). UV λ_{max}^{MeOH} nm (log ε): 211 (4.07). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1785, 1725 (–COO–). CD (MeOH) $\Delta\varepsilon$ (nm): −3.4 (263). The assignments of ^{1}H-NMR signals are summarized in Table 2. The ^{13}C-NMR signals were not measured clearly because of equilibrium between a hemiacetal form and an aldehyde form of **3**.

Oidiodendrolide B (**4**): Colorless needles, mp 247—249 °C (from chloroform–acetone). EI-MS *m/z* (%): 338.1338 (M^+, 338.1365 for $C_{17}H_{22}O_7$, 6), 278 (15), 109 (100). CI-MS *m/z* (%): 339 (M+1, 100). UV λ_{max}^{MeOH} nm (log ε): 208 (3.87), 316 (2.80). IR ν_{max}^{KBr} cm^{-1}: 3530 (OH), 1785, 1765, 1740 (–COO–). CD (MeOH) $\Delta\varepsilon$ (nm): −0.14 (267). The assignments of ^{1}H– and ^{13}C-NMR signals are summarized in Table 2.

Oidiodendrolide C (**5**): Colorless needles, mp 226—228 °C (from hexane–acetone). EI-MS *m/z* (%):290 (M^+, 7), 279 (14), 272 ($M-H_2O$, 10), 219 (91), 91 (100). CI-MS *m/z* (%): 291.1262 (M+1, 291.1232 for $C_{16}H_{19}O_5$, 100). UV λ_{max}^{MeOH} nm (log ε): 221 (4.10), 337 (4.36). IR ν_{max}^{KBr} cm^{-1}: 1785, 1725 (–COO–). CD (MeOH) $\Delta\varepsilon$ (nm): −7.9 (260). The assignments of ^{1}H- and ^{13}C-NMR signals are summarized in Table 2.

Oidiodendronic Acid (**7**): Colorless crystalline powder, mp 155—157 °C (from benzene–acetone). EI-MS *m/z* (%): 276.1343 (M^+, 276.1361 for $C_{16}H_{20}O_4$, 100), 258 ($M-H_2O$, 11), 230 (73), 215(19), 200 (50). UV λ_{max}^{MeOH} nm (log ε): 219 sh (4.08), 276 (4.36). IR ν_{max}^{KBr} cm^{-1}: 3200—2400 (CO_2H), 1720, 1700 (C=O, CO_2H). CD (MeOH) $\Delta\varepsilon$ (nm): −30.1 (270). The assignments of ^{1}H- and ^{13}C-NMR signals are summarized in Table 2.

Demethylation of 2 Potassium carbonate (40 mg) was added to a solution of **2** (49 mg) in MeOH (4 ml) and the mixture was stirred at room temperature for 90 min. The reaction mixture was neutralized with 10% HCl and extracted with chloroform, and the solvent was evaporated *in vacuo*. The residue was purified by LPLC using hexane–ethyl acetate (2 : 1) to obtain a demethyl derivative (**3**) (16 mg, 34%). This compound was identified as the naturally occurring oidiodendrolide A by comparison of the UV, IR, CD, and ^{1}H- and ^{13}C-NMR spectra and TLC behavior.

Antifungal Activity of the Metabolites of *Oidiodendron* spp. The MIC of the isolated metabolites was determined by the serial micro-broth dilution method, with incubation in SB agar at 25 °C for 36—48 h. *C. albicans* ATCC 90028, ATCC 90029 and 1463D, *C. tropicalis* IFM 46816, *C. parapsilosis* IFM 46863, *C. dubliniensis* CBS 7987, *C. kefyr* IFM 46921, *C. guilliermondii* IFM 46823, *Pichia anomala* IFM 47182, *Cryptococcus neoformans* ATCC 90112, *Exophiala dermatitidis* (yeast phase) were used as yeasts, and *Trichophyton mentagrophytes* (ROBIN) BLANCHARD var. *mentagrophytes* (ROBIN) BLANCHARD KCH 1155, *Aspergillus fumigatus* IFM 41243, *A. flavus* LINK: FRIES IFM 41934, *A. niger* H7160B were used as filamentous fungi. The results are summarized in Table 3.

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