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LEPTOSPHAERONES A AND B, NEW CYCLOHEXENONES FROM *LEPTOSPHAERIA HERPOTRICHOIDES*

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ABSTRACT.—Two previously unreported functionalized cyclohexenones, leptosphaerone A [**1**] and leptosphaerone B [**2**], differing in configuration at one of the stereogenic centers, have been isolated from the grass pathogenic fungus *Leptosphaeria herpotrichoides*. The known compounds 2,4-dihydroxy-3,6-dimethylbenzaldehyde, methyl 2,4-dihydroxy-6-methylbenzoate, methyl 2,4-dihydroxy-3,6-dimethylbenzoate, and 4-hydroxy-3,6-dimethyl-2-pyrone were also isolated. On one occasion mevalonic lactone was isolated.

We have previously reported on the metabolites of the grass-destroying fungus *Marasmius oreades* (1,2). *Leptosphaeria herpotrichoides* De Not. (Ascomycotina) [ATCC 38153; =*Phaeosphaeria herpotrichoides* (De Not.) L. Holm=*Trematosphaeria herpotrichoides* (De Not.) G. Wint.] is a common pathogen on grass and cereals found in northern Europe, northwestern US, and western Canada. It causes leaf spotting of wheat, barley, and rye (3), as well as root rot (4,5). We report herein on the metabolites produced when *L. herpotrichoides* is grown in liquid culture.

RESULTS AND DISCUSSION

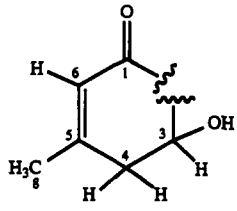
The fungus was grown in liquid shake culture on potato dextrose/yeast extract medium, and the metabolites were isolated by extraction of the filtered culture broth with CH_2Cl_2 followed by EtOAc. They were separated by flash chromatography and preparative tlc of the organic extracts.

Leptosphaerone A [**1**], obtained as a colorless solid, mp 78.0–82.5°, has the molecular formula $C_8H_{12}O_3$ as shown by hreims. Ir bands at 1672 and 1632 cm^{-1} and ^{13}C-nmr (Table 1) signals at 201.6 (s), 123.4 (d), 160.2 (s) ppm indicate the presence of an α,β-unsaturated ketone. A COSY spectrum (Table 1) extends this feature to fragment **A** which accounts for six of the eight carbon atoms in the molecule. The remaining portion of the molecule is represented by fragment **B** as demonstrated by the ^{1}H- and ^{13}C-nmr data [^{1}H δ 1.25, 3H; ^{13}C δ 77.3 (s), 17.7 (q)]. Since there are two D_2O exchangeable protons (3.9, 2.9 ppm) in the ^{1}H-nmr spectrum, two hydroxyl groups are present, one in fragment **A** and the other in fragment **B**. HMBC correlations from the methyl (δ 1.25 ppm) in fragment **B** to C-1 (201.6 ppm), C-2 (77.3 ppm), and C-3 (72.8 ppm) establish the connectivity between these two fragments to arrive at structure **1** (without stereochemistry) for leptosphaerone A. All other HMBC correlations are consistent with this structure.

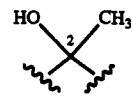
When leptosphaerone A [**1**] was acetylated (Ac_2O/pyridine) a diacetyl derivative was obtained. The ^{1}H-nmr spectrum of this diacetate **3** is similar to that of the parent compound **1**, with two additional signals for the acetyl methyl groups and with H-3 shifted downfield to 6.04 ppm. The diacetate shows HMBC correlations analogous to those of **1** (see Experimental).

Leptosphaerone B [**2**], obtained as a yellow oil, has the molecular formula $C_8H_{12}O_3$, identical with that of leptosphaerone A [**1**]. The nmr spectra (Table 1) and the ir spectrum are also very similar to those of leptosphaerone A [**1**]. The major difference in the ^{1}H-nmr spectrum is the coupling constants of H-3 (3.7 and 2.1 Hz) compared to those in leptosphaerone A (10.0 and 5.8 Hz).

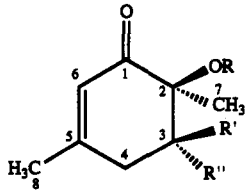
The relative stereochemistry of leptosphaerones A and B was established by a series of nOe experiments. Only



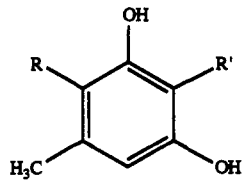
A



B



1 R=H, R′=H, R″=OH
2 R=H, R′=OH, R″=H
3 R=Ac, R′=H, R″=OAc



4 R=CHO, R′=Me
5 R=COOMe, R′=H
6 R=COOMe, R′=Me

leptosphaerone B shows enhancement of the signal of H-3α on irradiation of the Me-7 signal, while both compounds display nOe enhancement of the signal of H-4α on irradiation of the same methyl group signal (Figure 1).

The alternative possibility, that leptosphaerones A and B differ in configuration at C-2 rather than C-3, cannot be rigorously excluded. However, the fact that A and B show similar cd spectra suggests that the configuration at C-2 and the ring conformation remain the same in both compounds.

Three known aromatic compounds were also isolated and identified as 2,4-dihydroxy-3,6-dimethylbenzaldehyde [**4**] (6), methyl 2,4-dihydroxy-6-methylbenzoate [**5**] (7), and methyl 2,4-dihydroxy-3,6-dimethylbenzoate [**6**] (7). Other known compounds present were 4-hydroxy-3,6-dimethyl-2-pyrone (8) and mevalonic lactone (9). The latter was not isolated consistently. The amount

TABLE 1. ^{1}H- and ^{13}C-nmr Data[a] of **1** and **2** (in $CDCl_3$).

Position	Compound					
	1				**2**	
	^{1}H	COSY	HMBC	^{13}C	^{1}H	^{13}C
1				201.6, s		200.8, s
2				77.3, s		77.3, s
3	3.98, dd; 10.0, 5.8	H-4α, H-4β	C-2, C-7	72.8, d	4.13, dd; 3.7, 2.1	73.2, d
4β	2.62, dd; 18.2, 5.8	H-3, H-4α	C-2, -3, -5, -6, -8	37.6, t	2.55, br d; 17.6	36.3, t
4α	2.40, dddq; 18.2, 10.0, 2.6, 1.3	H-3, H-4β, H-6, H-8	C-3, C-5, C-6		2.66, br d; 17.6	
5				160.2, s		158.9, s
6	5.92, dq; 2.6, 1.3	H-4α, H-8	C-2, C-4, C-8	123.4, d	5.96, br s	122.2, d
7	1.25, s, 3H		C-1, C-2, C-3	17.7, q	1.28, s, 3H	22.9, q
8	2.00, br s, 3H	H-4α, H-6	C-4, C-5, C-6	24.4, q	1.90, br s, 3H	24.3, q
OH	2.9, br s; 3.9, br s				2.85, br s, 4.03, br s	

[a]^{1}H-nmr recorded at 400 MHz; ^{13}C-nmr recorded at 100 MHz.

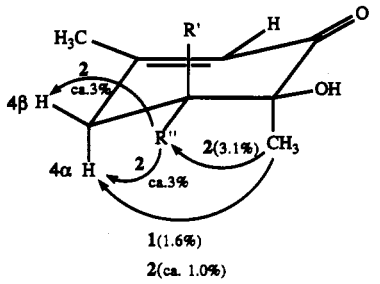


FIGURE 1. Selected nOe's of **1** and **2**.

decreased with the age of the stock culture, and it was not present in the fermentation described in the Experimental section.

Leptosphaerones A and B may be biogenetically related to the aromatic compounds **4** and **6** by loss of the oxygenated carbon substituent, epoxidation, and non-stereospecific ring opening of the epoxide.

The phytotoxicity of the metabolites is under investigation.

EXPERIMENTAL

CULTURE OF *L. HERPOTRICHOIDES* AND ISOLATION OF METABOLITES.—*L. herpotrichoides* was grown in potato dextrose/yeast extract liquid shake culture (5 liters; 20 g potato dextrose/1 g yeast extract per 1 liter H_2O; 4 weeks). The culture broth was separated from the mycelium by filtration, concentrated under reduced pressure to 0.5 liters, and sequentially extracted with CH_2Cl_2 (3×500 ml) and EtOAc (3×500 ml). Repeated Si gel chromatography (column 1–5% MeOH/CH_2Cl_2; preparative tlc 1% MeOH/CH_2Cl_2) of the CH_2Cl_2 extract (215 mg) yielded leptosphaerones A [**1**] (7.8 mg) and B [**2**] (1.8 mg), and compounds **4** (1.7 mg), **5** (2.5 mg), and **6** (3.2 mg). A similar procedure on the EtOAc extract (608 mg) led to the isolation of leptosphaerones A [**1**] (8 mg) and B [**2**] (12 mg), and compounds **4** (2.5 mg), **6** (4 mg), and **7** (3 mg).

Leptosphaerone A [**1**].—Compound **1** was obtained as a pale yellow oil which solidified at 0° forming long needles: mp 78.0–82.5°; $[\alpha]_D$ +1.9° (c=0.47, $CHCl_3$); cd $\Delta\epsilon_{240}$ −1.5, $\Delta\epsilon_{315}$ +0.4 (c=0.009, MeOH); ir (CH_2Cl_2) ν max 3600–3100, 2976, 2931, 1672, 1632, 1435, 1382, 1363, 1166, 1114, 1073, 1026, 997, 866, 741, 658 cm^{-1}; uv (MeOH) λ max 233 nm (8300); hreims *m/z* $[M]^+$ 156.0786 (5%, $C_8H_{12}O_3$, Δmmu=−0.1), 138.0680 (36%, $C_8H_{10}O_2$, Δmmu=−0.1), 113.0601 (17%, $C_6H_9O_2$, Δmmu=−0.2), 96.0572 (44%, C_6H_8O, Δmmu=−0.4), 83.0498 (62%, C_5H_7O, Δmmu=0.1), 74.0370 (100%, $C_3H_6O_2$, Δmmu=0.2); ^1H and ^{13}C nmr see Table 1.

Leptosphaerone B [**2**].—Compound **2** was obtained as a yellow oil: $[\alpha]_D$ +32° (c=0.47, MeOH); cd $\Delta\epsilon_{256}$ −0.25, $\Delta\epsilon_{313}$ +0.7 (c=0.024, MeOH); ir (MeOH) ν max 3600–3100, 2976, 2932, 2910, 1675, 1636, 1435, 1381, 1163, 1115, 1070, 893 cm^{-1}; uv (MeOH) λ max 233 nm (8700); hreims *m/z* $[M]^+$ 156.0789 (7%, $C_8H_{12}O_3$, Δmmu=−0.1), 138.0681 (28%, $C_8H_{10}O_2$, Δmmu=−0.1) 113.0603 (12%, $C_6H_9O_2$, Δmm=−0.1), 96.0575 (32%, C_6H_8O, Δmmu=0.0), 83.0495 (81%, C_5H_7O, Δmmu=0.2), 74.0367 (100%, $C_3H_6O_2$, Δmmu=0.1); cims *m/z* $[M+H+NH_3]^+$ 258 (96%), $[M+H]^+$ 241 (100%), $[M+H-HOAc]^+$ 181 (91%); ^1H and ^{13}C nmr see Table 1.

Leptosphaerone A diacetate [**3**].—Leptosphaerone A (1.8 mg) was kept in a mixture of Ac_2O (0.3 ml) and pyridine (0.4 ml) overnight, toluene (0.5 ml) was added, and the solvent was removed under vacuum to afford pure **3** as colorless oil: $[\alpha]_D$ +3.1° (c=0.16, MeOH); ir (MeOH) ν max 3550–2800, 1746, 1682, 1633, 1433, 1366, 1247, 1229, 1192, 1155, 1094, 1036; uv (MeOH) λ max 232 nm (7400); hreims *m/z* $[M-HOAc]^+$ 180.0787 (1%, $C_{10}H_{12}O_3$, Δmmu=0.0), 155.0709 (23%, $C_8H_{11}O_3$, Δmmu=0.1), 138.0681 (33%, $C_8H_{10}O_2$, Δmmu=0.0), 116.0474 (18%, $C_5H_8O_3$, Δmmu=0.0), 82.0418 (17%, C_5H_6O, Δmmu=0.0), 74.0316 (15%, $C_3H_6O_2$, Δmmu=0.3); ^1H nmr (200 MHz, $CDCl_3$) 1.20 (3H, s, H-7), 2.01 (3H, br m, H-8), 2.05, 2.12 (each 3H, s, 2×OAc), 2.45 (1H, dddq, J=18.0, 10.0, 2.4, 1.0, H-4β), 2.65 (1H, dd, J=18.0, 6.5, H-4α), 5.96 (1H, br m, H-6), 6.04 (1H, dd, J=10.0, 6.5, H-3); HMBC: C-2/H-4, -6, -7; C-3/H-4, -7; C-4/H-6, -8; C-5/H-4, -8; C-6/H-4, -8; C-8/H-4, -6.

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