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ANGUILLOSPORAL, A NEW ANTIBACTERIAL AND ANTIFUNGAL METABOLITE FROM THE FRESHWATER FUNGUS *ANGUILLOSPORA LONGISSIMA*

GEORGE G. HARRIGAN, BETH L. ARMENTROUT, JAMES B. GLOER,*

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242

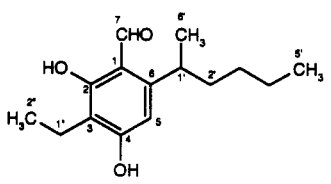
and CAROL A. SHEARER

Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

ABSTRACT.—Anguillosporal [**1**], a new antifungal and antibacterial metabolite, has been isolated from the freshwater fungus *Anguillospora longissima* (CS-869-1A). Its structure was determined as 2,4-dihydroxy-3-ethyl-6-(1′-methylpentyl)-benzaldehyde on the basis of ms, ^{1}H-nmr, and ^{13}C-nmr data.

Fungi isolated from marine and freshwater habitats have not been extensively surveyed as sources of new biologically active natural products. Our chemical investigations of fungi from these sources have resulted in the isolation of several new bioactive metabolites (1–5). Some fungi from aquatic environments are known to display antagonistic effects toward competitors (6). Because our studies of antagonistic coprophilous fungi have been successful in leading to the discovery of novel antifungal agents (7), we have recently expanded our efforts to specifically target antagonistic aquatic fungi as well. Our interest in this area led us to examine an antagonistic isolate of *Anguillospora longissima* (Sacc. & Syd.) Ingold (Hyphomycetes) obtained from a freshwater stream. This study afforded a new antimicrobial metabolite, which we have named anguillosporal [**1**].

Semi-prep. reversed-phase hplc of the EtOAc extract of an *A. longissima* liquid culture afforded compound **1** as the only antifungal component. A molecular formula of $C_{15}H_{22}O_3$ was established for this compound by hreims analysis. The ^{1}H- and ^{13}C-nmr spectral data (Table 1) were consistent with the presence of a tetrasubstituted benzaldehyde moiety, and the ^{1}H-nmr and ir spectra also demonstrated the presence of hydrogen-bonded and non-chelated OH groups. The former was placed ortho to the aldehyde group, while the ^{13}C-nmr shifts and the uv spectrum suggested that the latter be placed para to this group (8).



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The presence of an ethyl substituent and a 1′-methylpentyl group was deduced from the ^{1}H-, ^{13}C-, and DEPT nmr spectra. Selective INEPT (9) experiments were performed to establish the regiochemistry of the alkyl substituents and to verify the positions of the two OH groups. Irradiation of the benzylic methine signal at δ 3.32 resulted in polarization transfer to C-1, C-5, and C-6, placing the 1′-methylpentyl substituent at C-6, while irradiation of the ethyl group methylene signal at δ 2.62 produced enhancements of the C-2, C-3, and C-4 resonances, locating the ethyl substituent at C-3. Other selective INEPT correlations, together with chemical shift considerations, permitted complete carbon assignments, as listed in Table 1. Based on these data, the structure of anguillosporal [**1**] was established as 2,4-dihydroxy-3-ethyl-6-(1′-methylpentyl)-benzaldehyde. The absolute configuration at C-1′ was not determined.

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for Anguillosporal [**1**] in $CDCl_3$.

Position	δ_H (mult., J in Hz)	δ_C (mult.)	Selective INEPT correlations (C#)
1	—	112.1 (s)	
2	—	163.7 (s)	
3	—	114.2 (s)	
4	—	160.5 (s)	
5	6.27 (s)	105.1 (d)	1, 3, 4, 1′
6	—	151.8 (s)	
7	10.15 (s)	192.2 (d)	1, 2
OH-2	12.87 (s)	—	1, 2, 3
OH-4	5.49 (s)	—	
1′	3.32 (br sextet, 6.9)	32.2 (d)	1, 5, 6, 2′, 3′, 6′
2′	1.58 (m)	37.8 (t)	
3′	1.24 (m)	29.6 (t)	
4′	1.24 (m)	22.5 (t)	
5′	0.85 (br t, 6.9)	13.7 (q)	
6′	1.24 (d, 6.9)	21.9 (q)	
1″	2.62 (q, 7.5)	15.1 (t)	2, 3, 4, 2″
2″	1.13 (t, 7.5)	12.8 (q)	

[a]Multiplicities were determined by a DEPT experiment.

Other dihydroxybenzaldehydes have been reported from fungi, including compounds with substitution patterns analogous to that of **1** (10). However, the aliphatic side-chains present in **1** distinguish it structurally from other members of this class. Anguillosporal [**1**] appears to have a polyketide origin, although the presence of an ethyl group is somewhat unusual (10,11). Some polyketide-derived naphthoquinones bearing an ethyl branch on the polyketide chain have been proposed to arise from reduction of an acetyl substituent (11), which would, in turn, originate from acetyl-CoA. Antimicrobial assays conducted on **1** using the broth macrodilution method (12,13) afforded MIC values of 4 μg/ml against *S. aureus* (ATCC 29213) and 58 μg/ml against *C. albicans* (ATCC 90029). To our knowledge, anguillosporal [**1**] is the first secondary metabolite to be reported from a member of the genus *Anguillospora*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ on a Bruker WM-360 spectrometer at 360 MHz and 90 MHz, respectively, using residual solvent signals as internal references. Selective INEPT nmr experiments were carried out at 75 MHz on a Bruker AC-300 spectrometer with $^nJ_{CH}$ optimized for 7 Hz. Ms data were recorded at 70 eV on a VG ZAB-HF mass spectrometer.

FUNGAL MATERIAL.—The original culture of *A. longissima* was isolated from birchwood baits placed in Jordan Creek, a tributary of the Salt Fork River, Vermillion County, Illinois, and collected on 21 February 1990. The culture was assigned the accession number CS-869-1A in the C.A. Shearer culture collection at the University of Illinois at Urbana-Champaign.

EXTRACTION AND ISOLATION.—Five 2-liter Erlenmeyer flasks, each containing 400 ml of potato dextrose broth (Difco) were inoculated with several 1-cm^2 agar plugs taken from a 6-day-old stock culture maintained on potato dextrose agar. Flask cultures were incubated at 25–28° and aerated by agitation on an orbital shaker at 150 rpm for a period of 26 days. The culture filtrate was extracted with EtOAc and the organic phase was evaporated to give an oil (27.0 mg), which was subjected to semi-prep. reversed-phase hplc (5-μm Beckman Ultrasphere C_{18}, 1.0×25.0 cm; 4 ml/min; detection at 220 nm) using a MeCN-0.1% HCOOH gradient (20–100% over 20 min, and then 100% MeCN for 10 min). Compound **1** (8.0 mg) was collected at *R*, 21 min.

Anguillosporal [**1**].—Pale yellow oil: $[\alpha]_D$ −6.7° (c=1.5, $CHCl_3$); uv λ max (MeOH) 222 (log ε 4.19), 299 (4.24) nm; (+NaOH) 215 (4.18), 253 (4.13), 326 sh (4.26), 344 (4.30) nm; ir ν max ($CHCl_3$) 3568, 3261, 2965, 2932, 2874, 1621, 1256 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 1; eims *m/z* 250 (M^+, 62), 235 (8), 232 (10), 217

(16), 207 (53), 194 (38), 193 (28), 189 (100); hreims [M^+] at *m/z* 250.1551 ($C_{15}H_{22}O_3$ requires 250.1569).

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