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OBIONIN A: A NEW POLYKETIDE METABOLITE FROM THE MARINE FUNGUS LEPTOSPHAERIA OBIONES

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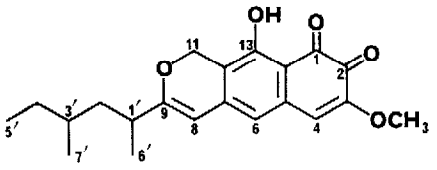
Abstract: Obionin A (1) has been isolated from liquid cultures of the marine fungus Leptosphaeria obiones by chromatography on Sephadex LH-20. Its structure was assigned primarily by selective INEPT and other NMR techniques.

Although many biologically active natural products have been isolated from marine invertebrates and terrestrial microorganisms, chemical studies of bacteria and fungi from marine habitats have been limited.[1-3] During our studies of natural products produced by fungi found in marine environments, we have encountered a novel metabolite produced by the marine fungus Leptosphaeria obiones (Crouan et Crouan) Saccoro, a halotolerant ascomycete isolated from coastal marsh grass. L. obiones is commonly found in the lower portions of coastal marsh grasses that are regularly immersed by the tides, and has been classified as an obligate marine fungus.[4] We wish to report here details of the isolation and structure determination of this new metabolite, which we have named obionin A.

A culture of L. obiones (SAP 14),[5] originally isolated from the salt marsh grass Spartina alterniflora in the coastal marshlands of Sapelo Island, Georgia, was used to inoculate three two-liter erlenmeyer flasks, each containing 400 mL of an artificial marine medium.[6] Flask cultures were incubated at 25-28°C and aerated by agitation on an orbital shaker at 200 rpm for 30 days. The air-dried mycelium from the pooled cultures was extracted exhaustively with ethyl acetate, which was subsequently dried ($MgSO_4$) and evaporated to afford 800 mg of a purple oil. The oil was chromatographed on Sephadex LH-20 with 4:1 CH_2Cl_2-hexane. Fractions of similar composition as determined by TLC analysis were pooled to afford 40 mg of obionin A (1)[7] as a brownish-red solid.

The HRFAB mass spectrum of obionin A suggested the formula $C_{21}H_{24}O_5$. ^{13}C NMR data (Table I) confirmed the presence of twenty-one carbons, including two carbonyl groups, ten other sp^2 carbons, and two sp^3 carbons directly attached to oxygen. The proton NMR spectrum (Table I) contained three vinylic or aromatic proton singlets, two aliphatic methyl doublets, one methyl triplet, and signals for a methoxy group, an isolated CH_2-O unit, a hydrogen-bonded phenol, and several additional aliphatic protons. A series of homonuclear proton decoupling experiments demonstrated that the aliphatic proton signals comprised a single side-chain, and

indicated the presence of two CH-CH_3 units and a CH_2CH_3 group. This information, along with the EIMS fragmentation pattern (major loss of C_5H_{10}) and a series of selective INEPT experiments[8] (Table II) allowed assignment of the side chain structure as a 1,3-dimethylpentyl group, and located it on an oxygen-substituted vinylic carbon.



1

Table I. Proton and Carbon NMR Data for Obionin A (1)

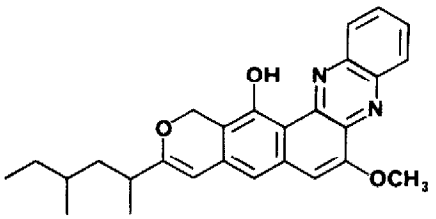
Position	^1H	^{13}C	Position	^1H	^{13}C
1	--	178.7	13	--	162.1
2	--	177.0	14	--	110.9
3	--	151.6	1'	2.42 (m)	36.5
4	6.28 (s)	113.8	2'	1.08, 1.60 (m)	41.5
5	--	134.9	3'	1.28 (m)	32.3
6	6.34 (s)	117.2	4'	1.28, 1.64 (m)	29.9
7	--	144.1	5'	0.84 (t; 7)	11.3
8	5.58 (s)	99.9	6'	1.15 (d; 7)	19.2
9	--	170.9	7'	0.85 (d; 7)	19.3
11	5.07 (d; 14)	62.4	3-OCH_3	3.80 (s)	55.6
	5.16 (d; 14)		13-OH	12.28 (s)	--
12	--	111.6			

*Spectra recorded in $CDCl_3$ at 360 and 90.7 MHz, respectively. All carbon multiplicities are in agreement with the assignments.

Table II. Selective INEPT Two- and Three-bond Correlations for Obionin A (1)

Proton signal irradiated	Carbon signals observed	Proton signal irradiated	Carbon signals observed
H-4	2,3,6,14	H-1'	2',6',8,9
H-6	4,5,8,12,14	H-5'/H-7'	2',3',4'
H-8	1',6,7,9,12	H-6'	1',2',9
H-11	7,9,12,13	OCH_3	3

The remainder of the structure was established as shown in 1 based on the results of a one-bond heteronuclear shift correlation experiment, along with selective INEPT two- and three-bond CH correlations listed in Table II. These data also permitted assignment of all of the proton and carbon signals in obionin A. The presence of an ortho-benzoquinone ring system was suggested by these experiments and by the UV spectrum of the natural product, which showed maxima at 280 and 464 nm[9] that shifted to 350 and 530 nm upon addition of base. Verification of the ortho-quinone structure was obtained by reaction of 1,2-phenylenediamine with obionin A to form the bright orange quinoxaline derivative 2,[10] which was identified by EIMS and NMR analysis.[11] All of these data were consistent with assignment of the structure of obionin A as 1. The stereochemistry of the side-chain in obionin A remains to be assigned. Obionin A contains the first reported 1H-naphtho[2,3-c]pyran 8,9-dione system, although 6,9-diones are known.[12] Obionin A can be classified as a regular nonaketide which has been modified by addition of two C_1-units to its side-chain.



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Although the culture of L. obiones was originally selected for study because of its potent brine shrimp toxicity, compound 1 does not account for that activity. In fact, studies of the bioactivity of obionin A have been hampered by a lack of water solubility. An ichthyotoxic hydroquinone metabolite has been reported from a brown alga which oxidizes in air to the less active ortho-quinone.[13] It remains to be seen whether a similar phenomenon might be responsible for the toxicity of the L. obiones extract. In a screen for potential CNS activity, obionin A inhibited binding of the dopamine D-1 selective ligand ^{3}H-SCH 23390[14] to bovine corpus striatum membrane with an IC_{50} of 2.5 μg/mL. Further studies of the biological activity of 1 and other L. obiones metabolites are under way.

The significant nutritional and environmental differences between marine and terrestrial habitats suggest that marine fungi may have developed secondary metabolic pathways which differ in some respects from those of other fungi. Furthermore, although the classification of many marine fungal isolates as true marine species is sometimes debated, such organisms often represent genera that have not been commonly explored for their ability to produce bioactive secondary metabolites. Prior to this account, only two reports of new metabolites from marine fungi had been published,[15,16] one of these from another Leptosphaeria species.[15]

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References and Notes

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5. The culture was provided by Dr. S. Y. Newell, University of Georgia Marine Station, Sapelo Island, Georgia.
6. Growth medium consisted of Instant Ocean (80% strength), 0.3% yeast extract, 0.3% malt extract, 1.5% D-glucose, and 1.0% soluble starch.
7. Obionin A has the following properties: mp 168-169°C; $[\alpha]_D$ +28.5° (c = 0.01; $CHCl_3$); IR ($CHCl_3$); 3684, 3020, 1684, 1642, 1598 cm^{-1}; UV (MeOH) 464 (ϵ 2090), 280 nm (ϵ 4640); changed to 530 (ϵ 1600), 350 (ϵ 2090), 264 (ϵ 5400) upon addition of NaOH; EIMS (70 eV) ions at m/z 356 (M^+, rel int 64%), 338 (2.9), 299 (2.9), 286 (45), 271 (4.8), 258 (100), 229 (36), 201 (45), 186 (28), 158 (21), 137 (27), 109 (8.7); ^1H NMR and ^{13}C NMR ($CDCl_3$), see Table I; HRFABMS (thioglycerol): obsd; 357.1702; calcd for $C_{21}H_{24}O_5$ + H, 357.1701.
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11. Compound 2; EIMS (70 eV) ions at m/z 428 (M^+; rel int 100%), 413 (4.5), 399 (4.3), 358 (21), 357 (17), 343 (18), 329 (69), 315 (20), 303 (24), 223 (54), 207 (37); ^1H NMR (360 MHz, $CDCl_3$) signals at 8.40 (br dd; 7.2, 1.8); 8.18 (br dd; 7.2, 1.5), 7.86 (ddd; 7, 7, 1.8), and 7.82 ppm (ddd; 7, 7, 1.5), corresponding to the proton signals of the newly formed quinoxaline ring system. All other proton signals were virtually identical to those of compound 1, except for slight downfield shifts for the aromatic and methoxy protons.
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