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ANTIMICROBIAL CONSTITUENT OF THE BROWN ALGA *SPOROCHNUS PEDUNCULATUS*

Lushantha S. Gunasekera, Amy E. Wright, Sarath P. Gunasekera*, Peter McCarthy, and John Reed

Harbor Branch Oceanographic Institution, Inc., 5600 U.S. 1, North, Fort Pierce, Florida 34946, USA

ABSTRACT

Bioassay-guided fractionation of the ethanol extract of the brown alga Sporochnus pedunculatus *led to the isolation of 2-(3'-methylbut-2'-enyl)-4-(1", 1"-dimethylprop-2"-enyl) phenol (1) as the only antimicrobial compound. This compound is a growth inhibitor of* Candida albicans, Cryptococcus neoformans, *and* Bacillus subtilis. *In this paper we report the isolation, identification, and biological activity of 1.*

INTRODUCTION

The compound **1** has been previously isolated from *Perithalia caudata* (Lab.), Womersley, a brown alga of the order Sporochnales, collected from the coast of southern Australia (Blackman *et al.*, 1979). Subsequently, the same group reported the isolation of an isomeric diprenylated phenol 2,4-*bis*(3-methylbut-2-enyl) from this brown alga (Blackman *et al.*, 1988). To our knowledge biological activity has not been reported for these two isomeric compounds. In our search for antifungal substances from marine organisms, an ethanol extract from a brown alga, identified as *Sporochnus pedunculatus* (Hudson) C. Agardh (Phaeophyta, Order Sporochnales) inhibited the growth of *Candida albicans*. This paper describes the isolation, identification and the biological activity of the antimicrobial constituent present in this organism.

Keywords: Sporochnus pedunculatus, brown alga, diprenylated phenol, antimicrobial activity, NMR data.

* Author to whom correspondence should be addressed.

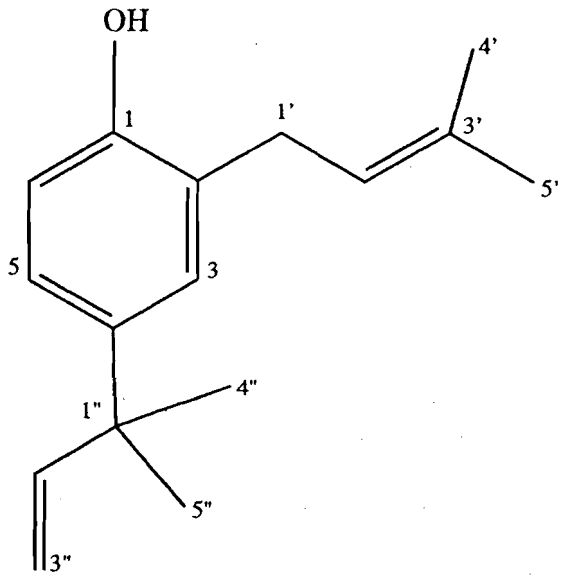

Fig. 1. 2-(3'-Methylbut-2'-enyl)-4-(1",1"-dimethylprop-2"-enyl)phenol (**1**).

MATERIALS AND METHODS

General Experimental Procedures

^{13}C Nmr spectra were measured on a Bruker AM-360 at 90.6 MHz. ^{1}H and all 2D spectra were measured on a Bruker AMX-500 at 500.13 MHz. All Nmr spectra were measured in $CDCl_3$. Chemical shifts were referenced to solvent $CDCl_3$ signal at 7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C. The 2D spectra were run non-spinning.

Plant Material

The brown alga *Sporochnus pedunculutus* was col-

lected by scuba off the southeast coast of Canary Islands at a depth of 3 m. A taxonomic voucher specimen is deposited in the Harbor Branch Foundation Herbarium (Catalog no. 7273, DBMR no. 5-VI-91-3-101).

Antimicrobial Bioassays

Minimum inhibitory concentrations (MICs) were determined by standard microdilution broth techniques (Jones, *et al.*, 1985) in a total volume of 50 µL. Growth media used were as follows: *Candida albicans* (ATCC 44506), Sabouraud dextrose broth (SDB) and RPMI-1640; *Cryptococcus neoformans* (ATCC 32045), Emmon's modification of Sabouraud dextrose broth; *Bacillus subtilis* (ATCC 6633), *Pseudornonas aeruginosa* (ATCC 27853), and *Escherichia coli* (HBOI strain), Mueller-Hinton broth supplemented with Ca^{2+} and Mg^{2+}. Plates were incubated at 37°C for either 24 h (*Can. albicans* and bacteria) or 48 h (*Cr. neoformans*). RPMI-1640 plates were incubated in a humidified atmosphere of 5% (v/v) CO_2. The MIC was determined as the lowest concentration of the drug which completely inhibited growth.

Extraction and Isolation

The freshly thawed brown alga (10 g, wet wt) was extracted three times with EtOH. The concentrated extract was then partitioned between EtOAc and H_2O. The EtOAc soluble fraction (0.2 g) showed activity against *Can. albicans* (MIC = 15.6 µg/ml; RPMI-1640 growth medium). This active fraction was chromatographed on Si gel (Kiesel gel 60 H) using a hexane/CH_2Cl_2 step gradient. The antifungal active fraction (30 mg, *Can. albicans* MIC = 3.1 µg/mL; RPMI-1640) eluted with hexane/20% CH_2Cl_2 and was further purified by passing through a Si Sep-Pak with the same solvent system to isolate **1**, as a pale yellow oil.

Diprenylated Phenol 1

Yield, 24 mg (0.24% wet wt.); NMR data see Table 1.

Biological Activity of 1

The compound **1** showed antimicrobial activity with the following MICs: *Can. albicans* RPMI-1640, 3.1 µg/mL; *Can. albicans* (SDB), 3.1 µg/mL; *Cr. neoformans*, 3.1 µg/mL; and *B. subtilis*, 6.2 µg/mL.

Table 1. ^{13}C- and ^{1}H-nmr Data[a] for **1** in $CDCl_3$.

	^{13}C[b]	^{1}H[c]	HMBC (^{1}H)
1	152.4 s		H-1', H-5, H-6
2	126.3 s		H-1', H-2', H-6
3	127.9 d	7.03 (d, 2.1)	H-1'
4	140.9 s		H-6,H-2",H-4",H-5"
5	125.2 d	7.05 (dd, 8.3, 2.1)	
6	115.3 d	6.71 (d, 8.3)	
1'	30.3 t	3.32 (d, 7.1)	H-2', H-3
2'	122.1 d	5.30 (t, 7.1)	H-1', H-4' H-5'
3'	134.5 s		H-1',H-4',H-5'
4'	17.9 q	1.75 (s)	H-2'
5'	25.7 q	1.76 (s)	H-2'
1"	40.5 s		H-5,H-3"
2"	148.5 d	5.98 (dd, 17.5, 10.4)	H-4"
3"	110.2 t	5.00 (d, 17.5)	
		4.98 (d, 10.4)	
4"	28.4 q	1.35 (s)	H-2"
5"	28.4q	1.35 (s)	H-2"

[a] Table entries are chemical shift, ppm from solvent (multiplicity, *J* in Hz).
[b] Assignments based on APT, DEPT and HMQC experiments.
[c] Assignments based on COSY experiment.

RESULTS AND DISCUSSION

An ethanol extract of *S. pedunculatus* yielded an extract that was partitioned between EtOAc and water. The biologically active EtOAc-soluble fraction was further purified by bioassay-guided cc over Si gel. The antimicrobial compound **1** was isolated as a pale brown oil using a Si Sep-Pak.

Using a combination of APT, DEPT and HMQC experiments the 16 resonance lines in the carbon spectrum were assigned to one aliphatic quaternary carbon, ten sp^2 hybridized carbons (four quaternary, five methine and one methylene), one aliphatic methylene and four methyls. The proton nmr spectrum showed the presence of two *ortho*-coupled and one *meta*-coupled aromatic protons forming an ABX system, which indicated the presence of a 1,2,4-trisubstituted benzene ring. The analysis of the proton spectrum in combination with the ^{1}H-COSY spectrum indicated the presence of two independent ABX spin systems. One spin system constituted a terminal methylene group and the other constituted an aliphatic methylene group coupled to an olefinic proton. These spin systems together with the two vinylic methyls, two tertiary methyls and the remaining unaccounted

for quaternary carbon suggested the presence of a 3-methylbut-2-enyl and a 1,1-dimethylprop-2-enyl functionality in the molecule. The comparison of the ^{13}C- and ^{1}H-chemical shift values (Table 1) with those reported in the literature (Blackman *et al.*, 1979) identifted the compound as 2-(3'-methylbut-2'-enyl)-4-(1",1"-dimethylprop-2"-enyl)phenol. The structure was confirmed by analyzing the long-range C-H correlation results (HMBC, Table 1).

The compound **1** showed significant *in vitro* antiffungal activity against the pathogenic fungi *Can. albicans* and *Cr. neoformans.* The compound also showed antibacterial activity against the Gram-positive bacterium *B. subtilis* but showed no activity against the Gram-negative bacteria *E. coli* and *P. aeruginosa.*

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REFERENCES

Blackman, A.L., Drager, C., and Wells, R.J. (1979). *Aust. J. Chem.* **32:** 2783-2786.

Blackman, A.L., Rogers, G.I., and Volkman, J.K. (1988). *Phytochemistry* **27:** 3686-3688.

Jones, R.N., Barry, A.L., Gavan, T.L., and Washington III, J.A. (1985). In: *Manual of Clinical Microbiology.* Lennette, E.H., Barlow, A., Hausler, W.J. and Shadomy, H.J., ed.), *American Society of Microbiology,* Washington, D.C., Chapter 101, pp. 972-977.

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